

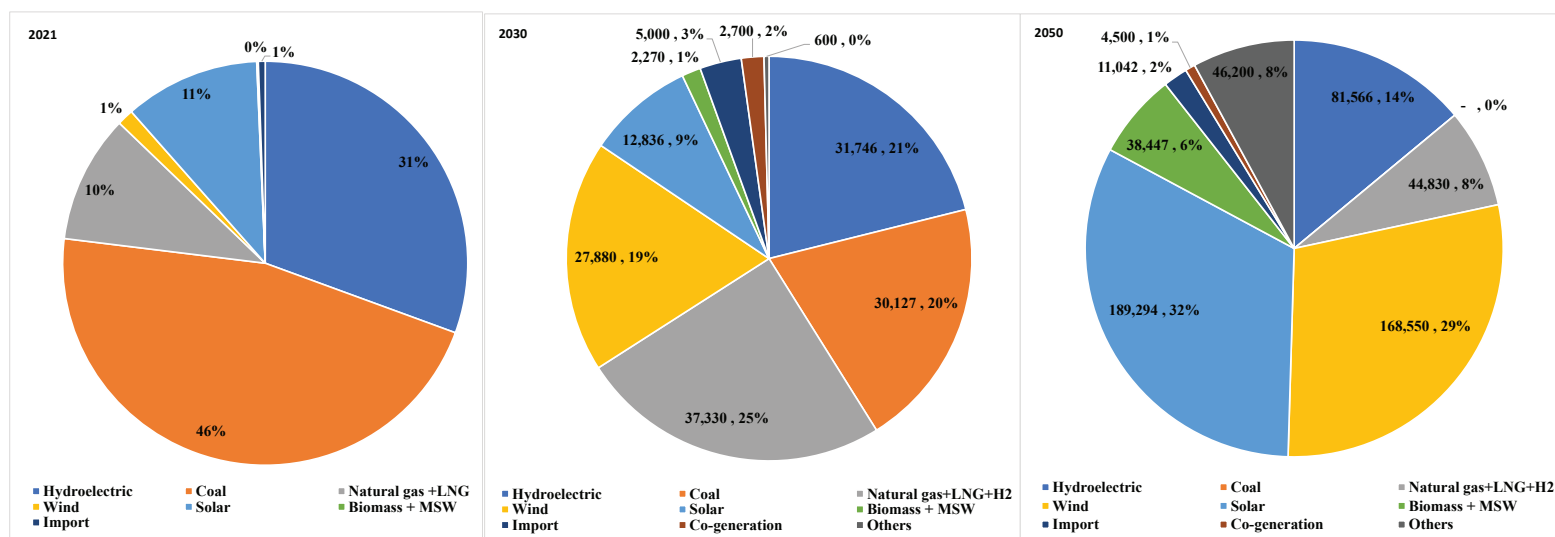


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Investigation of the status and the prospect of fuel pellet production and utilisation toward net zero emissions by 2050 in Vietnam

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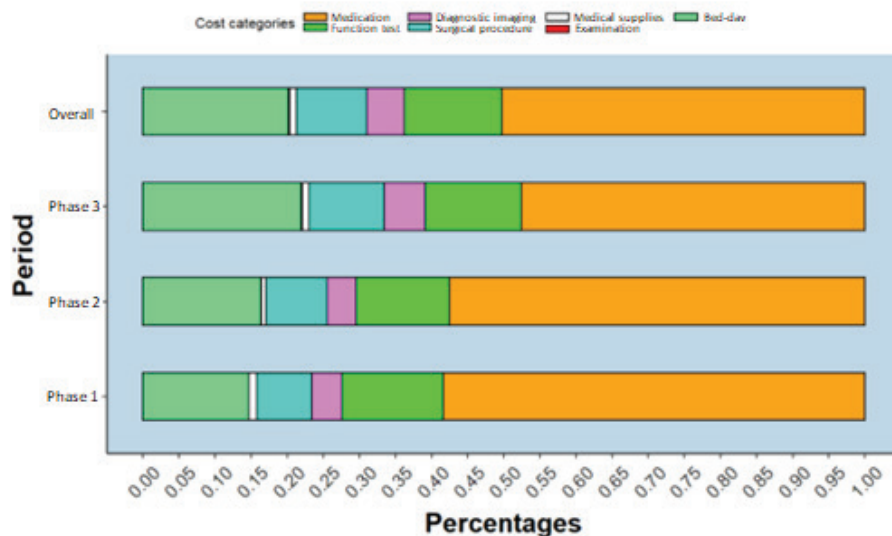
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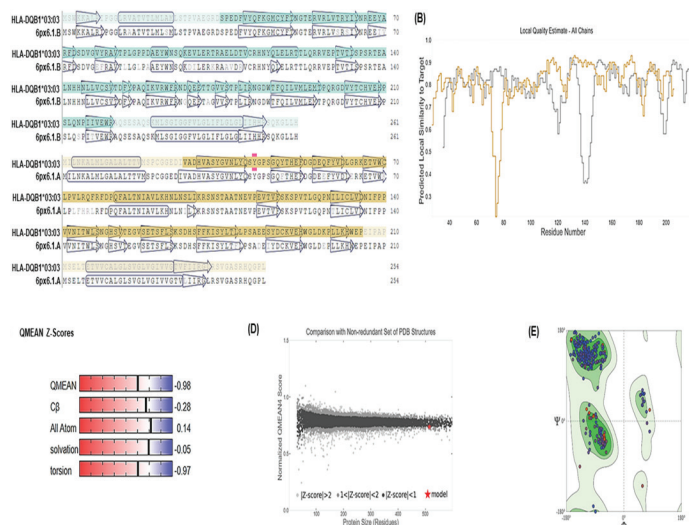
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Improving the convergence velocity of the balancing composite motion optimisation algorithm

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Abstract:

The balancing composite motion optimisation (BCMO), is a metaheuristic algorithm. However, the strengths of BCMO lie in its ability to extract information from the current generation and balance local and global searches. As a result, the convergence velocity of BCMO is superior to other optimisation methods, with a parameter-free and simplified computational procedure. This study proposes an enhanced algorithm, BCMO_A, designed to further increase the convergence velocity of the original BCMO. The proposed enhancement involves introducing an individual selection operator for the new generation. Three candidate options for the selection process were explored, showing minor differences. The best individuals from the two available populations were selected before interaction. The study also emphasises the importance of the terminal condition, which is based on the best value and the mean of objective functions in the current population. Numerical experiments were conducted on 23 classical benchmark functions, and the results were analysed using official statistical tests. The findings demonstrate that the original BCMO has been significantly improved, achieving better convergence speed, with optimised values reached earlier.

Keywords: algorithms, balancing composite motion optimisation, convergence velocity, metaheuristic, optimisation.

Classification numbers: 1.3, 2.3

1. Introduction

Recently, numerous metaheuristic optimisation algorithms have been proposed, yielding promising results across various fields. Natural evolution serves as the inspiration and foundation for several algorithms, such as the Genetic Algorithm (GA) [1], and later, Differential Evolution (DE) [2]. Other optimisation algorithms mimic natural phenomena, including Particle Swarm Optimisation (PSO) [3-5], Ant Colony Optimisation (ACO) [6, 7], Elephant Herding Optimisation [8], among others. Unlike conventional approaches, all metaheuristic algorithms employ an exchange mechanism between local search and global exploration (i.e., exploration and exploitation), with the solution evolution process commonly governed by random motion.

In such intelligent algorithms, local search focuses on a limited domain to identify the best solution within that domain, while global discovery explores a broader domain in search of an improved solution. These search mechanisms work in tandem to achieve the best solution, and the balance between them significantly impacts the quality

of optimisation algorithms. The defining characteristic of each metaheuristic optimisation algorithm is the method used to achieve this balance. Another critical attribute of metaheuristic algorithms is the convergence velocity of the analysis process, particularly for large-scale problems.

Introduced by T.L. Duc, et al. (2020) [9] with some preliminary applications [10], the BCMO is a novel optimisation algorithm that focuses on balancing local and global motion. The key feature of the algorithm is the determination of relative local and global motions, based on information extracted from the current population and the temporary global optimisation point (serving as a surrogate for the true solution of the problem). The BCMO algorithm has been tested and demonstrated superior performance compared to other algorithms. These features, observed in the original study, will be further elaborated upon in this study.

Early application of such algorithm has been applied for the practical optimizing problem in [10]. H.T. Duong, et al. (2020) [10] applied the algorithm to the axial load capacity of a concrete-filled steel tube column. A secondary

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deep learning model was successfully developed, based on the dataset generated from a large-scale implementation of BCMO (i.e., 1000 samples), providing a rough estimation of the optimisation design results.

Clearly, the convergence velocity and computational expense of the algorithm are crucial factors contributing to the success of such complex models, where a large number of optimised variables are required. Metaheuristic algorithms are well-known for their adaptability, allowing for further improvements. It has been observed in the literature that original algorithms can be significantly enhanced with either minor or major adjustments to improve their performance [11-13]. For instance, an adjustment was made by incorporating a Greedy Adaptive control parameter into DE [11]. In contrast to DE, BCMO has the advantage of being parameter-free, requiring only the number of individuals and generations to be preset. The Elephant Herding Optimization (EHO) [8] was modified using feasible rules to solve an optimisation problem with constraints [12]. Similarly, G. Liu, et al. (2016) [13] proposed an inertia weight update strategy to enhance search space coverage and speed for the real-time multiple object tracking problem. Numerous other examples of successful algorithm enhancements can be found in [14-20].

During the algorithm implementation process, the authors observed that BCMO could be adjusted and refined to achieve faster convergence and reduce computational expense. The adjustment involves supplementing the individual selection operator within the procedure and emphasising the importance of the terminal condition, which was mentioned in the original paper [9] but not included in the provided code. This paper is organised as follows: Section 2 summarises the fundamentals of the BCMO algorithm; Section 3 proposes approaches for improving the convergence velocity and speed of the original algorithm. Section 4 presents an extensive numerical comparison between the original and adjusted algorithms (i.e., BCMO versus BCMO_A, respectively).

2. The original balancing composite motion optimisation

The initial population of the BCMO is generated in a manner analogous to other metaheuristic algorithms, by randomly selecting design variables with uniform distribution within their corresponding bounds, as shown in Eq. 1:

$$x_i = x_i^L + \text{rand}(1, d) \times (x_i^U - x_i^L) \quad (1)$$

where x_i^U ; x_i^L are the upper and lower bounds of the i^{th} design variables; $\text{rand}(1, d)$ is a uniform random vector of size $d[0, 1]$.

The new position of the i^{th} ($i > 0$) individual of a given generation in the next generation can be searched by Eqs. 2 and 3:

$$x_i^{t+1} = x_i^t + v_i \quad (2)$$

$$v_i = v_{i/j} + v_j \quad (3)$$

Thus, the new position of the i^{th} individual in Eqs. 2 and 3 is similar to that in PSO, ACO, FA, and DE. The distinction of BCMO lies in the definition of the motion vector v_i , where v_i and v_j represent the motion of i and j relative to the global solution O ; v_{ij} is the relative motion of i^{th} individual to the j^{th} individual, and the j^{th} individual performs better than the i^{th} .

Clearly, v_i depends on two points, one of which is the global solution, or the actual solution to the problem, which has not yet been obtained. Therefore, an alternative for O is proposed by the so-called “instant global optimisation point” O_{in} .

If individuals in the population are ranked in descending order based on the objective function (for minimisation), j is smaller than i , or $i=j=1$. In the simplest case, O_{in} can be taken as the top-ranked individual of the population. However, this may lead to premature convergence due to the consistency of over several generations. To avoid premature termination, O_{in} must be updated by Eq. 4:

$$x_{O_{in}}^t = \begin{cases} u_1^t & \text{if } f(u_1^t) < f(x_1^t) \\ x_1^{t-1} & \text{otherwise} \end{cases} \quad (4)$$

where u_1^t is the trial vector, obtained by extracting information of the current population, as shown in Eq. 5:

$$u_1^t = u_c + v_{k1/k2}^t + x_{k2/1}^t \quad (5)$$

where u_c is the centre point of the design space $[LB, UB]$:

$$u_c = \frac{LB + UB}{2} \quad (6)$$

where $v_{k1/k2}^t$, $x_{k2/1}^t$ are the pseudo relative movements of the k_1^{th} individual to the k_2^{th} and k_2^{th} to the first individual, respectively. k_2^{th} is randomly chosen between $[2, NP]$, NP is the size of the population. The values of are obtained in the same way as v_{ij} and v_i .

With the new location of the individual calculated as in Eqs. 2 and 3, BCMO incorporates the local and global

searches, represented by v_j and v_{ij} , respectively. The global search is defined by Eq. 7:

$$v_j = \alpha_j (x_{oin} - x_j) \quad (7)$$

where α_j is the first-order derivative of the movement distance $x_{oin} - x_j$ and found by Eq. 8:

$$\alpha_j = L_{GS} * dv_j \quad (8)$$

where L_{GS} is the length of the global step size of the j^{th} individual, dv_j is the direction vector with a sign of 0.5:0.5 for positive:negative. L_{GS} and dv_j are found by Eq. 9, which depends on the random number TV_j uniformly distributed within $[0, 1]$:

$$L_{GS} = \begin{cases} e^{\frac{1}{dNP}r_j^2} & \text{if } TV_j > 0.5 \\ e^{\frac{-1}{d(1-NP)}r_j^2} & \text{otherwise} \end{cases} \quad (9)$$

$$dv_j = \begin{cases} rand(1, d) & \text{if } TV_j > 0.5 \\ -rand(1, d) & \text{otherwise} \end{cases} \quad (10)$$

where d is the number of dimensions or direction of S space, NP is the size of the population, $rand(1, d)$ is a random number within $[0, 1]$, and r_j is the distance from the j^{th} individual to O_{in} as in Eq. 11:

$$r_j = \|x_j - x_{oin}\| \quad (11)$$

Analogous to v_j , v_{ij} can be found as in Eqs. 12 and 13:

$$v_{i/j} = \alpha_{ij} (x_j - x_i) \quad (12)$$

$$\alpha_{ij} = L_{LS} \times dv_{ij} \quad (13)$$

where L_{LS} is fixed at 1 to balance the exploration and exploitation of the i^{th} individual; dv_{ij} is the direction vector of i^{th} and j^{th} individual as in Eq. 10.

The i^{th} individual may move towards or away from the O_{in} and j^{th} individual with better performance. If v_{ij} is positive, the i^{th} individual moves into the local exploration domain of the j^{th} individual, and vice versa. Similarly v_j can move away or towards the instant global optimisation point O_{in} after each generation. To balance the exploration and exploitation of each individual in the solution domain, the proportion of positive and negative values for v_{ij} and v_j should be equal.

Figure 1 provides the procedure of the original BCMO proposed in [9], along with numerical experiments conducted on 23 classical benchmark functions for optimisation, the CEC 2014 benchmark functions, and three real-life engineering design problems, all of which yielded promising results. The key advantages of BCMO can be summarised as follows:

(I) The motion of the individual to the new location depends on:

(Ia) information of the best individual at the current generation $[X]_{best}^t$ and

(Ib) the individual considered as the pseudo global point $[X]_{oin}^t$ and

(Ic) the reversal of the motion directions, with 50% balancing the local and global searches.

(II) Unlike other algorithms, BCMO is a parameter-free optimisation algorithm, reducing the need for fine-tuning parameters to obtain optimal solutions.

(III) The convergence velocity of BCMO is notably fast, especially for problems with a large number of design variables. This is crucial for the potential application of BCMO in developing deep learning models, where optimising a high-dimensional set of weights is required, and where multiple computationally expensive algorithms are involved.

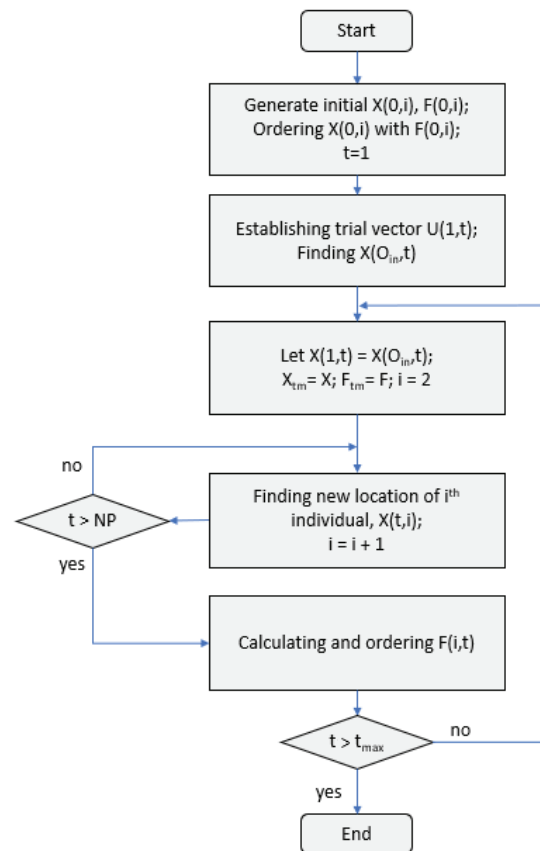


Fig. 1. Flow chart of the original balancing composite motion optimisation.

3. The adjusted algorithm balancing composite motion optimisation_A

3.1. Clarifying the terminal conditions

As with other metaheuristic algorithms, the original BCMO applied 2 terminal conditions simultaneously: a maximum number of interactions and an error value, with the algorithm terminating upon meeting either condition first. However, the code provided with the paper [9] did not include the error value condition. Without this condition, the calculation process may unnecessarily repeat. This can be observed in Eqs. 2 and 3, where the number of vectors v_j and v_{ij} depends on the relative locations of i^{th} and j^{th} and O_{in} as in Eqs. 7 and 8. When the algorithm nears convergence at a minimum point (whether local or global), the individuals move closer to one another, and v_i , v_{ij} will approach zero. In such cases, no motion occurs, yet the number of interactions increases.

To address this drawback, the terminal condition from [2] is proposed. This condition is based on the best and mean objective function values of individuals in the current population, as shown in Eq. 14:

$$\left| F(x)_{Min} - \frac{\sum_{i=1}^{NP} F(x)_i}{NP} \right| \leq \varepsilon \quad (14)$$

3.2. Implementing the selecting operator

Metaheuristic algorithms commonly employ a population selection operator before proceeding to the next iteration. The original BCMO did not discuss this operator. This study proposes incorporating a selection operation during the analysis process to improve the algorithm's convergence speed.

Due to the random nature of the local and global searches, some individuals may have poorer adaptability, affecting the overall population's performance. At the end of an iteration, two populations exist simultaneously (i.e., X_{t-1} and X_t). Using only one population, X_t , for the next iteration results in the loss of valuable information. This study proposes extracting that information by selecting the best NP individuals from the two populations (i.e., selecting from 2NP individuals).

In summary, the flow chart of the adjusted BCMO, termed BCMO_A (presented in Fig. 2), will include the terminal condition outlined in Eq. 14 and the selection operation. The following sections will present the numerical experiments.

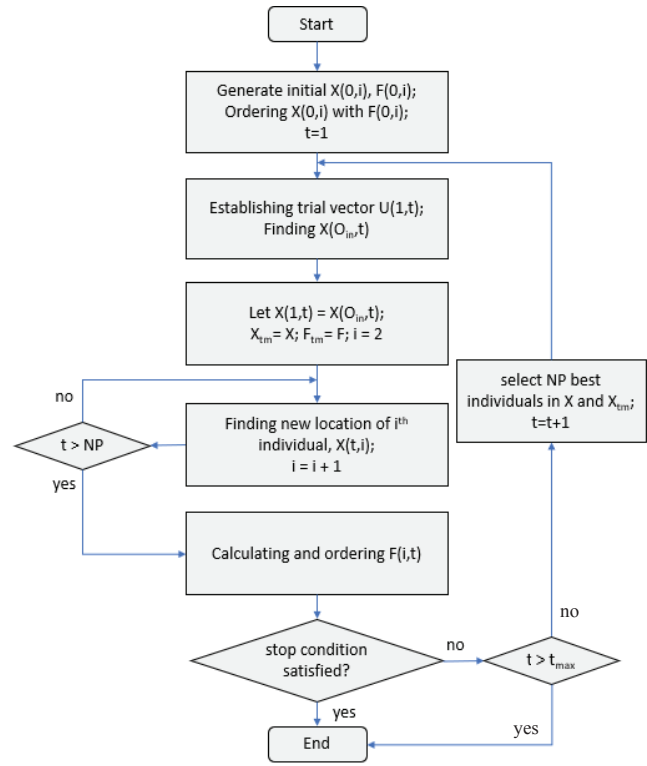


Fig. 2. Flowchart of the adjusted algorithm, balancing composite motion optimisation_A.

4. Numerical experiments

4.1. The validated optimisation problems

To validate the quality of the optimisation algorithm, benchmark functions as described in [2, 21-23] are commonly used. In this study, 23 conventional benchmark functions were employed, as listed in Table 1. The first group consists of single minimum functions, numbered from F01 to F07. Functions F08 to F13 have multiple minima, and functions F14 to F23 have fixed design variables.

4.2. Establishing code for balancing composite motion optimisation and balancing composite motion optimisation_A

Based on T.L. Duc, et al. (2020) [9] and the associated code provided, the program allows for the use of the flowchart in either Fig. 1 or 2. The results generated by the present code for the original BCMO are designated as BCMO* to differentiate them from the results in [9]. Table 2 compares the results of 23 selected functions between BCMO* and BCMO, using the same flowchart as in Fig. 1. Each implementation was conducted with NP=100 and N_{thmax} =1000 as the terminal condition. Such optimisation analyses were implemented on a computer with a configuration of CPU Intel Core i3, 2.53 GHz, 4 GB RAM.

Table 1. Benchmark functions.

ID	I. Functions with single minimum/maximum	nd	Boundaries	Theoretical solution
F01	Sphere model $f_1(x) = \sum_{i=1}^n x_i^2$	30	[-100, 100]	$F_{min} = f(0, 0, \dots, 0) = 0$
F02	Schewefel's problem 2.22 $f_2(x) = \sum_{i=1}^n x_i + \prod_{i=1}^n x_i $	30	[-10, 10]	$F_{min} = f(0, 0, \dots, 0) = 0$
F03	Schewefel's problem 1.2 $f_2(x) = \sum_{i=1}^n \left(\sum_{j=1}^i x_j^2 \right)$	30	[-100, 100]	$F_{min} = f(0, 0, \dots, 0) = 0$
F04	Schewefel's problem 2.21 $f_4(x) = \max_i [x_i , 1] \leq i \leq n$	30	[-100, 100]	$F_{min} = f(0, 0, \dots, 0) = 0$
F05	Generalised rosenbrock's function $f_1(x) = \sum_{i=1}^{n-1} [1000(x_{i+1} - x_i^2)^2 + (x_i - 1)^2]$	30	[-30, 30]	$F_{min} = f(1, 1, \dots, 1) = 0$
F06	Step function $f_6(x) = \lfloor x_i + 0.5 \rfloor^2 \quad [f(x) = \text{floor}(xi + 0.5)^2]$	30	[-100, 100]	$F_{min} = f(0, 0, \dots, 0) = 0$
F07	Quartic function with nose $f_7(x) = \sum_{i=1}^n i x_i^4 + \text{rand}$	30	[-1.28, 1.28]	$F_{min} = f(0, 0, \dots, 0) = 0$
II. Functions with multiple minima/maxima				
F08	Generalised schewefel's problem 2.26 $f_8(x) = \sum_{i=1}^n -x_i \sin(\sqrt{ x_i })$	30	[-500, 500]	$F_{min} = f(421, \dots, 421) = -12569.5$
F09	Generalised rastrigin function $f_9(x) = \sum_{i=1}^n [x_i^2 - 10 \cos(2\pi x_i) + 10]$	30	[-5.12, 5.12]	$F_{min} = f(0, 0, \dots, 0) = 0$
F10	Ackley's function $f_{10}(x) = -20 \exp \left(-0.2 \sqrt{\frac{1}{n} \sum_{i=1}^n x_i^2} \right) - \exp \left(\frac{1}{n} \sum_{i=1}^n \cos(2\pi x_i) \right) + 20 + e$	30	[-32, 32]	$F_{min} = f(0, 0, \dots, 0) = 0$
F11	Generalised griewank function $f_{11}(x) = \frac{1}{4000} \sum_{i=1}^n x_i^2 - \prod_{i=1}^n \cos \left(\frac{x_i}{\sqrt{i}} \right) + 1$	30	[-600, 600]	$F_{min} = f(0, 0, \dots, 0) = 0$
F12	Generalised penalised function $f_{12}(x) = \frac{\pi}{30} [10 \sin^2(\pi y_1) + \sum_{i=1}^{n-1} (y_i - 1)^2 [1 + 10 \sin^2(\pi y_{i+1}) + (y_n - 1)^2]] + \sum_{i=1}^{30} u(x_i, 10, 100, 4)$ $y_i = 1 + \frac{x_i - 1}{4}$ $u(x_i, a, k, m) = \begin{cases} k(x_i - a)^m & \text{if } x_i > a \\ 0 & \text{if } -a < x_i < a \\ k(-x_i - a)^m & \end{cases}$	30	[-50, 50]	$F_{min} = f(1, 1, \dots, 1) = 0$
F13	$f_{13}(x) = 0.1 [\sin^2(3\pi x_1) + \sum_{i=1}^n (x_i - 1)^2 [1 + \sin^2(3\pi x_i + 1)] + (x_n - 1)^2 [\sin^2(2\pi x_n)]] + \sum_{i=1}^n u(x_i, 5, 100, 4)$ $u(x_i, a, k, m) = \begin{cases} k(x_i - a)^m & \text{if } x_i > a \\ 0 & \text{if } -a < x_i < a \\ k(-x_i - a)^m & \end{cases}$	30	[-50, 50]	$F_{min} = f(1, 1, \dots, 1) = 0$
III. Functions with multiple minima/maxima and fixed design variables				
F14	Shekel's foxholes function $f_{14}(x) = \left[\frac{1}{500} + \sum_{j=1}^{25} \frac{1}{\sum_{i=1}^2 (x_i - a_{ij})^6} \right]^{-1}$ $a_{ij} = [-32, -32, -32, -32, -32, -16 \dots 32, 32, 32]$	2	[-65, 65]	$F_{min} = f(-32, -32) = 0.998004$

ID	I. Functions with single minimum/maximum	nd	Boundaries	Theoretical solution
F15	Kowalik's function	4	[-5, 5]	$F_{min}=f(0.1928, 0.1908, 0.1231, 0.1358)=0.0003075$
	$f_{15}(x) = \sum_{i=1}^{11} \left[a_i - \frac{x_1(b_i^2 + b_i x_2)}{b_i^2 + b_i x_3 + x_4} \right]^2$ $a=[0.1957 \ 0.1947 \ 0.1735 \ 0.1600 \ 0.0844 \ 0.0627$ $0.0456 \ 0.0342 \ 0.0323 \ 0.0235 \ 0.0246];$ $b=[4 \ 2 \ 1 \ 1/2 \ 1/4 \ 1/6 \ 1/8 \ 1/10 \ 1/12 \ 1/14 \ 1/16];$			
F16	Six-hump camel-back function $f_{16}(x) = 4x_1^2 - 2.1x_1^4 + \frac{1}{3}x_1^6 + x_1x_2 - 4x_2^2 + 4x_2^4$	2	[-5, 5]	$F_{min}=f(0.08983, -0.7126)=f(-0.08983, 0.7126)=-1.0316285$
F17	Branin function $f_{17}(x) = \left(x_2 - \frac{5.1}{4\pi^2}x_1^2 + \frac{5}{\pi}x_1 - 6 \right)^2$ $+ 10 \left(1 - \frac{1}{8\pi} \right) \cos x_1 + 10$	2	[-5, 5]	$F_{min}=f(-3.142, 12.275);(3.142, 2.275);(9.425,2.275)=0.397887$
F18	Goldstein-price function $f_{18}(x) = [1 + (x_1 + x_2 + 1)^2(19 - 14x_1 + 3x_1^2 - 14x_2 + 6x_1x_2 + 3x_2^2)] \times [30(2x_1 - 3x_2)^2(18 - 32x_1 + 12x_1^2 + 48x_2 - 36x_1x_2 + 27x_2^2)]$	2	[-2,2]	$F_{min}=f(0, -1)=3$
F19	Hartman's family $f_{19}(x) = -\sum_{i=1}^4 c_i \exp \left[-\sum_{j=1}^n a_{ij} (x_j - p_{ij})^2 \right]$ $a = \begin{bmatrix} 3 & 10 & 30 \\ 0.1 & 10 & 35 \\ 3 & 10 & 30 \\ 0.1 & 10 & 35 \end{bmatrix}; \quad p = \begin{bmatrix} 0.3689 & 0.1170 & 0.2673 \\ 0.4699 & 0.4387 & 0.7470 \\ 0.1091 & 0.8732 & 0.5547 \\ 0.03815 & 0.5743 & 0.8828 \end{bmatrix};$ $c=[1; 1.2; 3; 3.2]^T$	3	[0,1]	$F_{min}=f(0.114; 0.556; 0.852)=-3.86$
F20	$f_{20}(x) = -\sum_{i=1}^4 c_i \exp \left[-\sum_{j=1}^n a_{ij} (x_j - p_{ij})^2 \right]$ $a = \begin{bmatrix} 10 & 3 & 17 & 3.5 & 1.7 & 8 \\ 0.05 & 10 & 17 & 0.1 & 8 & 14 \\ 3 & 3.5 & 1.7 & 10 & 17 & 8 \\ 17 & 8 & 0.05 & 10 & 0.1 & 14 \end{bmatrix}; \quad c=[1; 1.2; 3; 3.2]^T$ $p = \begin{bmatrix} 0.1312 & 0.1696 & 0.5569 & 0.0124 & 0.8283 & 0.5886 \\ 0.2329 & 0.4135 & 0.8307 & 0.3736 & 0.1004 & 0.9991 \\ 0.2348 & 0.1451 & 0.3522 & 0.2883 & 0.3047 & 0.6650 \\ 0.4047 & 0.8828 & 0.8732 & 0.5743 & 0.1091 & 0.0381 \end{bmatrix}$	6	[0, 1]	$F_{min}=f(0.201; 0.150; 0.477; 0.275; 0.311; 0.657)=-3.32$
21	Shekel's family $f_{(21)}(x) = -\sum_{i=1}^5 [(x_i - a_i)(x_i - a_i)^T + c_i]^{-1}$ $a = \begin{bmatrix} 4 & 4 & 4 & 4 \\ 1 & 1 & 1 & 1 \\ 8 & 8 & 8 & 8 \\ 6 & 6 & 6 & 6 \\ 3 & 7 & 3 & 7 \\ 2 & 9 & 2 & 9 \\ 5 & 5 & 3 & 3 \\ 8 & 1 & 8 & 1 \\ 6 & 2 & 6 & 2 \\ 7 & 3.6 & 7 & 3.6 \end{bmatrix}; \quad c = \begin{bmatrix} 0.1 \\ 0.2 \\ 0.2 \\ 0.4 \\ 0.4 \\ 0.6 \\ 0.3 \\ 0.7 \\ 0.5 \\ 0.5 \end{bmatrix}$	4	[0, 10]	$F_{min,21}=f(4.00004, 4.00013, 4.00004, 4.00013)=-10.1532;$
22	Shekel's family $f_{(22)}(x) = -\sum_{i=1}^7 [(x_i - a_i)(x_i - a_i)^T + c_i]^{-1}$ $a = \begin{bmatrix} 4 & 4 & 4 & 4 \\ 1 & 1 & 1 & 1 \\ 8 & 8 & 8 & 8 \\ 6 & 6 & 6 & 6 \\ 3 & 7 & 3 & 7 \\ 2 & 9 & 2 & 9 \\ 5 & 5 & 3 & 3 \\ 8 & 1 & 8 & 1 \\ 6 & 2 & 6 & 2 \\ 7 & 3.6 & 7 & 3.6 \end{bmatrix}; \quad c = \begin{bmatrix} 0.1 \\ 0.2 \\ 0.2 \\ 0.4 \\ 0.4 \\ 0.6 \\ 0.3 \\ 0.7 \\ 0.5 \\ 0.5 \end{bmatrix}$	4	[0, 10]	$F_{min,22}=f(4.00057,4.00069, 3.99949, 3.99961)=-10.403;$
23	Shekel's family $f_{(23)}(x) = -\sum_{i=1}^{10} [(x_i - a_i)(x_i - a_i)^T + c_i]^{-1}$ $a = \begin{bmatrix} 4 & 4 & 4 & 4 \\ 1 & 1 & 1 & 1 \\ 8 & 8 & 8 & 8 \\ 6 & 6 & 6 & 6 \\ 3 & 7 & 3 & 7 \\ 2 & 9 & 2 & 9 \\ 5 & 5 & 3 & 3 \\ 8 & 1 & 8 & 1 \\ 6 & 2 & 6 & 2 \\ 7 & 3.6 & 7 & 3.6 \end{bmatrix}; \quad c = \begin{bmatrix} 0.1 \\ 0.2 \\ 0.2 \\ 0.4 \\ 0.4 \\ 0.6 \\ 0.3 \\ 0.7 \\ 0.5 \\ 0.5 \end{bmatrix}$	4	[0, 10]	$F_{min,23}=f(4.00075, 4.00059, 3.99949, 3.99961)=-10.5364$

Table 2. Validating results of the present code.

No. F	BCMO ¹	BCMO*	No. F	BCMO	BCMO*
F01	2.180E-42	1.240E-39	F13	5.100E-03	5.126E-03
F02	3.172E-24	3.977E-25	F14	9.980E-01	9.980E-01
F03	2.937E-30	9.636E-73	F15	3.991E-04	4.601E-04
F04	1.686E-15	3.035E-123	F16	-1.032E+00	-1.032E+00
F05	2.749E+01	2.748E+01	F17	3.979E-01	3.979E-01
F06	2.284E-06	0.000E+00	F18	3.000E+00	3.000E+00
F07	1.960E-02	2.235E-04	F19	-3.863E+00	-3.863E+00
F08	-9.256E+03	-9.242E+03	F20	-3.274E+00	-3.259E+00
F09	0.000E+00	0.000E+00	F21	-7.740E+00	-7.475E+00
F10	8.882E-16	4.441E-16	F22	-9.474E+00	-7.455E+00
F11	0.000E+00	0.000E+00	F23	-9.684E+00	-8.653E+00
F12	1.040E-02	1.037E-02			

¹: results from [9]. BCMO: balancing composite motion optimisations. BCMO*: the results generated by the present code for the original BCMO to differentiate them from the results.

For statistically testing the difference between results from T.L. Duc, et al. (2020) [9] and those of our present code, the Wilcoxon test was conducted, as shown in Table 3. With a p-value of 0.792, the null hypothesis “there is no difference between the results of the two coding programs” - cannot be rejected.

Table 3. Wilcoxon test on the original and adjusted balancing composite motion optimisations.

Methods	Sign test			Wilcoxon test				
Parameter	R ⁻	R ⁺	R ⁰	R ⁻	R ^{avg}	R ⁺	R ^{avg}	p_value
BCMO* - BCMO	9	8	6	60	6.67	93	11.63	0.792

BCMO*: are the results generated by the present code for the original BCMO to differentiate them from the results.

4.3. Options of the selection operation

As discussed earlier, at the end of each interaction, two populations, $[X]_{t-1}$ and $[X]_t$, are generated and there are three potential options that can be used as the selection operator to obtain $[X]_{t+1}$. They are:

- Option 1: From the $2 \times NP$ individuals in $[X]_t$ and $[X]_{t-1}$ at the beginning of an iteration, the NP best individuals are selected for the next iteration $[X]_{t+1}$;

- Option 2: Pairs of NP 2 individuals, each from $[X]_t$ and $[X]_{t-1}$, are compared, and the individual with better performance is selected for $[X]_{t+1}$;

- Option 3: From the $2 \times NP$ individuals, the best NP/2 individuals from each population are selected for the next iteration, $[X]_{t+1}$.

The chosen option is determined based on statistical analyses for the 23 benchmark functions (Table 4). Three metrics are used to assess each option in determining the final choice:

Table 4. Statistical features of options for selection operator.

F	F_{opt}^*	Option 1			Option 2			Option 3		
		F_{opt_mean}	N_{opt}	Time	F_{opt_mean}	N_{opt}	Time	F_{opt_mean}	N_{opt}	Time
F1	0.000E+00	4.430E-10	13	0.294	5.398E-14	0	240.0	0.000E+00	30	0.357
F2	0.000E+00	2.313E-09	16	0.380	2.304E-11	0	292.0	0.000E+00	30	0.536
F3	0.000E+00	3.193E-11	7	0.337	8.230E-19	0	203.0	0.000E+00	30	0.432
F4	0.000E+00	2.737E-11	7	0.096	6.976E-19	0	106.0	0.000E+00	30	0.312
F5	0.000E+00	2.674E+01	0	3.633	2.736E+01	0	1000.0	2.898E+01	0	0.610
F6	0.000E+00	0.000E+00	30	0.967	0.000E+00	30	438.0	0.000E+00	30	0.210
F7	0.000E+00	2.673E-04	0	5.785	3.939E-03	0	5.97	5.943E-04	0	6.549
F8	-1.043E+04	-6.474E+03	0	3.275	-8.272E+03	0	1000.0	-3.277E+03	0	0.793
F9	0.000E+00	4.184E-10	12	0.284	8.911E-14	16	212.0	0.000E+00	30	0.359
F10	0.000E+00	2.710E-09	0	0.514	5.375E-11	0	318.0	4.441E-16	0	0.639
F11	0.000E+00	6.621E-10	10	0.379	3.962E-14	6	212.0	0.000E+00	30	0.527
F12	0.000E+00	1.381E-05	0	3.680	1.037E-02	0	984.0	1.621E+00	0	0.813
F13	0.000E+00	4.324E-01	0	4.494	1.080E-01	0	1000.0	5.399E+00	0	0.812
F14	9.980E-01	3.016E+00	10	0.265	1.197E+00	24	168.0	8.105E+00	0	0.335
F15	3.075E-04	1.457E-03	0	2.645	6.247E-04	2	912.0	2.410E-02	0	0.279
F16	-1.032E+00	-1.032E+00	30	0.093	-1.032E+00	30	100.0	-9.192E-01	0	0.283
F17	3.979E-01	3.979E-01	29	0.097	3.979E-01	30	102.0	5.498E-01	0	0.264
F18	3.000E+00	3.000E+00	30	0.088	3.000E+00	30	106.0	7.773E+00	0	0.276
F19	-3.863E+00	-3.863E+00	30	0.118	-3.863E+00	30	105.0	-3.793E+00	0	0.301
F20	-3.322E+00	-3.233E+00	11	0.259	-3.251E+00	12	190.0	-2.601E+00	0	0.334
F21	-1.015E+01	-7.984E+00	20	0.199	-8.560E+00	22	176.0	-1.581E+00	0	0.381
F22	-1.040E+01	-7.826E+00	19	0.191	-9.207E+00	25	163.0	-1.622E+00	0	0.337
F23	-1.054E+01	-9.245E+00	25	0.025	-9.353E+00	25	190.0	-1.846E+00	0	0.374

F_{opt}^* : represents the theoretical results for the corresponding benchmark function.

1. The mean of optimised results after 30 analyses on the same benchmark function, F_{opt_mean} .
2. The number of times the adjusted BCMO algorithm reaches the theoretical results, N_{opt} .
3. The mean computational time. F_{opt}^* represents the theoretical results for the corresponding benchmark function. Statistical results of the three options, after running each benchmark function 30 times, are presented in Table 4, with a maximum number of generations set to 1000 and an error value ε is $1.e-8$. Paired statistical tests with options 1-2 and 1-3 are given in Table 5. The adjusted BCMO with chosen options is designated as BCMO_A.

Table 5. Statistical tests of the difference between Option 1 to Options 2 and 3.

Method	Parametric	Option 1 - Option 2			Option 1 - Option 3		
		F_{mean}	N_{opt}	Time	F_{mean}	N_{opt}	Time
Sign test	R ⁻	4	6	22	15	6	17
	R ⁺	14	6	1	7	10	6
	R ⁰	5	11	0	1	7	0
Wilcoxon test	R ⁻	42	34	275	225	46	154
	Mean(R ⁻)	10.5	5.67	12.5	15	7.67	9.06
	R ⁺	129	44	1	28	90	122
	Mean(R ⁺)	9.21	7.33	1.0	4	9	20.33
	P_Value	0.606	0.693	0.004	0.974	0.105	0.317

It can be seen in Table 5 that F_{mean} of Option 1 versus Option 2 and Option 1 versus Option 3 are statistically similar, with the corresponding p-values consistently greater than the critical value of 0.05 (i.e., 0.606 and 0.974, respectively). Likewise, for N_{opt} , no significant differences are seen, although the p-value of Option 1 versus Option 3 is somewhat lower at 0.105 but still exceeds the critical p-value threshold. The only noticeable difference arises in the computation time for Option 1 compared to Option 2, with a p-value of 0.004. In the case of Option 1 versus Option 3, no statistically significant difference in time is observed. Therefore, Option 1 was selected for the selection operation in this study.

4.4. Comparing original and adjusted balancing composite motion optimisation

The results comparing the original and adjusted BCMO algorithms are presented in Table 6. For each benchmark function, both the original algorithm (BCMO) and the adjusted algorithm (BCMO_A) were run 30 times. The recorded data include: the mean value of optimised objective functions (i.e., F_{mean} and F_{mean_A} for BCMO and BCMO_A respectively), the best of optimised objective functions (i.e., F_{best} and F_{best_A} for BCMO and BCMO_A respectively), the number of times the algorithm reach the theoretical optimised value (i.e., N_{opt} and N_{opt_A} for BCMO and BCMO_A, respectively, maximum=30), and the computational time required for the algorithm on the same computer configuration as mention in Section 4.2 (i.e., T and T_A for BCMO and BCMO_A, respectively). The T_A/T ratio is also provided to offer a quick overview of the improvement in computation time due to the selection operator.

It can be seen that in most cases (except for F7), the additional operations successfully reduced computation time, from 0.8739 (F15) to 0.0266 (F23).

For functions with a single minimum or maximum (i.e., F1 to F7), F_{mean} values of BCMO are superior to those of BCMO_A. However, the probability of converging to the theoretical optimised value is higher for BCMO_A than for BCMO. For functions with multiple minima or maxima, the selection operation does not significantly affect the capacity of the algorithm to find the global minimum or maximum. The most significant observation in Table 6 is the difference in computation time between the two algorithms. The adjusted BCMO_A, with the added selection operation, converges faster than the original algorithm. The only exception in computation time occurs with F7, where both BCMO and BCMO_A stop due to reaching the maximum number of generations. Formal statistical tests on the differences between the two algorithms are presented in Table 7. The results indicate that: (1) there is no statistical difference in the solution-searching capacity or the ability to reach the theoretical solutions between BCMO and BCMO_A, and (2) BCMO_A significantly reduces computation time compared to the original BCMO. Thus, while preserving the quality of solution searching, BCMO_A demonstrates improved convergence speed.

Table 6. Comparison of balancing composite motion optimisation and balancing composite motion optimisation_A (NP=100; $N_{thmax}=1000$; $\epsilon=1.0E-8$).

Function	F_{opt}^*	BCMO_A				BCMO				T_A/T
		F_{mean_A}	F_{best_A}	N_{opt}	T_A (s)	F_{mean}	F_{best}	N_{opt}	T (s)	
F1	0.000E+00	4.430E-10	0.000E+00	13	0.294	6.641E-18	4.419E-28	0	1.737	0.1693
F2	0.000E+00	2.313E-09	0.000E+00	16	0.380	7.306E-13	3.208E-17	0	2.142	0.1774
F3	0.000E+00	4.966E-11	0.000E+00	5	0.337	6.334E-21	5.835E-55	0	0.600	0.5617
F4	0.000E+00	4.806E-11	0.000E+00	5	0.096	2.141E-20	8.155E-31	0	0.632	0.1519
F5	0.000E+00	2.674E+01	2.625E+01	0	3.633	2.745E+01	2.732E+01	0	4.227	0.8595
F6	0.000E+00	0.000E+00	0.000E+00	30	0.967	0.000E+00	0.000E+00	30	2.580	0.3748
F7	0.000E+00	2.673E-04	6.980E-07	0	5.785	1.244E-02	1.560E-03	0	5.623	1.0288
F8	-1.043E+04	-6.474E+03	-9.012E+03	0	3.275	-9.353E+03	-1.067E+04	0	5.778	0.5668
F9	0.000E+00	4.184E-10	0.000E+00	12	0.284	0.000E+00	0.000E+00	30	1.890	0.1503
F10	4.441E-16	2.710E-09	4.441E-16	0	0.514	1.244E-12	4.441E-16	0	3.060	0.1680
F11	0.000E+00	6.621E-10	0.000E+00	10	0.379	6.291E-17	0.000E+00	29	2.664	0.1423
F12	0.000E+00	1.982E-06	2.894E-08	0	3.680	6.911E-03	3.313E-03	0	7.483	0.4918
F13	0.000E+00	4.324E-01	2.412E-08	0	4.494	5.502E-03	3.495E-07	0	7.726	0.5817
F14	9.980E-01	3.016E+00	9.980E-01	10	0.265	9.980E-01	9.980E-01	30	0.900	0.2944
F15	3.075E-04	5.658E-04	3.075E-04	4	3.090	5.211E-04	3.075E-04	19	3.536	0.8739
F16	-1.032E+00	-1.032E+00	-1.032E+00	30	0.093	6.282E-13	-1.032E+00	0	2.150	0.0433
F17	3.979E-01	3.979E-01	3.979E-01	29	0.097	3.979E-01	3.979E-01	30	0.485	0.2000
F18	3.000E+00	3.000E+00	3.000E+00	30	0.088	3.000E+00	3.000E+00	30	0.516	0.1705
F19	-3.863E+00	-3.863E+00	-3.863E+00	30	0.118	-3.863E+00	-3.863E+00	30	0.601	0.1963
F20	-3.322E+00	-3.233E+00	-3.322E+00	11	0.259	-3.255E+00	-3.322E+00	13	1.061	0.2441
F21	-1.015E+01	-7.984E+00	-1.015E+01	20	0.199	-7.483E+00	-1.015E+01	18	0.85	0.2341
F22	-1.040E+01	-7.184E+00	-1.040E+01	16	0.191	-8.330E+00	-1.040E+01	21	0.88	0.2170
F23	-1.054E+01	-9.245E+00	-1.054E+01	25	0.025	-8.391E+00	-1.054E+01	21	0.94	0.0266

BCMO: balancing composite motion optimisations. F_{opt}^* : represents the theoretical results for the corresponding benchmark function.

Table 7. Statistical tests on the performance of balancing composite motion optimisation versus balancing composite motion optimisation_A.

Metric \ Test method	Sign test			Wilcoxon test				
	R^-	R^+	R^0	R^-	R^-_{avg}	R^+	R^+_{avg}	$p-value$
F_{mean_A}/F_{mean}	6	13	4	77	12.83	113	12.83	0.606
F_{best_A}/F_{best}	10	1	12	55	5.5	11	11	0.659
T_A/T	21	2	0	273	13	3	1.5	0.0017

5. Conclusions

The original BCMO, which balances global and local searches, is a promising optimisation algorithm, particularly for optimising functions with large design variables. Its major advantage is its parameter-free nature, which makes it both practical and convenient for users. This study proposed a modification to the original BCMO by adding a selection operation to improve the algorithm. The effectiveness of three candidate selection methods for choosing the new

population from the $2 \times NP$ individuals was investigated. Numerical experiments comparing the performance of BCMO and BCMO_A show that while maintaining the original algorithm's quality, the proposed adjustment significantly improves convergence speed.

CRediT author statement

Nang Duc Bui: Conceptualisation, Supervision; Thang Quan Nguyen: Conceptualisation, Methodology, Writing - Original draft preparation; Hieu Chi Phan: Conceptualisation, Validation, Writing - Original draft preparation; Dung-Nhan Nguyen Bui: Conceptualisation, Writing - Reviewing and Editing.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] Z. Michalewicz (1996), *Genetic Algorithms + Data Structures = Evolution Programs*, Springer, DOI: 10.1007/978-3-662-03315-9.
- [2] K.V. Price, R.M. Storn, J.A. Lampinen (2006), *Differential Evolution: A Practical Approach to Global Optimization*, Springer, DOI: 10.1007/3-540-31306-0.
- [3] Q. Bai (2010), "Analysis of particle swarm optimization algorithm", *Computer and Information Science*, **3**(1), pp.180-184, DOI: 10.5539/cis.v3n1p180.
- [4] R.C. Eberhart, Y. Shi (2001), "Particle swarm optimization: Developments, applications and resources", *Proceedings of The 2001 Congress on Evolutionary Computation (IEEE Cat. No. 01TH8546)*, **1**, pp.81-86, DOI: 10.1109/CEC.2001.934374.
- [5] J. Kennedy, R.C. Eberhart, Y. Shi (2001), *Swarm Intelligence*, Elsevier, DOI: 10.1016/B978-1-55860-595-4.X5000-1.
- [6] M. Dorigo, M. Birattari, T. Stutzle (2006), "Ant colony optimization", *IEEE Computational Intelligence Magazine*, **1**(4), pp.28-39, DOI: 10.1109/MCI.2006.329691.
- [7] M. Dorigo, G.D. Caro (1999), "Ant colony optimization: A new meta-heuristic", *Proceedings of The 1999 Congress on Evolutionary Computation-CEC99 (Cat. No. 99TH8406)*, DOI: 10.1109/CEC.1999.782657.
- [8] G.G. Wang, S. Deb, X.Z. Gao, et al. (2016), "A new metaheuristic optimisation algorithm motivated by elephant herding behaviour", *International Journal of Bio-Inspired Computation*, **8**(6), pp.394-409, DOI: 10.1504/IJBIC.2016.081335.
- [9] T.L. Duc, Q.H. Nguyen, H.N. Xuan (2020), "Balancing composite motion optimization", *Information Sciences*, **520**, pp.250-270, DOI: 10.1016/j.ins.2020.02.013.
- [10] H.T. Duong, H.C. Phan, T.T. Le, et al. (2020), "Optimization design of rectangular concrete-filled steel tube short columns with balancing composite motion optimization and data-driven model", *Structures*, **28**, pp.757-765, DOI: 10.1016/j.istruc.2020.09.013.
- [11] M. Leon, N. Xiong (2016), "Adapting differential evolution algorithms for continuous optimization via greedy adjustment of control parameters", *Journal of Artificial Intelligence and Soft Computing Research*, **6**(2), pp.103-118, DOI: 10.1515/jaiscr-2016-0009.
- [12] A. Alihodzic, E. Tuba, R.C. Hrosik, et al. (2017), "Unmanned aerial vehicle path planning problem by adjusted elephant herding optimization", *2017 25th Telecommunication Forum (Telfor)*, pp.1-4, DOI: 10.1109/TELFOR.2017.8249468.
- [13] G. Liu, Z. Yeung, H.W.F. Chung, et al. (2016), "A new weight adjusted particle swarm optimization for real-time multiple object tracking", *International Conference on Neural Information Processing*, Springer, pp.643-651, DOI: 10.1007/978-3-319-46672-9_72.
- [14] E. Tuba, M. Tuba, E. Dolicanin (2017), "Adjusted fireworks algorithm applied to retinal image registration", *Studies in Informatics and Control*, **26**(1), pp.33-42, DOI: 10.24846/v26i1y201704.
- [15] B.S. Yıldız, S. Kumar, N. Pholdee, et al. (2022a), "A new chaotic Lévy flight distribution optimization algorithm for solving constrained engineering problems", *Expert Systems*, **39**(8), DOI: 10.1111/exsy.12992.
- [16] S. Kumar, B.S. Yildiz, P. Mehta, et al. (2023), "Chaotic marine predators algorithm for global optimization of real-world engineering problems", *Knowledge-Based Systems*, **261**, DOI: 10.1016/j.knsys.2022.110192.
- [17] B.S. Yıldız, S. Kumar, N. Panagant, et al. (2023), "A novel hybrid arithmetic optimization algorithm for solving constrained optimization problems", *Knowledge-Based Systems*, **271**, DOI: 10.1016/j.knsys.2023.110554.
- [18] B.S. Yıldız, P. Mehta, N. Panagant, et al. (2022b), "A novel chaotic Runge Kutta optimization algorithm for solving constrained engineering problems", *Journal of Computational Design and Engineering*, **9**(6), pp.2452-2465, DOI: 10.1093/jcde/qwac113.
- [19] P. Mehta, B.S. Yıldız, S.M. Sait, et al. (2023), "A novel hybrid Fick's law algorithm-quasi oppositional-based learning algorithm for solving constrained mechanical design problems", *Materials Testing*, **65**(12), pp.1817-1825, DOI: 10.1515/mt-2023-0235.
- [20] D. Gürses, P. Mehta, S.M. Sait, et al. (2023), "A multi-strategy boosted prairie dog optimization algorithm for global optimization of heat exchangers", *Materials Testing*, **65**(9), pp.1396-1404, DOI: 10.1515/mt-2023-0082.
- [21] E.P. Adorio, U. Diliman (2005), *MVF-multivariate Test Functions Library in C for Unconstrained Global Optimization*, <http://www.geocities.ws/eadorio/mvf.pdf>, accessed 21 January 2024.
- [22] K. Hussain, M.N.M. Salleh, S. Cheng, et al. (2017), "Common benchmark functions for metaheuristic evaluation: A review", *JOIV: International Journal on Informatics Visualization*, **1**(4-2), pp.218-223, DOI: 10.30630/joiv.1.4-2.65.
- [23] M. Hellwig, H.G. Beyer (2019), "Benchmarking evolutionary algorithms for single objective real-valued constrained optimization - A critical review", *Swarm and Evolutionary Computation*, **44**, pp.927-944, DOI: 10.1016/j.swevo.2018.10.002.

A model of factors influencing safety behaviour and awareness among Vietnamese construction workers

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Abstract:

Ensuring labour safety remains a critical concern within the construction industry, particularly in Vietnam, where workers face various challenges related to awareness and behaviour. This study explores the factors influencing workers' safety behaviour and perception. Key factors were identified through a review of existing literature and consultations with experts. Data were collected via questionnaires distributed to three respondent groups, differentiated by job roles, project stakeholders, and years of experience. The data were analysed using appropriate statistical methods. The findings reveal 23 factors, categorised into three groups: worker characteristics, management capacity, and working conditions. The study found no significant differences in the assessment of these factors' effects on worker safety behaviour across the respondent groups. Using exploratory factor analysis (EFA), these initial factors were consolidated into principal groups, accounting for over 60% of the total variance. Practically, the study provides insights into the content and implications of each factor and group. These findings can assist safety managers in better understanding worker behaviour and attitudes, thereby facilitating the development of appropriate policies.

Keywords: construction worker, factor model, safety behaviour.

Classification numbers: 1.3, 2.3

1. Introduction

The labour force is of paramount importance in the development of the market economy and international economic integration. Prioritising workplace safety and workers' health has significant implications for human resource development, enhances the quality of labour within companies, and fosters a sustainable economy within the Vietnamese construction industry [1]. There have been notable improvements in ensuring labour safety in Vietnam, with increasing awareness and responsibility among organisations and agencies yielding positive results [2]. However, it remains essential to establish robust labour protection measures alongside productive labour activities. Moreover, occupational accident statistics reveal complex patterns in workers' adherence to safety regulations [3].

The construction industry plays a critical role in the national economy but faces significant challenges in ensuring labour safety, characterised by distinct difficulties [2]. Workers in this sector often contend with unpredictable conditions, such as exposure to harsh weather, heavy physical labour, and remote worksite locations [4]. These

factors, coupled with the absence of fixed workplaces, contribute to an increased risk of accidents and health hazards, including occupational diseases [1]. Notably, the construction sector accounts for 14% of all labour accidents, attributable to factors such as a lack of emphasis on establishing safe working procedures, inadequate safety solutions by companies, and insufficient or poor-quality safety training for workers [5].

Previous study identified the primary causes of accidents that negatively impact labour productivity in construction [6]. These include low safety awareness among workers, inadequate training, insufficient provision of protective equipment, the use of outdated equipment, and engagement in unsafe practices. Furthermore, another study assessed how management characteristics and methodologies influence workers' safety performance [7].

The above discussion highlights the critical role of construction workers in occupational accidents. However, previous studies have not comprehensively evaluated the factors affecting construction workers' safety behaviour and awareness. It is thus increasingly important to investigate

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workers' behaviour, attitudes, and practices. This study aims to explore the impact of various factors on workers' safety behaviour and examine differences in the assessment of these factors' influence on safety behaviour across respondent groups differentiated by job roles, project stakeholders, and years of experience. The specific research objectives are as follows:

- To identify the factors that impact workers' safety behaviour in the workplace.
- To determine the extent of each factor's impact on workers' safety behaviour.
- To investigate any variations and their significance in assessing each factor's effect on workers' safety behaviour across different respondent groups.
- To develop a model of the principal factors influencing workers' safety behaviour.
- To provide recommendations based on the findings to improve safety behaviour in the workplace.

2. Literature review

2.1. Typical domestic research

Previous study examined the persistently high rate of workplace safety incidents, which result in substantial human and economic losses [8]. The study identified various factors influencing workers' behaviour and adherence to occupational safety measures, with particular emphasis on the pivotal roles of individual employee characteristics and managerial influence. It quantified the time lost due to work-related incidents and highlighted common causes of accidents. These issues underscore the crucial role of labour safety management officials, emphasising the need for clearly defined responsibilities and the importance of fostering a safety-oriented work culture among employees.

Other studies focused on organisational factors, including hygiene practices and occupational safety protocols, such as site organisation, worker selection, and safety training procedures [9]. The study proposed technical measures for the appropriate arrangement of equipment and materials to prevent accidents during construction, with a primary focus on fall prevention and working at heights. These measures were suggested to mitigate the risk of accidents and improve overall site safety.

T.K. Trang (2017) [10] explored the relationship between safety culture and organisational performance at the Northern power corporation (NPC). By employing a combination of document synthesis, analysis, and

questionnaires distributed to NPC employees, the study clarified the concept and content of safety culture and assessed its impact on organisational efficiency. The findings revealed a positive correlation between safety culture and the company's productivity, as improved safety practices contributed to reducing accidents, minimising costs, enhancing employee performance, and increasing company profits. The questionnaire results were categorised into four sections: the current state of safety culture at NPC, its impact on employee efficiency, its influence on NPC's overall effectiveness, and the relationship between employee efficiency and the company's overall performance in relation to safety culture.

B.T. Tung (2016) [11] addressed the issue of poor compliance with labour safety practices at construction sites in Ho Chi Minh City, Vietnam. The study presented a model consisting of four groups of variables associated with inadequate occupational safety practices at these sites. These variables included deficiencies in organisational management, the implementation and inspection of safety measures, irregular maintenance, and a lack of proper labour protection equipment. The study also highlighted insufficient tools and electrical safety measures, as well as a lack of awareness and compliance with safety practices among both management and construction workers. Tung emphasised the urgent need to raise safety awareness among workers to improve compliance with safety protocols.

2.2. Typical abroad research

M. Shin, et al. (2014) [12] investigated the use of System Dynamics to model workers' safety attitudes and behaviours. Their study examined the effects of three conditions aimed at improving safety: first, providing workers with information on safe work behaviours to foster habits conducive to safety, thereby preventing desensitisation to accident risks; second, sharing accident information across job roles to reduce incidents by addressing workers' tendency to overestimate their ability to control risks; and third, increasing workers' concern about accidents, leading to a more accurate assessment of accident likelihood.

D. Fang, et al. (2015) [13] explored the impact of supervisory influence on worker safety behaviour in construction projects, aiming to identify managerial behaviours that affect safety. Supervisors, being the most frequent interactors with construction workers among management levels, were studied in terms of two aspects: coaching and preventive actions, and reactive and supportive actions. A 3-month questionnaire survey conducted in Hong Kong's construction industry was used to gather data.

Results from confirmatory factor analysis and structural equation modelling revealed both direct and indirect effects of supervisory behaviour on worker safety behaviour, with responsive and supportive actions found to have a direct impact.

B.H. Guo, et al. (2016) [14] focused on predicting occupational safety behaviour in the construction industry through the development and testing of an integrated model. Data from 215 construction workers in New Zealand were collected via questionnaire, and eight competing models were tested using structural equation modelling (SEM). The results indicated significant relationships, with management safety commitment positively linked to social support and production pressure. Production pressure emerged as a key factor directly impacting safety behaviour and compliance, while social support also influenced safety behaviour, along with safety knowledge and motivation.

B. Choi, et al. (2017) [15] examined group norms and individual standards of construction workers regarding safe behaviour from a social identity perspective. The study aimed to determine the influence of group norms on workers' standards for safety behaviour and the moderating role of social identity in this relationship. Empirical data were collected from 82 construction workers and nine project managers across three sites and analysed using correlation and regression analyses. The findings highlighted varying levels of social identity among construction workers, associated with different groups (e.g., workgroup, company, project), with social identity moderating the effect of group norms on personal safety standards.

K.P. Cigularov, et al. (2010) [16] investigated the role of error management and communication environments in workplace safety, gathering data from 235 union construction workers employed by 15 contractors across the Midwest and Northwest regions of the United States. The study underscored the importance of positive occupational safety communication and management in improving workplace safety, with the implications discussed for organisational safety research and practice.

S.L. Morrow, et al. (2010) [17] examined the relationship between psychological safety climate dimensions and safety behaviour in the railway industry. The study aimed to confirm the correlation between workers' perceptions of occupational safety psychology and safety behaviour, exploring aspects such as work safety management, colleague influence on safety behaviour, and the impact of pressure on safety compliance.

I. Hansez, et al. (2010) [18] explored safety behaviour by examining the effects of job demands, job pressure, and perceived management commitment to safety. Their research highlighted the influence of job demands and resources on safety behaviour, emphasising the impact of work-related stress and the significance of management's perceived commitment to safety.

L. Zhang, et al. (2016) [19] studied the interactive perceptions of construction safety behaviour from workers' perspectives, employing SEM and exploratory factor analysis (EFA) to elucidate the cause-and-effect relationships between factors influencing worker safety. Their findings underscored the positive influence of management- and system-oriented supervision and leadership on construction workers' psychological well-being and safety behaviour.

S. Clarke (2012) [20] explored the effects of pressure factors on construction worker safety behaviour through meta-analysis, examining the relationships between stressors, safety behaviours, and outcomes. The findings revealed that hindering factors negatively impacted safety compliance and outcomes, while certain pressurising factors had a positive influence on safety performance.

I. Mohammadfam, et al. (2017) [21] developed a Bayesian network model to enhance worker safety behaviour based on research conducted in power plant construction projects in Iran. The study identified several factors influencing safety behaviour, including management commitment, a supportive environment, the safety management system, employee participation, safety knowledge, attitude, motivation, resource allocation, and work pressure.

R.M. Choudhry (2014) [22] investigated construction site safety based on workers' behaviour, proposing a management method to improve occupational safety. The approach involved supervisors recognising and praising safe behaviours, engaging observers in discussions with workers, distributing training materials, providing feedback to management, and displaying safety performance charts. The study demonstrated significant improvements in safety performance across various categories, highlighting the effectiveness of the proposed approach in enhancing workplace safety.

2.3. Research gaps

Previous studies have provided valuable insights into the factors affecting construction workers' safety behaviour, significantly improving project stakeholders' understanding of workplace safety. However, most of these studies focus on individual-level factors, such as attitudes, beliefs,

and perceptions, while there is a paucity of research into broader contextual factors, including organisational culture, project demands, and societal influences. Furthermore, existing research predominantly examines top-down safety management approaches, with limited attention given to the role of worker involvement in safety decision-making processes. Additionally, psychosocial factors, such as job stress, job satisfaction, and social support, which can profoundly influence safety behaviour, have not been extensively studied. Consequently, there is currently no comprehensive model that evaluates the impact of these broader contextual and psychosocial factors on construction workers' safety behaviour.

2.4. Summary and description of influential factors

Twenty-one factors were identified through a comprehensive literature review. Additionally, interviews with three experts were conducted for two main purposes: first, to evaluate the factors identified from the literature, and second, to solicit additional factors based on their practical experience. The results indicated unanimous agreement among the experts on the twenty-one factors from the literature, and they collectively proposed eleven additional factors. Table 1 summarises the thirty-two potential factors influencing construction workers' safety behaviour and awareness. These factors are categorised into three clusters: worker-related, management capacity-related, and working conditions-related aspects.

Factor A2 "Experience": Experience is crucial for construction workers. Those with extensive project involvement and training are well-versed in labour safety regulations. Experienced workers, having encountered various construction scenarios, possess a deeper understanding of how subjective judgments, lack of awareness, and inexperience can compromise safety. In team environments, experienced workers play a pivotal role, often staying with companies long-term and receiving further training to enhance their skills. Their accumulated experience gives them a heightened awareness of behaviours impacting workplace safety.

Factor A6 "Tenure with the company": Tenure is vital in evaluating workers' adherence to safety principles and regulations. Human resources departments assess employees who consistently demonstrate exemplary compliance and safety practices. Additionally, competitive wages and incentive packages contribute to workers' material and psychological well-being, fostering long-term commitment to the company.

Table 1. Factors affecting construction worker's safety behaviour and awareness.

Group	Factors	References
<i>A. Workers themselves-related</i>		
A1	Age	[23]
A2	Experience	Proposed by experts
A3	Health status during work	[23]
A4	Marital status	[24]
A5	Education level	[11]
A6	Tenure with the company	Proposed by experts
A7	Gender	[24]
A8	Knowledge of labour safety	[11, 12, 21, 24]
A9	Trust in management effectiveness	Proposed by experts
A10	Smoking or alcohol consumption habits	Proposed by experts
A11	History of violating labour safety regulations	[11]
A12	Level of risk tolerance	Proposed by experts
<i>B. Management capacity-related</i>		
B1	Sanctions for labour safety violations	[18-20]
B2	Failure to enforce working and rest hours strictly	Proposed by experts
B3	Awareness of labour safety supervision and management	[9]
B4	Manager's experience	[19]
B5	Regular inspection of labour safety conditions and risks on construction sites	[10, 14]
B6	Absence of commitment to occupational safety in construction sites	[9, 10]
B7	Policy for safety activities	[21, 22]
B8	Lack of strict supervision of tool usage	[14, 17]
B9	Unreasonable worker arrangement	[13]
B10	Inefficient labour safety training programs	[9, 10, 13, 22, 25]
<i>C. Working conditions-related</i>		
C1	Lack of innovation in safety and construction techniques	[9-11]
C2	Challenging construction site conditions	Proposed by experts
C3	Polluted environment (dust, noise, toxic gases)	Proposed by experts
C4	Unfavourable organization of construction sites	[9]
C5	Absence of signs, instructions, rules on safety techniques, and protective equipment for workers	[14, 17]
C6	Poor quality of safety equipment	[26]
C7	Failure to meet workers' daily needs and personal hygiene	Proposed by experts
C8	Negative working conditions	[14, 19, 21]
C9	Natural disasters and epidemics	Proposed by experts
C10	Extreme weather conditions	Proposed by experts

Factor A9 “Trust in management effectiveness”: Worker trust in management’s ability to enforce safety regulations directly influences compliance rates. Disagreement with safety regulations, such as the necessity of safety straps and helmets when working at heights, can arise from perceived inconvenience on construction sites, affecting workers’ adherence to safety protocols.

Factor A10 “Smoking or alcohol consumption habits”: Workers who frequently use stimulants, cigarettes, or alcohol experience deteriorating health and decreased alertness over time, which impacts their awareness during construction work. For example, habitual alcohol consumption after work hours can lead to liver and kidney health issues, physical exhaustion, and insufficient sleep, subsequently affecting mental acuity the following morning. This lack of alertness can hinder adherence to safety regulations. A worker driven by a smoking habit may disregard safety protocols to satisfy personal needs, creating potential hazards such as careless cigarette disposal, which poses significant risks of fire, explosion, and other occupational safety concerns.

Factor A12 “Level of risk tolerance”: Risk tolerance significantly influences workers’ awareness and adherence to occupational safety protocols. When employees understand the potential hazards of workplace accidents, they are more inclined to follow safety guidelines strictly. For instance, when working at heights, using protective belts and safety locks is essential to reduce the risk of falls and fatalities. Recognising these risks compels workers to prioritise safety measures to protect themselves, their families, and mitigate the financial burdens associated with accidents. This study underscores the importance of risk awareness and acceptance in promoting occupational safety.

Factor B2 “Failure to enforce working and rest hours strictly”: Companies that fail to enforce regulations on working hours, rest breaks, and internal procedures often exhibit negligence towards labour safety regulations, significantly reducing overall compliance levels.

Factor C2 “Challenging construction site conditions”: High-altitude and confined construction sites can impede workers’ movements, leading to frustration and decreased awareness of safety behaviours.

Factor C3 “Polluted environment (dust, noise, and toxic gases)”: A polluted work environment negatively affects workers’ physical and mental well-being, which in turn impacts their compliance with safety regulations. For example, inadequate control of dust and toxic gas emissions during construction projects can cause respiratory issues, diminishing workers’ health and cognitive function, thus impairing their ability to follow safety protocols. Similarly, prolonged exposure to loud machinery noises can disrupt

concentration and awareness, compromising safety behaviour.

Factor C7 “Failure to meet workers’ daily needs and personal hygiene”: Failing to meet workers’ basic needs, such as access to personal hygiene and adequate accommodation, can lead to health issues and dissatisfaction, which in turn affect their psychological state and adherence to safety protocols. For instance, unsanitary living conditions and insufficient access to clean water in construction camps can compromise workers’ health and well-being, leading to psychological distress and reducing their commitment to safety behaviours.

Factor C9 “Natural disasters and epidemics”: Natural disasters and epidemics contribute to health concerns and negative outlooks, reducing workers’ concentration and awareness of safety protocols. For example, during the COVID-19 pandemic, restrictions on movement and uncertainties about personal safety negatively impacted workers’ mental health, leading to decreased vigilance in implementing safety measures on construction sites.

Factor C10 “Extreme weather conditions”: Adverse weather conditions, such as intense sunlight or thunderstorms, can negatively impact workers’ health, morale, and compliance with safety regulations. For example, prolonged exposure to intense sunlight may cause dizziness and fatigue, prompting workers to overlook safety measures.

3. Methodology

Selecting an appropriate methodology is essential for addressing research problems effectively. This study employed a structured questionnaire and expert surveys to collect data. A five-point Likert scale was used, ranging from 1, representing “no,” to 5, representing “extremely.” The questionnaire comprised two sections. The first section collected the personal information of the respondents, while the second section evaluated the impact of the thirty-two factors (as listed in Table 1) on workers’ safety behaviour.

This approach leveraged the expertise and insights of qualified professionals in construction management. The purpose was to gather scientific data, record feedback, and evaluate factors, which served as the basis for refining the research problem. Survey participants were selected from project stakeholders, including investors, consultants, and contractors, all of whom possess extensive work experience and hold senior positions. The surveys were conducted from March to May 2023.

A previous study provided the following formula for determining an appropriate sample size [27]:

$$N = \frac{(Z \times S_x)^2}{E^2} \quad (1)$$

where N: sample size; S_x : standard error of the samples; E: reliability of the samples; Z: value obtained from the normal distribution.

In this study, with an error range of $E \leq 10\%$ and a confidence level of 90%, the minimum required number of samples is 96.

The literature review provided the theoretical foundation for the research problem and synthesised information from previous studies. The research hypotheses were as follows:

- The null hypothesis: H_0 = There are no differences in assessing the effect of the proposed factors on worker safety behaviour between respondent groups in terms of working conditions, project stakeholders, and experience years.
- The alternative hypothesis: H_A = There are differences in assessing the effect of the proposed factors on worker safety behaviour between respondent groups in terms of working conditions, project stakeholders, and experience years.

Processing and analysing research data is a crucial step in any scientific study. This involves several key stages: (1) identifying the research problem; (2) collecting, processing, and analysing data; and (3) reporting the analysis results. Clearly defining the research problem facilitates faster and more accurate data collection. At the heart of data analysis lies statistical inference, which entails extrapolating understanding from a random sample to comprehend the entire population, a process known as inductive inference. Data must undergo analysis via statistical tests to ensure the reliability of the conclusions drawn. Initially, data are raw; through analytical processing, they transform into information and eventually knowledge, which is the ultimate goal of all research endeavours.

In this study, survey data was cleaned and coded using Excel. They were then analysed using SPSS software by descriptive analysis, reliability testing using Cronbach's alpha ($\alpha > 0.7$), hypothesis testing using analysis of variance (ANOVA), and exploratory factor analysis (EFA).

4. Results and discussion

4.1. Respondent's characteristics

Table 2 presents the statistics regarding 320 participants involved in the mass survey. This survey aimed to include a diverse range of respondents with characteristics relevant to the research topic.

Table 2. Descriptive statistics of survey respondents.

Type	Characteristics	Frequency	%
Work positions	1. Managers	63	19.7
	2. Site engineers	192	60.0
	3. Others	65	20.3
Project stakeholders	1. Investors	52	16.2
	2. Supervisors	79	24.7
	3. Contractors	150	46.9
	4. Others	39	12.2
Experience years	1. Less than 3	68	21.2
	2. Between 3-5	111	34.7
	3. Between 5-10	87	27.2
	4. Greater than 10	54	16.9

Regarding work positions, the primary survey participants included 63 managers (project managers, site managers, and team managers), representing 19.7% of the total; 192 site engineers, accounting for 60%; and 65 others (foremen and board of directors), representing 20.3%. Among the respondents, construction contractors formed the largest group, with 150 individuals making up 46.9% of the participants, followed by supervision consultants (79 individuals, 24.7%), investors (52 individuals, 16.2%), and others (state agencies and management consultants) (39 individuals, 12.2%). Most survey participants (78.7%) had three or more years of experience in the construction industry. These results demonstrate that the characteristics of the respondents were appropriate to address the research questions effectively.

4.2. Reliability test

The results of the Cronbach's alpha (α) analysis are shown in Table 3. The analysis indicates that all factors have α coefficients greater than 0.7. However, nine factors were excluded due to having a total variable correlation coefficient of less than 0.3. The excluded variables are A1, A2, A4, A6, and A7 from the worker-related group, B2 and B6 from the management capacity group, and C7 and C8 from the working conditions group.

Table 3. The coefficients of Cronbach's alpha.

Group	α
Workers themselves	0.786
Management capacity	0.848
Working conditions	0.828

4.3. Hypothesis test

An ANOVA test was conducted to examine the differences in the assessment of respondent groups regarding the effect of the proposed factors on construction workers' safety behaviours. The test results show that for most factors, the significance value is greater than 0.05, indicating that the null hypothesis is accepted. However, in nine cases, the null hypothesis was rejected due to significance values lower than 0.05. These cases include A10 (sig.=0.040), B10 (sig.=0.033), C2 (sig.=0.017), and C10 (sig.=0.011) for the working position group; A3 (sig.=0.001), A10 (sig.=0.019), B3 (sig.=0.015), B7 (sig.=0.001), B9 (sig.=0.048) for the project stakeholder group; and B8 (sig.=0.035) for the experience year group.

Table 4. The post hoc test results for working position groups.

Respondent	Subsets for A10		Subsets for B10		Subsets for C2		Subsets for C10	
	1	2	1	2	1	2	1	2
Managers	3.077	-	3.127	-	3.349	-	3.175	-
Site engineers	3.343	3.343	3.464	3.464	3.600	3.600	-	3.599
Others	-	3.556	-	3.662	-	3.776	-	3.677
Sig.	0.244	0.410	0.135	0.497	0.267	0.520	1.000	0.883

Table 5. The post hoc test results for project stakeholder groups.

Respondent	Subsets for A3		Subsets for A10		Subsets for B3		Subsets for B7		Subsets for B9
	1	2	1	2	1	2	1	2	1
Investors	2.942	-	3.115	3.115	3.462	3.462	3.269	3.269	3.481
Supervisors	3.304	3.304	-	3.494	3.101	-	3.165	-	3.266
Contractors	-	3.633	3.420	3.420	3.560	3.560	-	3.693	3.693
Others	3.154	3.154	2.949	-	-	3.667	-	3.667	3.564
Sig.	0.220	0.054	0.065	0.194	0.102	0.735	0.943	0.104	0.134

Table 6. The post hoc test results for experience year groups.

Respondent	Subsets for B8	
	1	
Less than 3	3.441	
Between 3-5	3.667	
Between 5-10	3.287	
Greater than 10	3.259	
Sig.	0.082	

Since respondents assessed these nine cases differently, a post hoc Tukey HSD test was conducted to examine the significant differences within each homogeneous subset. The results of the Tukey HSD test are presented in Tables 4, 5, and 6. These results indicate that all significance values exceed 0.05. However, differences were observed in only a few factors between the two subsets of respondent groups. Therefore, there is a relative consensus among the respondents' assessments for each factor, allowing the data to be used for further analysis.

4.4. Exploratory factor analysis (EFA)

Given the excellent reliability and differentiation test results, an EFA was conducted. Table 7 presents the analysis of factors influencing awareness and implementation of occupational safety. The Kaiser-Meyer-Olkin (KMO) measure is 0.879, exceeding the acceptable threshold of 0.5, and the Bartlett test yields a significance level of less than 0.05. Furthermore, the factor loading coefficients exceed 0.5, the extracted variance index is 4.480 (>1), and the total extracted variance is 60.007% (>50%). These results confirm that the scales and variables used to measure factors influencing awareness and implementation of occupational safety are suitable within the EFA model.

Table 7. Condition check results of exploratory factor analysis.

Variance extraction breakpoint	Total variance extracted	Kaiser-Meyer-Olkin coefficient	Sig.
4.789	20.823	0.879	0.000
4.533	40.530		
4.480	60.007		

4.5. Discussion on each grouped factor

The rotated matrix results reveal that the factors influencing awareness and implementation of occupational safety are categorised into three distinct groups, and no new concepts emerged during the analysis, indicating consistency with the research data (Table 8).

Factor C1 demonstrates the highest loading factor of 0.892 among the working conditions-related factors, indicating a strong influence on the variables. Conversely, C9 shows the lowest loading factor of 0.581, suggesting a less pronounced impact. In the context of occupational safety performance among construction workers, the absence of innovation in safety techniques and construction methods (C1) has a more direct influence than factors related to natural disasters and diseases, such as C9, which are relatively infrequent occurrences.

Table 8. Factor loading through Varimax rotation.

Factor	Loading		
	Component 1 'Working conditions'	Component 2 'Management capacity'	Component 3 'Workers themselves'
C1	0.892		
C2	0.892		
C3	0.770		
C4	0.769		
C5	0.768		
C6	0.740		
C10	0.591		
C9	0.581		
B5		0.806	
B1		0.790	
B3		0.775	
B4		0.771	
B9		0.715	
B8		0.701	
B10		0.651	
B7		0.649	
A12			0.861
A11			0.835
A10			0.808
A9			0.761
A8			0.757
A5			0.752
A3			0.727

Factor C10, relating to geographical conditions, reflects a specific climate with two distinct seasons and relatively comfortable conditions for construction workers accustomed to the local climate. Factors C3, C4, C5, and C6 have an average loading coefficient of approximately 0.7, indicating a notable influence, though not as pronounced as C1 and C2. These factors, which are frequently encountered on construction sites, include hazardous noise and dust, which persist due to asynchronous urbanisation and incomplete infrastructure development.

Factor B5, with the highest loading coefficient of 0.806 within the organisational work-related factors, highlights the importance of managing occupational safety through regular inspections and hazard identification. Effective safety management requires consistent monitoring and discipline to address unsafe practices, particularly during elevated work tasks.

Factors B10 and B7, concerning "Policy for safety activities" and "Occupational safety training programmes," receive relatively lower ratings, which can be attributed to

varying reward structures across companies and irregularity in the organisation of safety training programmes.

Loading coefficients ranging from 0.790 to 0.701 are considered appropriate for the remaining factors related to organisational work safety management capacity. In the employee-related factors group, A10, A11, and A12 exhibit the highest loading coefficients, indicating poor discipline, stimulant consumption, and risk tolerance among workers. Factors with lower loadings, such as A3, A5, A8, and A9, reflect workers' education levels, trust in management, and their impact on safety compliance.

5. Conclusions and recommendations

Construction workers operate in environments heavily influenced by working conditions, many of which pose significant risks to their health and safety. Without proper preventive measures, these conditions can lead to accidents on construction sites. Presently, the issue of occupational accidents on such sites remains complex, largely due to workers' behaviour and awareness regarding compliance with labour safety laws. Therefore, there is an urgent need for solutions to address these challenges and enhance the safety performance of construction workers.

This study provides a comprehensive overview of occupational safety and examines the factors impacting construction workers' awareness and compliance with safety protocols. The study identifies 23 factors categorised into three groups: worker-related factors (7 factors), organisational safety management factors (8 factors), and working conditions-related factors (8 factors). These factors are deemed influential in shaping construction workers' safety awareness and practices in projects. Based on the ANOVA test, the study found that most respondents assessed the effect of each factor on workers' safety behaviour similarly, with only a few cases showing significant differences at the 0.05 level. Additionally, based on the EFA, the extracted factor model accounts for over 60% of the original factors' variance, indicating that the model is highly representative.

Furthermore, the study delves into the role and significance of these identified factors within the context of the Vietnamese construction industry. This analysis serves as a crucial foundation for proposing recommendations and strategies to enhance the effectiveness of safety measures among construction workers. However, the study acknowledges certain limitations, including subjectivity among survey participants, the need for further validation of factor grouping, and the explanation for eliminating variables due to systematic factors. Additionally, the study notes that some total variable correlations require further explanation, which necessitates additional scrutiny. Future research can address these shortcomings to advance the field.

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COMPETING INTERESTS

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REFERENCES

- [1] T.H. Quan (2023), *Application of System Dynamics to Assessing The Safety Performance of Construction Workers*, Master thesis, Ho Chi Minh City University of Technology and Education.
- [2] N.M. Nga, H.D. Khanh (2016), "Examining, analyzing and proposing relevant solutions for enhancing safety perception of workers on construction sites", *Journal of Construction*, pp.51-57 (in Vietnamese).
- [3] General Statistics Office of Vietnam (2023), <https://www.gso.gov.vn>, accessed 18 April 2023 (in Vietnamese).
- [4] P.M. Tin, H.D. Khanh (2017), "Developing a model for assessing factors causing unsafe phenomenon when executing the work at height", *Journal of Construction and Urban*, **54**, pp.75-78 (in Vietnamese).
- [5] N. Duong (2022), "The construction industry has the most occupational accidents", *VnEconomy*, <https://vneconomy.vn/nganh-xay-dung-xay-ra-tai-nan-lao-dong-nhieu-nhat.htm>, accessed 9 April 2023 (in Vietnamese).
- [6] L.T. Van, D.T.X. Lan (2002), "Safety problems at construction sites in Ho Chi Minh City", *The Proceedings of The 8th Conference on Science and Technology, Ho Chi Minh City University of Technology*, pp.21-27 (in Vietnamese).
- [7] T.H. Tuan, D.T.X. Lan (2009a), "Analyzing factors affecting the performance of construction workers' labour safety", *The Proceedings of The 11th Conference on Science and Technology, Ho Chi Minh City University of Technology*, pp.297-302 (in Vietnamese).
- [8] T.H. Tuan (2009b), "Analysis of factors affecting the implementation of occupational safety of construction workers", *Journal of Science, Can Tho University*, **12**, pp.162-170 (in Vietnamese).
- [9] N.H.G. Hoang (2016), *Proposing Technical Measures for Occupational Safety in Construction in Vinh Long Province*, Master's Thesis, Hanoi University of Architecture (in Vietnamese).
- [10] T.K. Trang (2017), "A study on the impact of safety culture on organizational performance at Northern Power Corporation", *Industry and Trade Magazine*, <https://tapchicongthuong.vn/bai-viet/nghien-cuu-anh-huong-cua-van-hoa-an-toan-den-hieu-qua-hoat-dong-to-chuc-tai-tong-cong-ty-dien-luc-mien-bac-46676.htm>, accessed 9 April 2023 (in Vietnamese).
- [11] B.T. Tung (2016), "Solutions to improve poor safety performance at construction sites in Ho Chi Minh City", *Builders Magazine*, <https://nguoixaydung.com.vn/>, accessed 18 April 2023 (in Vietnamese).
- [12] M. Shin, H.S. Lee, M. Park, et al. (2014), "A system dynamics approach for modelling construction workers safety attitudes and behaviors", *Accident Analysis & Prevention*, **68**, pp.95-105, DOI: 10.1016/j.aap.2013.09.019.
- [13] D. Fang, C. Wu, H. Wu (2015), "Impact of the supervisor on worker safety behavior in construction projects", *Journal of Management in Engineering*, **31**(6), DOI: 10.1061/(ASCE)ME.1943-5479.000035.
- [14] B.H. Guo, T.W. Yiu, V.A. González (2016), "Predicting safety behavior in the construction industry: Development and test of an integrative model", *Safety Science*, **84**, pp.1-11, DOI: 10.1016/j.ssci.2015.11.020.
- [15] B. Choi, S. Ahn, S. Lee (2017), "Construction workers group norms and personal standards regarding safety behavior: Social identity theory perspective", *Journal of Management in Engineering*, **33**(4), DOI: 10.1061/(ASCE)ME.1943-5479.000051.
- [16] K.P. Cigularov, P.Y. Chen, J. Rosecrance (2010), "The effects of error management climate and safety communication on safety: A multi-level study", *Accident Analysis & Prevention*, **42**(5), pp.1498-1506, DOI: 10.1016/j.aap.2010.01.003.
- [17] S.L. Morrow, A.K. McGonagle, M.L.D. Steinkamp, et al. (2010), "Relationships between psychological safety climate facets and safety behavior in the rail industry: A dominance analysis", *Accident Analysis & Prevention*, **42**(5), pp.1460-1467, DOI: 10.1016/j.aap.2009.08.011.
- [18] I. Hansez, N. Chmiel (2010), "Safety behavior: Job demands, job resources, and perceived management commitment to safety", *Journal of Occupational Health Psychology*, **15**(3), pp.267-278, DOI: 10.1037/a0019528.
- [19] L. Zhang, Q. Liu, X. Wu, et al. (2016), "Perceiving interactions on construction safety behaviors: Workers' perspective", *Journal of Management in Engineering*, **32**(5), DOI: 10.1061/(ASCE)ME.1943-5479.000045.
- [20] S. Clarke (2012), "The effect of challenge and hindrance stressors on safety behavior and safety outcomes: A meta-analysis", *Journal of Occupational Health Psychology*, **17**(4), pp.387-397, DOI: 10.1037/a0029817.
- [21] I. Mohammadfam, F. Ghasemi, Q. Kalatpour, et al. (2017), "Constructing a Bayesian network model for improving safety behavior of employees at workplaces", *Applied Ergonomics*, **58**, pp.35-47, DOI: 10.1016/j.apergo.2016.05.006.
- [22] R.M. Choudhry (2014), "Behavior-based safety on construction sites: A case study", *Accident Analysis & Prevention*, **70**, pp.14-23, DOI: 10.1016/j.aap.2014.03.007.
- [23] Y. Gao, V.A. González, T.W. Yiu (2020), "Exploring the relationship between construction workers' personality traits and safety behavior", *Journal of Construction Engineering and Management*, **146**(3), DOI: 10.1061/(ASCE)CO.1943-7862.0001763.
- [24] D. Fang, Y. Chen, L. Wong (2006), "Safety climate in construction industry: A case study in Hong Kong", *Journal of Construction Engineering and Management*, **132**(6), pp.573-584, DOI: 10.1061/(ASCE)0733-9364(2006)132:6(573).
- [25] N.T. Viet, H.N. Viet (2021), "Building a framework for assessing the safety management on construction sites in Ho Chi Minh City", *Journal of Science and Technology, Industrial University Ho Chi Minh City*, **53A**, pp.16-18 (in Vietnamese).
- [26] C.M. Tam, S.X. Zeng, Z.M. Deng (2004), "Identifying elements of poor construction safety management in China", *Safety Science*, **42**(7), pp.569-586, DOI: 10.1016/j.ssci.2003.09.001.
- [27] W.W. Daniel (1999), *Biostatistics: A Foundation for Analysis In The Health Sciences*, 7th Edition, New York: John Wiley & Sons, 755pp.

Direct hospitalisation cost of patients with chronic obstructive pulmonary disease, covered by the National Health Insurance at Vinh Phuc General Hospital from 2015 to 2019

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Abstract:

Chronic obstructive pulmonary disease (COPD) is a treatable disease with high prevalence and morbidity, associated with a significant economic burden. This study aimed to investigate the direct hospitalisation costs of patients with COPD covered by the National Health Insurance (NHI) in Vietnam. A retrospective study was conducted at Vinh Phuc General Hospital from 2015 to 2019, involving 1,629 patients. Sociodemographic features, clinical characteristics, and hospitalisation costs were collected. A multivariate generalised linear regression model was used to identify factors associated with hospitalisation costs. The mean total hospitalisation cost per patient was 430.74 USD (SD=618.39). Medication costs accounted for the highest proportion, followed by bed-day, procedures, function tests, imaging diagnosis, functional exploration, examination, and medical supplies. Antibiotics had the highest medication cost, at 99.37 USD (SD=126.24). The average number of hospitalisation days was 10.1, closely related to the direct cost. Age and comorbidities also affected hospitalisation costs.

Keywords: chronic obstructive pulmonary disease, cost, hospitalisation cost, Vinh Phuc General Hospital.

Classification numbers: 1.3, 3.2

1. Introduction

Chronic obstructive pulmonary disease is a major cause of morbidity and mortality worldwide [1, 2]. COPD is the third leading cause of death globally, causing 3.23 million deaths in 2019 [3]. Most detailed information regarding its prevalence, morbidity, and mortality comes from high-income countries, yet 90% of COPD-related deaths occur in low- and middle-income countries [4]. In Vietnam, the prevalence of COPD was 7.1% in 2010, with 10.9% in men and 3.9% in women [5]. COPD imposes a significant burden on the healthcare system, not only due to mortality and disability but also due to substantial economic costs. In the United States, the estimated direct costs of COPD were \$32.1 billion in 2010, expected to reach \$49.0 billion by 2020 [6]. In China, the direct medical cost of COPD ranged from 72 to 3,565 USD per capita per year, accounting for 33.33 to 118.09% of the local average annual income [7]. In Vietnam, research at Bach Mai Hospital showed mean hospitalisation costs for COPD and acute exacerbations of COPD were 14.36 and 18.3 million VND, respectively [8]. However, most COPD patients are managed at grassroots hospitals, with limited research on its economic impact in Vietnam. This study aimed to (1) explore the demographic

and clinical characteristics of the COPD patient population, (2) examine the direct cost of COPD hospitalisation, and (3) identify factors potentially associated with these costs.

2. Methods

2.1. Study design

A retrospective cross-sectional study was designed to estimate the hospitalisation cost of COPD treatment in a grassroots hospital. Medical records of patients with a code of J44 (ICD-10 version) admitted to Vinh Phuc General Hospital between 2015 and 2019 were reviewed. Data on medical costs for COPD were collected for five years following the primary diagnosis. Unit costs during the study period were provided by the hospital's financial department. The direct hospitalisation cost was analysed from the perspective of the healthcare system as the health insurance provider, including costs of medications and materials used in clinical practice for COPD patients covered by NHI. Direct non-medical costs (e.g. travel, accommodation, time) and indirect costs (e.g. lost income or premature death due to the disease) were not included.

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2.2. Subject population

Eligibility criteria included: (1) diagnosed with COPD (ICD-10 code J44), (2) treated at Vinh Phuc General Hospital, and (3) covered by NHI from 1st January 2015 to 31st December 2019.

2.3. Data collection

All hospital discharge papers involved in the analysis were extracted from the hospital management system database. Data collected included sociodemographic features (age, gender, living location), clinical characteristics (treated department, cause of hospitalisation, comorbidities, and duration of hospitalisation), and direct cost components (costs of bed-day, tests, imaging diagnosis, procedures, medication, equipment, and supplies).

2.4. Statical analysis

Data were analysed using R-base software version 4.0.2 and R Studio version 1.3.1073. Descriptive statistics (mean (SD), median (min, max) for numerical variables; and frequency, percent for categorical variables) were used to assess sociodemographic and clinical variables. Inferential statistics assessed differences between patients with and without comorbidities on these variables, including department, cause of hospitalisation, days of treatment, days of treatment for exacerbation, and direct cost with covariance of days of treatment. Chi-square tests were used for department and cause of hospitalisation variables; Wilcoxon tests for days of treatment and days of treatment for exacerbation; and a Gamma regression model with “days of treatment” covariance was selected for the direct hospitalisation cost variable, with results reported with 95% CI.

Direct hospitalisation cost was separated into three phases according to Circular No. 37/TT-BYT (phase 1: before 1/3/2016; phase 2: from 1/3/2016 to 1/7/2016; and phase 3: after 1/7/2016). The Kruskal-Wallis test and the Wilcoxon test (post hoc, using the Bonferroni method to adjust p-values) examined differences in direct hospitalisation cost between the three phases. Statistical significance was set at 0.05.

A multivariate generalised linear regression model, with Gaussian family and log-link function, identified factors associated with the COPD hospitalisation cost. Based on the purpose of selecting a model to find factors related to the direct cost of treatment and while transforming the “direct treatment costs” variable to log “direct treatment costs,” the graph shape of the log variable followed a Gaussian distribution. Therefore, the generalised linear model with the log association function for the output variable following a Gaussian distribution was applied and validated

using the normality test of residuals method and statistical significance was acknowledged based on the exponential of coefficients and its 95% CI.

2.5. Ethical approval

The study protocol was reviewed and approved by the Science Committee of Vinh Phuc General Hospital.

3. Results

3.1. Sociodemographic characteristics

A total of 1,629 patients were diagnosed with COPD from 2015 to 2019 at the hospital. The mean age was 71.4 years (SD=11.1), with most patients aged over 40. The majority were male (78.1%) and lived in rural areas (77.3%). More than half of the patients had comorbidities (59.6%, N=971), with hypertension being the most common (44.2%), followed by pneumonia (31.7%), heart failure (18.6%), diabetes (13.3%), and coronary artery disease (4.1%) (Table 1).

Table 1. Demographic and clinical characteristics (N=1,629).

Characteristics	N
Age	
<40 years old	3
≥40 years old	1626
Gender	
Male	1,272 (78.1%)
Female	357 (21.9%)
Place of residence	
Rural	1260 (77.3%)
Urban	369 (22.7%)
Days of treatment	
Mean (SD)	9.57 (5.39)
Median [min; max]	9.00 [0.7; 50.0]
Comorbidity	
No comorbidity	658 (40.4%)
Have 1 comorbidity	638 (39.1%)
Have 2 comorbidities	247 (15.2%)
Have ≥3 comorbidities	86 (5.3%)
Comorbidities (n=971)	
Hypertension	429 (44.2%)
Pneumonia	308 (31.7%)
Heart failure	181 (18.6%)
Diabetes	129 (13.3%)
Coronary artery disease	40 (4.1%)

The majority of patients were treated in the internal medicine department (87.4%), with exacerbation being the primary cause of hospitalisation (90.9%). The average duration of treatment was 9.57 days (SD=5.39). The average number of exacerbations per patient with and without comorbidities was 1.47 and 1.41, respectively. The average number of ICU admissions per patient with and without comorbidities was 1.08 and 1.07, respectively (Table 2).

Table 2. Clinical characteristics of the research sample.

	With comorbidity (N=971)	Without comorbidity (N=658)	Absolute difference 95%CI
<i>Department</i>			
ICU	12.6%	13.7%	-1.1%
Internal	87.4%	86.3%	-5.1-2.8%
<i>Cause of hospitalisation</i>			
Exacerbation	90.9%	94.5%	-3.6%
Others	9.1%	5.5%	-6.6-(-0.6)%
<i>Days of treatment</i>			
Mean (SD)	10.1(5.51)	8.84(5.14)	1.00
Median	9.00	8.00	1.00-2.00
[min; max]	[0.747; 50.0]	[0.990; 41.0]	
<i>Days of exacerbation treatment</i>			
Mean (SD)	10.1(5.54)	8.76(5.08)	1.00
Median	9.00	8.00	1.00-2.00
[min; max]	[1.00; 50.0]	[0.990; 41.0]	

3.2. Average hospitalisation cost

The mean total cost for patients with COPD is illustrated in Table 3. The total cost was 430.74 USD (SD=618.39). The mean cost for patients with comorbidities (494.71 USD (SD=742.07)) was higher than for those without comorbidities (342.46 USD (SD=349.71)). The average duration of treatment for these two groups was 10.10 days and 8.84 days, respectively. After accounting for the effect of the “days of treatment” variable, the mean cost for the group without comorbidities was 0.847 times that of the group with comorbidities, a statistically significant difference (95% CI: 0.796-0.905). The mean cost of an ICU admission was 1138.69 USD (SD=1394.58), higher than the average cost for exacerbation treatment at 435.01 USD (SD=622.65). The mean costs for patients with 1, 2, and more than 3 comorbidities were 426.48, 558.68, and 780.45 USD, respectively. The highest average cost of treatment among patients with comorbidities was for pneumonia, at 699.42 USD per treatment, followed by diabetes, hypertension, heart failure, and coronary artery disease with averages of 580.01, 498.98, 481.92, and 398.33 USD, respectively.

Table 3. Direct cost of chronic obstructive pulmonary disease treatment.

Unit: USD*

	Mean	SD	Median	Min	Max
Mean total of patient					
Population (N=1629)	430.74	618.39	285.31	11.73	9254.53
With comorbidity (N=971)	494.71	742.07	310.47	22.90	9254.53
Without comorbidity (N=658)	342.46	349.71	252.47	11.73	3441.66
Ratio between 2 groups: with and without comorbidity				0.847 with 95%CI 0.796-0.905	
Mean cost per ICU admission					
Population (N=212)	1138.69	1394.58	720.74	26.19	9254.53
With comorbidity (N=122)	1484.14	1641.93	1074.72	26.19	9254.53
Without comorbidity (N=90)	665.30	754.86	303.65	30.32	3441.66
Mean cost per exacerbation treatment					
Population (N=1505)	435.01	622.65	290.00	11.73	9254.53
With comorbidity (N=883)	503.24	754.86	315.17	22.90	9254.53
Without comorbidity (N=622)	341.18	343.31	259.72	11.73	3441.66
Cost for group of patient with comorbidity					
1 comorbidity (N=638)	426.48	533.09	305.36	22.90	5288.30
2 comorbidities (N=247)	558.68	908.39	307.49	43.07	9254.53
More than 3 comorbidities (N=86)	780.45	1300.75	385.53	81.88	8273.63
Hypertension (N=429)	498.98	784.72	302.80	23.16	8273.63
Pneumonia (N=308)	699.42	1108.84	361.23	26.87	9254.53
Heart failure (N=181)	481.92	742.07	308.34	43.07	7676.57
Diabetes (N=129)	580.01	908.39	321.14	75.91	7676.57
Coronary artery disease (N=40)	398.33	631.18	263.99	61.84	4200.79

*The currency used was the US dollar (US dollar exchange rate on January 2020; 1 USD=23,193 VND).

3.3. Cost categories

In general, the percentages of bed-day cost, procedures, imaging diagnosis, and functional tests all increased from phase 1 to phase 3. The differences in direct cost between phase 1 vs phase 2 and phase 1 vs phase 3 were statistically significant ($p<0.05$). Medication costs accounted for the highest proportion of hospitalisation costs (over 50%) in all phases. The highest rate of medication cost was for antibiotics, with a mean of 99.37 USD (SD=126.24). Equipment and supplies costs had the lowest percentage, at less than 1% (Table 4, Fig. 1).

Table 4. Medication cost categories.

Unit: USD.

Average medication cost					
	Mean	SD	Median	Min	Max
<i>Patients hospitalised due to exacerbation</i>					
Bronchodilators	17.95	22.94	11.86	0	344.17
Antibiotic	99.37	126.24	63.12	0	1313.55
Corticosteroid	23.16	15.05	21.66	0	102.78
Other drugs	37.66	66.96	20.21	0	955.31
<i>Patients hospitalised due to other causes</i>					
Bronchodilators	13.73	20.26	3.75	0	73.78
Antibiotic	107.47	207.27	49.04	0	1223.99
Corticosteroid	21.11	16.42	18.21	0	81.03
Other drugs	55.02	102.35	22.90	0	528.83
<i>Total population</i>					
Bronchodilators	17.70	22.82	11.43	0	344.17
Antibiotic	99.80	131.78	62.69	0	1313.55
Corticosteroid	23.07	15.14	21.45	0	102.78
Other drugs	38.60	69.52	20.26	0	955.31

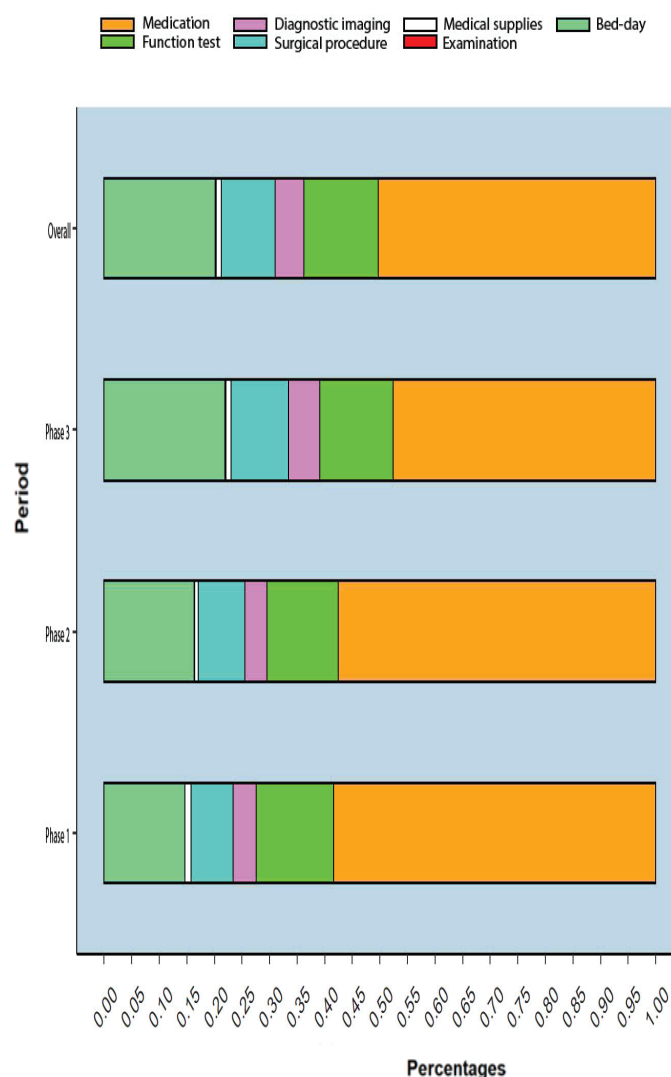


Fig. 1. Distribution of cost components according to phases.

3.4. Associated factors with chronic obstructive pulmonary disease hospitalisation cost

In the multivariate regression model (Table 5), female patients had a mean hospitalisation cost 0.823 times that of male patients (95% CI: 0.766-0.884). Urban patients had hospitalisation costs 0.845 times that of rural patients (95% CI: 0.797-0.897). Patients treated in the internal medicine department had treatment costs 0.400 times that of patients admitted to ICU (95% CI: 0.377-0.425). Patients without comorbidity had an average cost 0.840 times that of patients with coexisting conditions (95% CI: 0.778-0.996). With

each year's increase in age, the mean cost increased by 1.001 times (95% CI: 0.998-1.003), and with each additional day of treatment, the average cost of treatment increased by 1.061 times (95% CI: 1.059-1.063).

Approximately 95% CI of the variables "gender", "place of residence", "department classification", "days of treatment", and "comorbidity" did not contain value 1, indicating these variables correlate with the total cost of treatment. There was no correlation between the variables "age", "cause of hospitalisation" and total cost of treatment.

Table 5. Results from multivariate regression analysis.

Independent variables	Exp (regression coefficient)	95%CI
Gender (Female vs male)	0.823	0.766-0.884
Place of residence (Urban vs rural)	0.845	0.797-0.897
Age	1.001	0.998-1.003
Cause of hospitalisation (Others vs exacerbation)	1.071	0.979-1.172
Department classification (Internal vs ICU)	0.400	0.377-0.425
Days of treatment	1.061	1.059-1.063
Comorbidity (without vs with)	0.840	0.778-0.906
Diabetes	0.801	0.747-0.858
Hypertension	0.783	0.743-0.825
Heart failure	0.999	0.930-1.074
Coronary artery disease	0.890	0.772-1.026
Pneumonia	0.721	0.680-0.764

4. Discussion

The study was conducted at Vinh Phuc General Hospital from 2015 to 2019. The results showed that the mean age of patients covered by NHI was 71.4 (SD=11.1), with most patients over 40 years old (99.8%). This result was consistent with previous studies conducted at Dong Nai and Bach Mai Hospitals in Vietnam [8, 9]. COPD is thus a chronic and progressive disease with symptoms commonly expressed in those aged over 40.

Our study indicated a higher prevalence of male patients (78.1%) compared to females, consistent with prior research. L. Ekerljung, et al. (2014)'s study [5] in northern Vietnam showed that among individuals aged >50 years, 23.5% of men and 6.8% of women had COPD [5]. According to T.Q. Vo, et al. (2018) [9], COPD prevalence is higher among males, with samples predominantly male (86.0-92.2%). This aligns with the fact that smoking, a major COPD risk factor, is more prevalent among men in Vietnam. Although gender is not a direct factor of disease, which is instead related to smoking habits, the distribution of the disease is indirectly affected by gender.

Most COPD patients had at least one comorbidity (59.6%). Hypertension was the most common (26.3%), followed by pneumonia (18.9%), heart failure (11.1%), diabetes (7.9%), and coronary artery disease (2.5%). Similar

findings were reported in studies at Bach Mai Hospital with the rates of hypertension, diabetes mellitus, congestive heart failure, coronary artery diseases, cor pulmonale, and gastroesophageal reflux disease (GERD) being 19.3, 12.3, 15.8, 5.3, 22.8, and 7.0%, respectively [8]. These results correspond to reality, as COPD patients are of advanced age which is associated with these comorbidities due to impaired functioning, especially in cardiovascular and metabolisms.

The mean duration of treatment was 9.57 days (SD=5.39), higher for patients with comorbidities. This aligns with a study at E Hospital, showing a mean duration of 10.77 days [10]. COPD patients had longer treatment durations compared to the overall hospital average of 6 days. The average number of exacerbations per patient was 1.44 (SD=1.11), with a maximum of 10 hospitalisations. ICU admissions averaged 1.08 times (SD=0.266), highlighting the severe burden of COPD, particularly exacerbation management.

Circular No. 37/2015/TTLT-BYT-BTC led to fluctuating treatment costs from 2015 to 2019. The mean direct hospitalisation cost per treatment course was 430.74 USD (SD=618.39), equivalent to 10.01 (SD=14.5) million VND, with minimum and maximum costs of 11.73 and 9254.53 USD (0.28 million and 217 million VND). This cost aligns with another study at 74 Central Hospital, Vietnam (2014), reporting a mean hospitalisation cost of 8.3 million VND [11]. Our mean cost per day was more than 45 USD, about 1 million VND was roughly twofold higher than the cost of COPD at the National Lung Hospital in 2019 (about 0.5 million VND) [12]. The reason for this could be due to many factors such as the difference in prices of medication, changing supplies each year, different service techniques fees, the difference in hospital levels, and disease status of the population. Generally, the hospitalisation cost of COPD is quite incredible, nearly 2.5 times as high as the minimum wage in Vinh Phuc province.

The mean direct hospitalisation cost for exacerbation was 10.2 (SD=17.7) million VND with a duration of treatment of 9.54 (SD=5.34) days. This result was nearly 2 times higher than the mean cost per day of treatment in a study at the National Lung Hospital (0.507 million VND/day) [12] while 2 times lower than that of the study conducted at Bach Mai Hospital (the mean cost was 18.3 million VND at

2.1 million VND/day) [8]. Meanwhile, our figure was quite similar to another study at E Hospital, which showed that the average direct treatment cost for exacerbation of COPD was 9.1 million VND [10]. The differences between health systems and rates among hospitals and countries made it difficult to directly compare our results and those from other hospitals. However, collectively, the hospitalisation cost for exacerbation could cause an economic burden for patients and their families as well as for society. COPD exacerbation requires effective disease control and management, as well as prevention to reduce hospitalisation costs.

Medication costs accounted for the highest proportion of hospitalisation costs (over 50%) in all phases according to Circular No. 37/2015/TTLT-BYT-BTC. Our research result was similar to the research conducted in Bach Mai Hospital, which showed that the medication cost accounted for the highest proportion (53.9% of total cost) [8]. Another study showed that the spending on medications, blood, and infusion was the highest, accounting for 75% [12]. In addition, in the current study, our results showed that the medication often used for patients with COPD at Vinh Phuc General Hospital included antibiotics, bronchodilators, and corticosteroids. The spending on antibiotics of these medications was the highest with 99.37 USD per course of treatment. Our research result was similar to a previous study at two provincial facilities showing that antibiotic costs accounted for the highest proportion (54.5% of inpatient drug costs) [9]. This could be explained by the most common cause of an exacerbation being an infection in the lungs or airways, and treatment of infection for moderate to severe exacerbations is a top priority along with maintaining the patient's ventilation function.

The cost components differed significantly between phase 1 and phases 2 and 3. This indicates that specific allowances and salaries significantly impact medical expenses. Our study used generalised linear models with a log-link function, validated using the normality test of residuals. While not perfect, this method is acceptable. According to J.L. Moran, et al. (2007) [13], the GLM using the inverse Gaussian distribution may be advantageous for cost data analysis. Our results found that gender, place of residence, department classification, comorbidity, and treatment duration correlated with higher hospitalisation costs, consistent with other studies. For example, a study

in Turkey found that the number of co-morbidities and the duration of hospitalisation increased the cost significantly [14]. Results from a 2008 Chinese study found that cost positively correlated to the duration of hospitalisation and the number of hospitalisation co-morbidities [15].

The study's strength lies in the analysis of COPD patients' expenses using real data from a large sample size of 1,629 patients, allowing the results to inform policymaking and lay the foundation for future research on economic evaluation. Our study was conducted on patients at Vinh Phuc General Hospital, a provincial grade 1 general hospital. To our knowledge, no similar study has been published from a provincial hospital in Vietnam, making our research a valuable representation of direct medical treatment costs for COPD in such settings and laying the groundwork for larger-scale studies with broader applicability.

However, this study has certain limitations. Firstly, it only measured direct medical costs and did not assess the burden of indirect costs. Secondly, the retrospective data collection method limited the research team's ability to obtain information on the treatment efficacy of each patient. Consequently, the study could not classify the severity of COPD for each patient according to the GOLD criteria, a crucial factor impacting the direct cost of COPD treatment. Furthermore, potential bias may have arisen due to the inclusion of patients with multiple comorbidities. The ICD code for COPD may not accurately capture all exacerbations of COPD, and certain diseases not yet assigned ICD codes but similar in condition to COPD could be misclassified in the insurance payment software under code J44.

5. Conclusions

This analysis not only provides epidemiological evidence of COPD but also estimates the economic burden of COPD on the patient population at Vinh Phuc General Hospital. This is a critical first step for future pharmacoeconomic studies of COPD treatment regimens. The hospitalisation cost was significantly high among patients with COPD, especially males, those with longer treatment durations, and those with comorbidities. The study suggests that strategies to prevent COPD and mitigate its effects should be widely implemented in the community to reduce the economic impact of COPD and improve the quality of life for affected individuals.

CRediT author statement

Nguyen Viet Phu: Methodology, Investigation; Nguyen Thi Mai Phuong: Writing - Original draft preparation, Reviewing and Editing; Dang Huu Duc: Data curation, Software; Vuong Trong Hieu: Software, Validation; Tran Thi Hang: Data collection and Synthesis; Nguyen Thien Phong: Methodology, Writing discussion; Le Hong Phuc: Methodology consultation; Pham Nu Hanh Van: Conceptualisation, Methodology, Supervision.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] M. Miniati, S. Monti, I. Pavlickova, et al. (2014), "Survival in COPD: Impact of lung dysfunction and comorbidities", *Medicine (Baltimore)*, **93**(12), DOI: 10.1097/MD.0000000000000076.
- [2] J.A. Okely, S.O. Shaheen, A. Weiss, et al. (2017), "Wellbeing and chronic lung disease incidence: The survey of health, ageing and retirement in Europe", *PLOS ONE*, **12**(7), DOI: 10.1371/journal.pone.0181320.
- [3] World Health Organization (2020), "The top 10 causes of death", <https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death>, accessed 8 May 2023.
- [4] S.M. May, J.T.C. Li (2015), "Burden of chronic obstructive pulmonary disease: Healthcare costs and beyond", *Allergy Asthma Proc.*, **36**(1), pp.4-10, DOI: 10.2500/aap.2015.36.3812.
- [5] L. Ekerljung, E. Ronmark, K. Larsson, et al. (2014), "Prevalence of COPD by disease severity in men and women in northern Vietnam", *COPD*, **11**(5), pp.575-581, DOI: 10.3109/15412555.2014.898039.
- [6] E.S. Ford, L.B. Murphy, O. Khavjou, et al. (2015), "Total and state-specific medical and absenteeism costs of COPD among adults aged ≥ 18 years in the United States for 2010 and projections through 2020", *Chest*, **147**(1), pp.31-45, DOI: 10.1378/chest.14-072.
- [7] B. Zhu, Y. Wang, J. Ming, et al. (2018), "Disease burden of COPD in China: A systematic review", *Int. J. Chron. Obstruct. Pulmon. Dis.*, **13**, pp.1353-1364, DOI: 10.2147/COPD.S161555.
- [8] Q.C. Ngo, T.T. Bui, V.G. Vu, et al. (2018), "Direct hospitalization cost of patients with acute exacerbation of chronic obstructive pulmonary disease in Vietnam", *Int. J. Environ. Res. Public Health*, **16**(1), DOI: 10.3390/ijerph16010088.
- [9] T.Q. Vo, T.C.N. Phung, T.Q. Vu, et al. (2018), "Cost trend analysis of chronic obstructive pulmonary disease among Vietnamese patients: Findings from two provincial facilities 2015-2017", *Journal of Clinical and Diagnostic Research*, **12**(6), pp.92-98, DOI: 10.7860/JCDR/2018/36668.11715.
- [10] X.T. Bui, T.T. Ngo, K.L. To (2020), "Analysis of direct treatment cost for exacerbation of chronic obstructive pulmonary disease in e hospital from October 2019 to March 2020", *VNU Journal of Science: Medical and Pharmaceutical Science*, **36**(2), pp.85-93, DOI: 10.25073/2588-1132/vnumps.4242.
- [11] T.C. Thu (2014), *The Direct Hospitalization Cost of Chronic Obstructive Pulmonary Disease, Pneumonia and Lung Cancer At 74 Central Hospital*, Master Thesis, Hanoi University of Public Health (in Vietnamese).
- [12] X.P. Vu, V.T. Duong, T.H. Nguyen (2012), "Inpatient cost of COPD patient in national lung hospital in 2009", *J. Med. Pract.*, **804**, pp.51-53.
- [13] J.L. Moran, P.J. Solomon, A.R. Peisach, et al. (2007), "New models for old questions: Generalized linear models for cost prediction", *J. Eval. Clin. Pract.*, **13**(3), pp.381-389, DOI: 10.1111/j.1365-2753.2006.00711.x.
- [14] T. Ornek, M. Tor, R. Altin, et al. (2012), "Clinical factors affecting the direct cost of patients hospitalized with acute exacerbation of chronic obstructive pulmonary disease", *Int. J. Med. Sci.*, **9**(4), pp.285-290, DOI: 10.7150/ijms.4039.
- [15] Y.H. Chen, W.Z. Yao, B.Q. Cai, et al. (2008), "Economic analysis in admitted patients with acute exacerbation of chronic obstructive pulmonary disease", *Chin. Med. J. (Engl)*, **121**(7), pp.587-591.

Influences of ageing parameters on the microstructure and mechanical properties of extruded 6063 and 6005 alloys

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Abstract:

The influences of ageing time and ageing temperature on the microstructure and mechanical properties were investigated for the extruded 6063 and 6005 alloys. Artificial ageing was performed on the alloy extrusions. The ageing process for 6063 aluminium alloy extrusions was studied at temperatures of 190, 205, and 220°C for durations of 90, 150, and 210 minutes, while extruded 6005 alloys were treated at temperatures of 150, 175, and 200°C for 4, 8, and 12 hours. The results demonstrate that the coarse AlMeSi particles formed during extrusion evolved from granular to rod-like particles (AlFeSi) as the ageing temperature or time increased for both aluminium alloy extrusions, with spherical particles (Al(Mn,Cr,Mg)Si) observed in the 6005 aluminium alloy extrusions. The mechanical properties of the extrusions at room temperature were highly sensitive to the ageing process. The optimal tensile strength, elongation, and hardness for the extruded 6063 and 6005 alloys were achieved at 190°C after 150 minutes and at 175°C after 8 hours, respectively.

Keywords: ageing, aluminium alloy, mechanical properties, microstructure.

Classification numbers: 2.1, 2.2, 2.3

1. Introduction

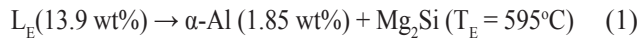
Aluminium alloys are highly desirable for structural applications due to their high strength-to-weight ratio, ease of machining and extrusion, excellent corrosion resistance, thermal and electrical conductivity, and recyclability. It is estimated that 78% of all extracted aluminium remains in use today, and recycling aluminium requires only 5% of the energy used in its original extraction [1]. These properties have driven the increased use of aluminium in the automotive industry. The two most commonly used aluminium alloys, 6005 and 6063, are alloyed with magnesium and silicon. As heat-treatable alloys, they can undergo post-extrusion heat treatments to increase strength via precipitation hardening. Alloy 6005 is a benchmark structural alloy known for its high strength, toughness, and ease of anodisation and machining. While 6063 alloy is not as tough or strong as 6005, it offers superior surface appearance and formability [2]. Although 7xxx series alloys are another option for structural aluminium, their poor extrudability makes them cost-prohibitive for many applications [1].

Due to their excellent extrusion performance, good welding characteristics, high corrosion resistance, and moderate strength, the 6063 and 6005 aluminium alloys

are widely used across industries such as machinery manufacturing, aerospace, aviation, and transportation, including in high-speed trains, subways, and urban railways [2, 3]. These alloys can be extruded to produce complex sectional shapes with thin walls and quenched via air or water mist cooling, making them one of the most commonly used materials for vehicle body construction [4-6]. However, 6005 alloy exhibits high quench sensitivity, requiring ageing treatments post-extrusion to achieve stable microstructures and mechanical properties [7-9]. To quickly achieve high-strength extrusions, artificial ageing is commonly employed in industrial production.

The appropriate ageing process and microstructure are key to achieving superior comprehensive mechanical properties in extruded 6063 and 6005 alloys. These alloys belong to the excess Si-strengthened Al-Mg-Si series. In the ternary Al-Mg-Si system, Mg₂Si particles form via a ternary peritectic reaction. However, in the pseudo-binary Al-Mg₂Si system, obtained from a vertical section of the Al-Mg-Si ternary system at a fixed Mg:Si atomic ratio of 2:1 (equivalent to 1.73:1 when the same ratio is expressed as a quotient of weights [10], Mg₂Si particles form as equilibrium reaction products from a eutectic reaction [11]:

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where the concentrations are expressed in weight percent of Mg_2Si . Given that the Mg limits the solubility of Mg_2Si in $\alpha\text{-Al}$, while the Si has no effect on it, a weight ratio of $\text{Mg}:\text{Si} \leq 1.73$ is desirable when designing industrial alloys in order to ensure the maximum amount of this compound in solution [12]. Assuming that all the Mg is present in the Mg_2Si form, the amount of Si needed to form Mg_2Si precipitates for the compositional range of Mg in 6xxx alloys is given by [10]:

$$[\text{Si}]_{\text{Mg}_2\text{Si}} = [\text{Mg}]_{\text{total}} \times \frac{A_{\text{Si}}}{2x A_{\text{Mg}}} \quad (\text{wt}\%) \quad (2)$$

where $[\text{Si}]_{\text{Mg}_2\text{Si}}$ is the amount of Si expressed in wt% present in the Mg_2Si compound; $[\text{Mg}]_{\text{total}}$ corresponds to Mg interval values for 6xxx alloys; and A_{Si} and A_{Mg} are the atomic weights of Si and Mg, respectively.

The Mg_2Si concentration, $[\text{Mg}_2\text{Si}]$, can be calculated according to:

$$[\text{Mg}_2\text{Si}] = [\text{Si}]_{\text{Mg}_2\text{Si}} + [\text{Mg}]_{\text{total}} \quad (\text{wt}\%) \quad (3)$$

As far as alloying is concerned, it appears that the amount of the so-called excess Si (Si_{ex}) is important as it affects the precipitation kinetics in Al-Mg-Si alloys. Si_{ex} leads to finer and denser precipitates by enhancing the nucleation kinetics of GP zones. It has been reported that the excess Si in the alloy enhances the nucleation of the hexagonal $\beta\text{-Mg}_2\text{Si}$ on Si atom grids, which are composed of hexagonal sub cells with lattice parameters $a=b=4.05 \text{ \AA}$ and $c=4.05 \text{ \AA}$ [13]. For a given Mg and Fe content, the amount of Si_{ex} depends on which intermetallic ($\alpha\text{-AlFeSi}$ or $\beta\text{-AlFeSi}$) is present [10]:

$$[\text{Si}]_{\text{xs}} = [\text{Si}]_{\text{alloy}} - [\text{Si}]_{\alpha\text{-AlFeMnSi}} - [\text{Si}]_{\text{Mg}_2\text{Si}} \quad (4)$$

$$[\text{Si}]_{\alpha\text{-AlFeMnSi}} = [\text{Fe}+\text{Mn}]_{\text{alloy}} \times \frac{A_{\text{Si}}}{3x(A_{\text{Mg}}+A_{\text{Mn}})} = 0.0664 [\text{Fe}+\text{Mn}]_{\text{alloy}} \quad (5)$$

As a heat-treatable aluminium alloy, precipitation strengthening is the primary strengthening mechanism in these alloys [14]. The precipitation phases vary with different ageing temperatures and times. At low temperatures and short times, solute atom clusters (GP zones) are difficult to form, resulting in low post-ageing strength (under-ageing). Conversely, at high temperatures and extended times, the critical nucleus size increases, leading to low strength after ageing (over-ageing).

Prior to the ageing process, the alloy undergoes solution treatment at elevated temperatures to dissolve the second-phase particles and maintain the elements in a single-phase (α) region. This is followed by quenching, which prevents the precipitation of the second phase, trapping the alloying elements in the α phase and producing a supersaturated solid solution (SSSS). During the ageing process, these alloying elements diffuse from the SSSS to form strengthening precipitates [15]. This nucleation is driven by the reduction

in Gibbs free energy as the thermodynamically unstable SSSS transforms into a more stable two-phase state. For 6xxx aluminium alloys, the artificial ageing temperature/time range is typically between 165 and 205°C for 2 to 16 hours, which is considered optimal for mechanical properties across a variety of applications [16].

While several types of precipitates can form during ageing, three main categories are typically considered, depending on the nature of the interface between the precipitates and the matrix. Coherent precipitates form when the atomic lattice of the precipitate aligns perfectly with the matrix lattice across the interface. Semi-coherent precipitates occur when the majority of the lattices match across the interface, though the mismatch is sufficient to accommodate dislocations. Lastly, incoherent precipitates do not align with the matrix at all [17].

Smaller precipitates with narrow inter-particle spacing generally provide optimal strengthening by creating strain fields that interact with dislocations, thereby hindering their motion. As precipitate size increases, the energy required for dislocation cutting also increases, leading to dislocation bowing around the precipitates. Additionally, as the inter-particle spacing grows, dislocations can more easily bow around the precipitates, forming a dislocation loop that obstructs other dislocations and favours the dislocation-cutting mechanism. To achieve optimal alloy strength, the aim is to determine the ageing time and temperature at which the cutting and bowing mechanisms result in equivalent strength. At this point, the alloy reaches its maximum strength [18].

Previous studies primarily focused on optimising composition, the extrusion process, quenching sensitivity, and welding in 6063 and 6005 alloys [6, 7, 19-23]. However, limited research has been conducted on the ageing process [22-29], particularly regarding short-term, single-stage ageing, which is advantageous in reducing production cycles and costs. To enhance the mechanical properties of extrusions while minimising energy consumption, the short-term ageing process can be optimised to achieve the best age-hardening effect in extruded 6063 and 6005 alloys. In this study, the influences of ageing temperature and time on the microstructure and mechanical properties of extruded 6063 and 6005 alloys were investigated under various short-term, single-stage ageing conditions.

2. Materials and methods

The 6063 and 6005 aluminium alloys in the form of extruded profiles with a thickness of 18 mm and a width of 80 mm were supplied by VietY Aluminium Company Limited. The chemical compositions of the experimental alloys, compared with the standard 6063 and 6005 alloys, are presented in Table 1.

Table 1. Chemical composition of 6063 and 6005 aluminium alloys.

Alloy	Si	Fe	Cu	Mn	Mg	Cr	Zn	Ti	Al
6063	0.486	0.236	0.028	0.023	0.547	0.012	0.022	0.023	Remain
6005	0.853	0.235	0.036	0.024	0.675	0.014	0.027	0.023	Remain

The extruded profiles were produced following the online solid solution and quenching process. The extrusion coefficient ranged between 18 and 20, with an extrusion speed of 5 mm/s and an extrusion temperature of 510–540°C. Online air cooling quenching was applied for the 6063 alloys, while water cooling quenching was employed for the 6005 alloys during the extrusion process.

The ageing treatments for the 6063 and 6005 aluminium alloys are illustrated in Figs. 1 and 2. The 6063 alloys were aged at 190, 205, and 220°C for 90, 150, and 210 minutes, respectively. Meanwhile, the 6005 alloys were aged at 150, 175, and 200°C for 4, 8, and 12 hours, respectively.

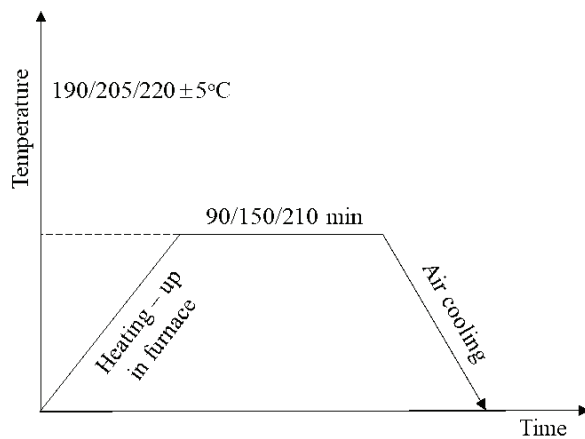


Fig. 1. Aging conditions of 6063 aluminium alloy.

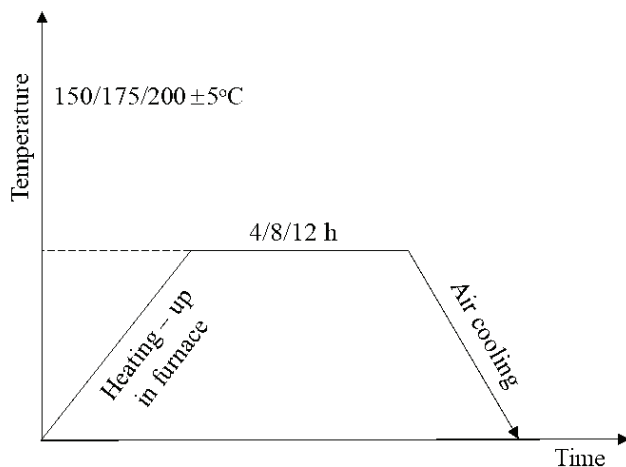


Fig. 2. Aging conditions of 6005 aluminium alloy.

The extruded profiles were sectioned transversely for microstructure observation and longitudinally for mechanical properties testing. The final tensile strength values were averaged from three tests, conducted at a strain rate of 1/20 mm/s using a YMM-5 testing apparatus. The tensile specimens were prepared according to ASTM E557M standards [2].

Hardness was measured at room temperature using a Brinell hardness testing machine with a 62.5 N load. The final hardness value was the average of five tests performed at various points evenly distributed from the outermost point to the centre of the specimen.

The microstructure of the samples was examined using both optical and scanning electron microscopes (SEM) with an energy-dispersive X-ray spectroscopy (EDS) detector. The samples were initially polished with 1200-grit paper and then polished using an alumina-containing colloidal suspension. Following polishing, the samples were inspected under both pre- and post-etching conditions. The etching process was conducted using Keller's solution (85 ml H₂O, 10 ml HNO₃, and 5 ml HF) for 30 seconds. The samples were then rinsed with deionised water and alcohol, dried, and examined under optical and scanning electron microscopes.

3. Results and discussion

Figure 3 presents optical microscopy images of the 6063 and 6005 aluminium alloys. Specifically, the former was aged at 205°C for 150 minutes, while the latter was aged at 175°C for 8 hours. The particle sizes in both alloys were inhomogeneous, with the largest particles measuring approximately 100 µm. Overall, altering the ageing temperature and time for either aluminium alloy did not significantly affect grain size.

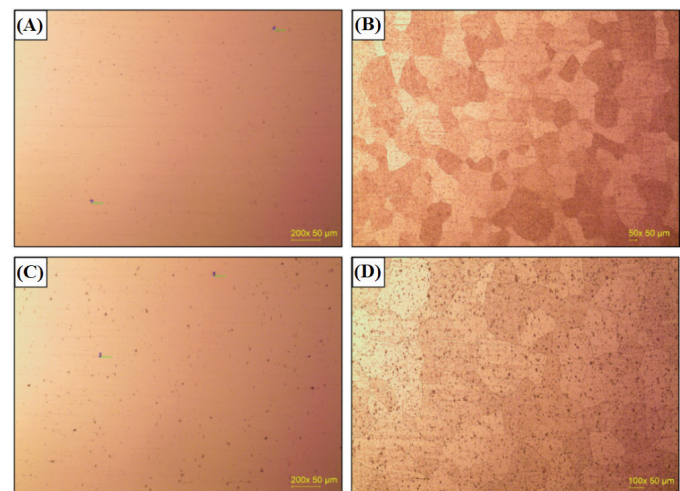


Fig. 3. (A, B) Optical microscope images of 6063 aluminium alloy aged at 205°C and held for 150 minutes, before and after etching, respectively; (C, D) 6005 aluminium alloy aged at 175°C and held for 8 hours, before and after etching, respectively.

During the high-temperature ageing process, precipitates developed at an accelerated rate. Through SEM and EDS analyses (Figs. 4 and 5) of the 6063 alloy, it was observed that precipitates formed spherical structures containing Cr and Fe at lower ageing temperatures, as shown in the EDS analysis in Fig. 4. These precipitates were identified as AlFeSi and AlCrSi particles, with a size of approximately 1 μm . However, increasing the ageing temperature altered the precipitate form, with AlFeSi particles observed as rod-like structures, and some spherical precipitates containing Ni, Cr, and Mn, as shown in Fig. 5.

SEM images and EDS analysis of the 6005 aluminium alloy, which was aged at 175 and 200°C, are presented in Figs. 6 and 7. The results show that, when aged at lower temperatures, precipitates formed in relatively small sizes, with Mg_2Si particles as spherical structures or AlFeSi as bar-like forms (Fig. 6). As the ageing temperature increased, the precipitate size increased slightly, while their shape remained unchanged (Fig. 7). The spherical precipitates contained Mn, Mg, or Cr, and their sizes ranged from 0.5 to 1 μm . AlFeSi particles were also observed as rod-like structures, similar to those in the 6063 aluminium alloy.

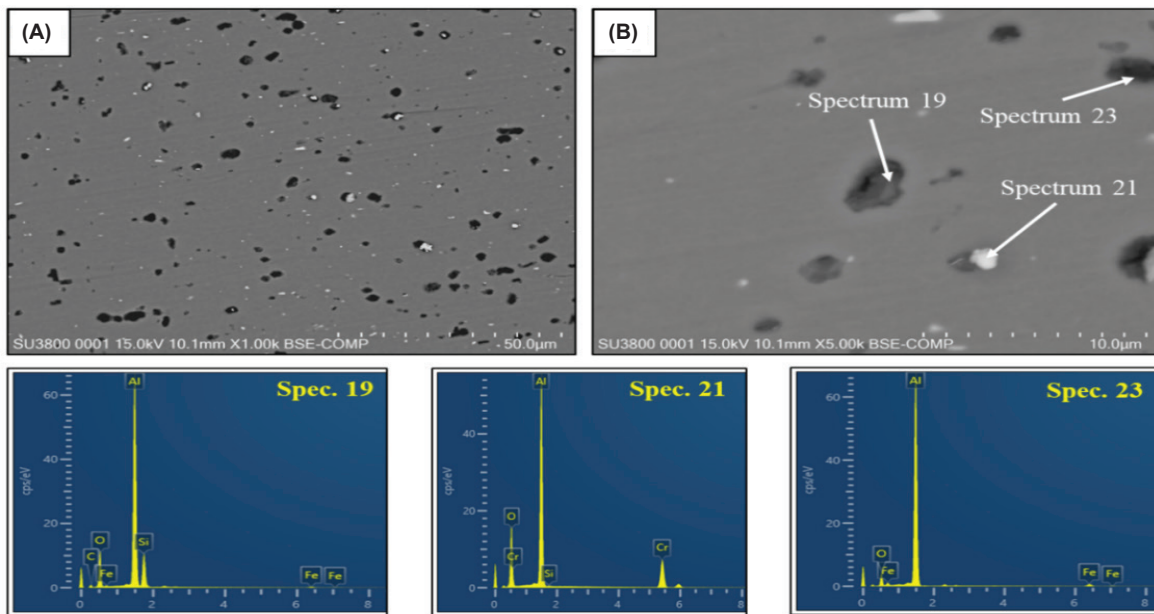


Fig. 4. SEM image of 6063 aluminium alloy aged at 190°C and held for 150 minutes, before etching (A) low magnification, (B) high magnification and EDS analysis for each spectrum.

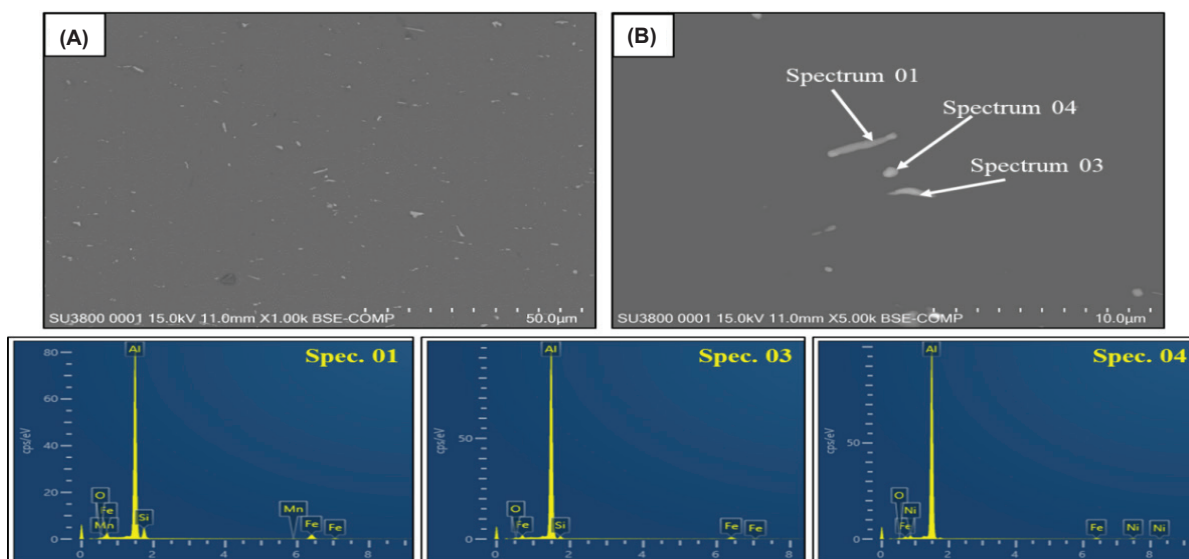


Fig. 5. SEM image of 6063 aluminium alloy aged at 205°C and held for 150 minutes, before etching (A) low magnification, (B) high magnification and EDS analysis for each spectrum.

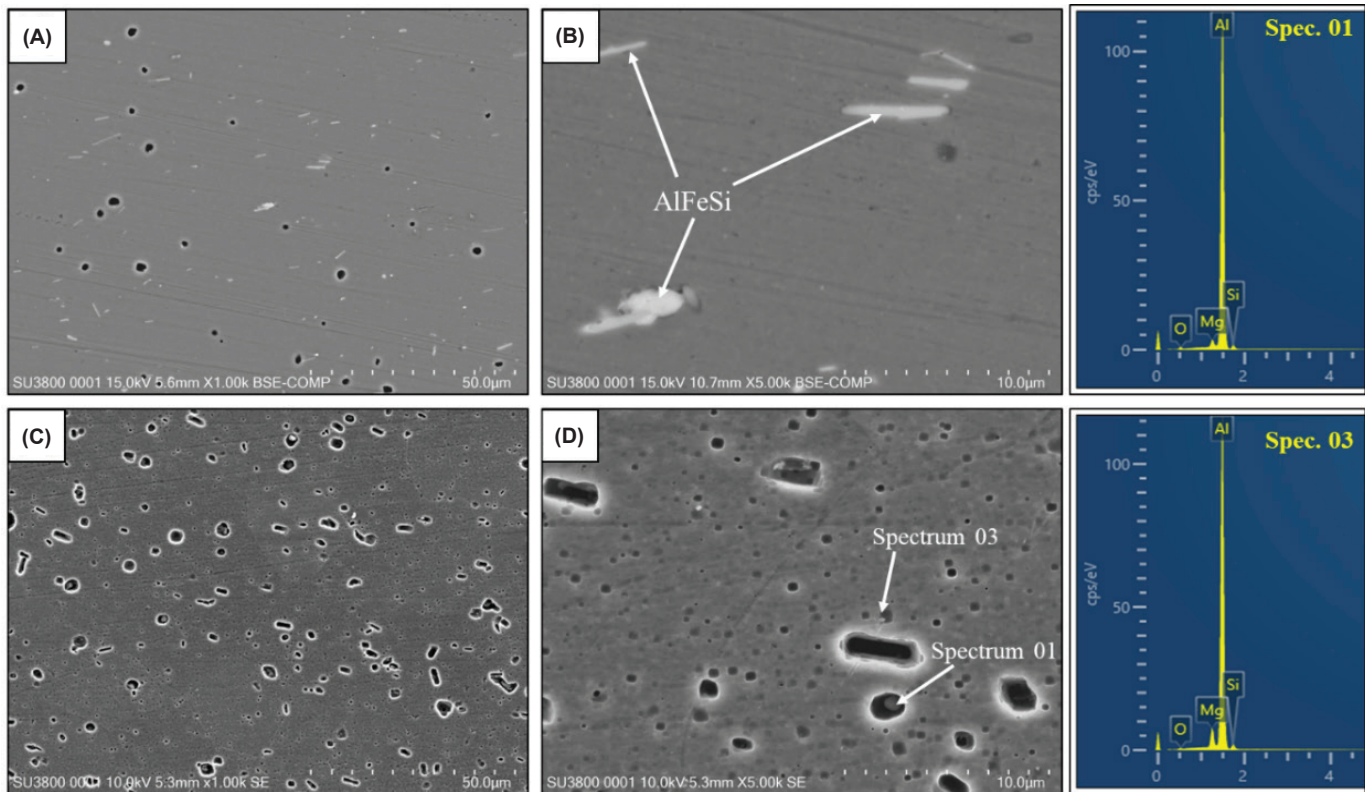


Fig. 6. SEM images and EDS analysis of 6005 aluminium alloy aged at 175°C and held for 8 hours, (A, B) before etching and (C, D) after etching.

The precipitates described above had a direct influence on the mechanical properties of the 6063 and 6005 aluminium alloys. Their formation during the ageing process enhanced the strength of the alloys due to the interaction between dislocation motion and the precipitates.

Peak ageing is one of the ageing treatment methods extensively used in current industrial applications. It produces the highest density of precipitates in the matrix, thereby maximising the strength of the alloys. The kinetics of ageing is controlled by the diffusion of solute atoms, which is strongly dependent on the temperature and duration of the ageing process. Precipitates clearly play a crucial role in determining the mechanical properties of the 6005 and 6063 aluminium alloys. The strengthening effect is closely related to the type, interface, morphology, density, and size of the precipitates. Coarse particles larger than 3 μm are typically formed during the extrusion process, comprising Fe-containing inclusions such as AlFeSi and Al(Fe,Cr)Si [8, 30]. As

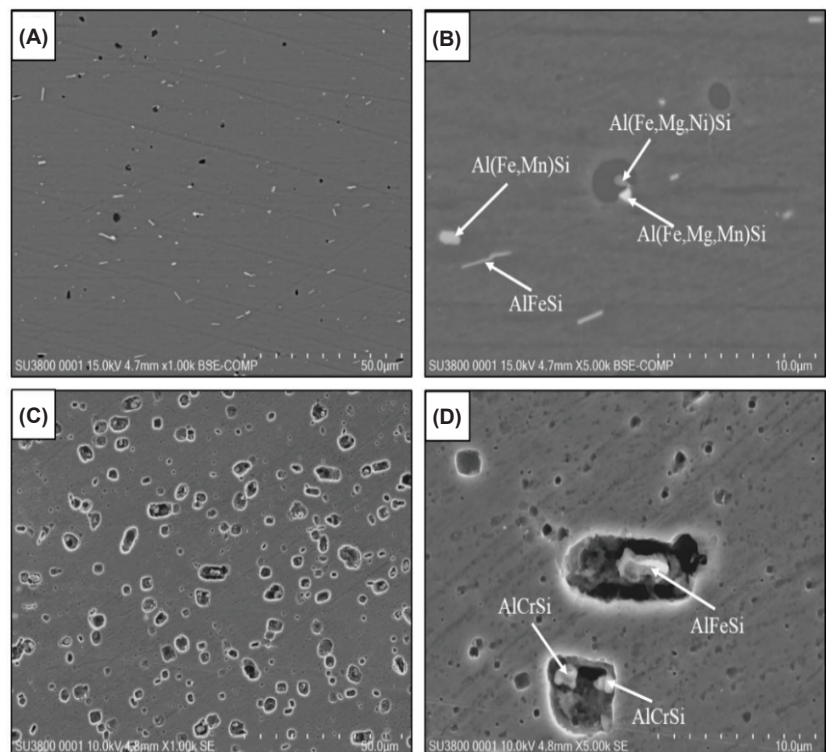


Fig. 7. SEM images of 6005 aluminium alloy aged at 200°C and held for 8 hours (A, B) before etching and (C, D) after etching.

shown in Figs. 2 to 7, increasing the time and temperature during ageing caused slight changes in the size and morphology of the coarse particles due to thermal diffusion at the phase interface. Submicron precipitates are expected to form during the ageing process [31]. Under short-term, single-stage ageing, the quantity and size of the submicron precipitates depended on the temperature and duration of the process.

Figures 8 and 9 illustrate the influence of temperature and holding time during the ageing process on the hardness of the 6063 and 6005 aluminium alloys. For the 6063 alloy (Fig. 8), hardness gradually increased with extended ageing time. After 2.5 hours of ageing, the hardness remained unchanged regardless of the temperature. In contrast, the hardness of the 6005 alloy decreased as the ageing time increased, with a more pronounced decline at lower temperatures compared to higher temperatures (Fig. 9). The hardness values obtained for the 6005 and 6063 alloys through this ageing process aligned with commercial standards.

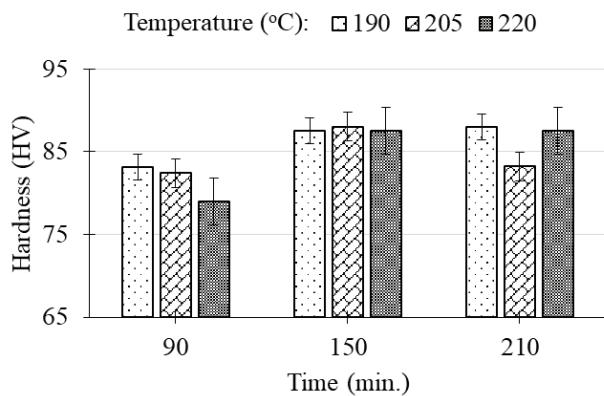


Fig. 8. The dependence of 6063 aluminium alloy hardness on temperature and hold time of ageing.

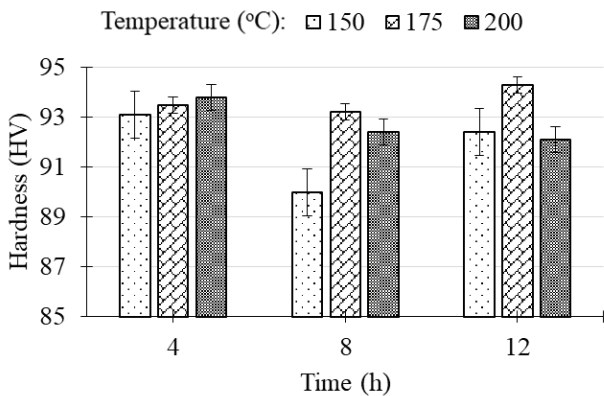


Fig. 9. The dependence of 6005 aluminium alloy hardness on temperature and hold time of ageing.

The rapid age-hardening response observed after 1.5 hours of ageing at 190°C for the 6063 alloy (Fig. 8) and 4 hours at 175°C for the 6005 alloy (Fig. 9) reflects the initial fast formation of GP zones and Mg-Si co-clusters, which were trapped during water-mist cooling. This precedes the formation of needle-shaped β'' -strengthening precipitates, which are partially or fully coherent with the matrix, significantly improving the mechanical properties as seen in the ageing curves. The participation of Mg and Si solute atoms in these precipitation events reduces the availability of solute atoms for further precipitation, leading to a reduction in strengthening over time. Additionally, the slow increase in hardness between 2.5 to 3.5 hours for the 6063 alloy and 8 to 12 hours for the 6005 alloy could be attributed to the formation of incoherent and larger, less effective rod-shaped β' and β precipitates. The lower hardness values observed after ageing, as shown in Figs. 8 and 9, could be attributed to supersaturation or low vacancy concentrations during the extrusion process, which may have impeded the formation of significant strengthening phases during ageing [32-35].

Figures 10 and 11 show the tensile strength of the 6063 and 6005 aluminium alloys as a function of ageing parameters. As ageing time increased, tensile strengths of both alloys increased at the lowest temperature (190°C) but decreased at higher temperatures. Notably, the tensile strength of the 6063 alloy aged at 205°C reached a peak of 248.02 MPa at 2.5 hours before declining. In the case of the 6005 alloy, despite initial differences, tensile strengths converged at values above 250 MPa after 12 hours of ageing, irrespective of temperature (Fig. 11). The highest tensile strength for the 6005 alloy, 268.53 MPa, was achieved by ageing at 175°C for 4 hours.

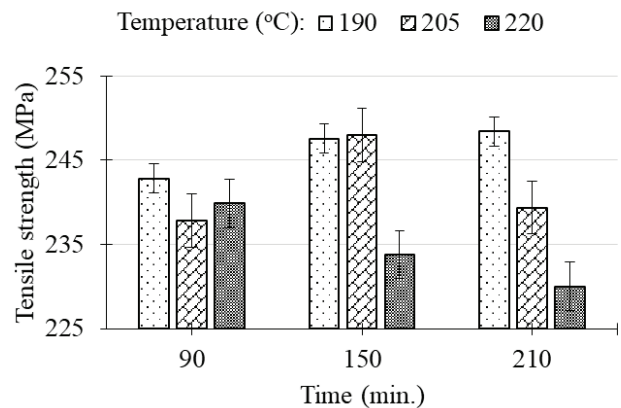


Fig. 10. The tensile strength of the 6063 aluminium alloy as a function of ageing temperature and holding time.

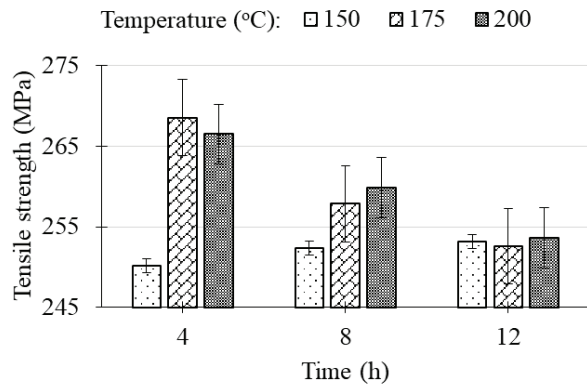


Fig. 11. Tensile strength of the 6005 aluminium alloy as a function of ageing temperature and holding time.

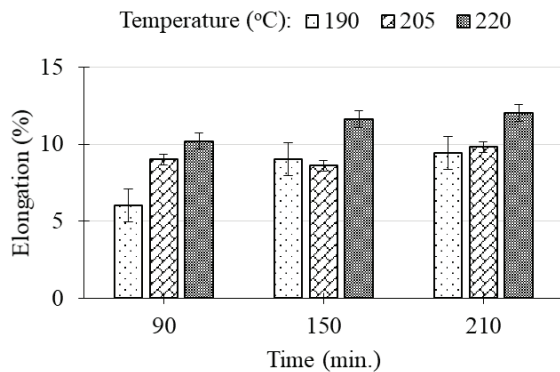


Fig. 12. Elongation of the 6063 aluminium alloy as a function of ageing time and temperature.

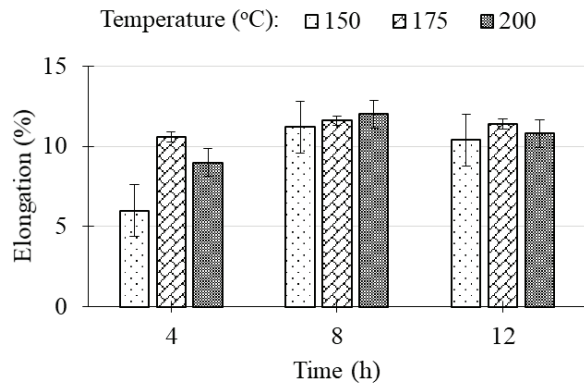


Fig. 13. Elongation of the 6005 aluminium alloy as a function of ageing time and temperature.

Figures 12 and 13 show the influence of time and temperature on the elongation of the 6063 and 6005 alloys. Both alloys exhibited increased elongation with longer ageing times and higher temperatures. The 6063 alloy achieved its maximum elongation of 12% after 3.5 hours of ageing at 220 °C. Similarly, the 6005 alloy attained the highest elongation (12%) after 8 hours at any of the tested ageing temperatures, with a slight reduction after 12 hours of ageing.

The results above indicate that the mechanical properties of the aluminium alloys are closely linked to their microstructure. Increasing the temperature and ageing time led to grain growth, promoted the formation of precipitates, and had a significant effect on the material's mechanical properties. The small size of the ageing precipitates allowed dislocations to easily cut through them, which facilitated dislocation movement along the channel and reduced the ductility of the alloy [7]. The evolution of coarse Al(Fe,Cr) Si precipitates from granular to rod-like particles is likely to cause stress concentrations, particularly when AlFeSi particles form at grain boundaries during high-temperature ageing. These particles can further reduce the strength of the extruded alloys [36].

4. Conclusions

This study investigated the effects of temperature and ageing time on the microstructure and mechanical properties of extruded 6063 and 6005 alloys. Precipitates formed rapidly during the high-temperature ageing process in both alloys. These precipitates included rod-like AlFeSi particles, while spherical precipitates consisted of fine Mg₂Si particles and Cr-, Ni-, and Mn-containing particles, with sizes ranging from 0.5 to 1 µm. The presence of these precipitates in the matrix significantly enhanced the mechanical properties of the extruded 6063 and 6005 alloys.

The mechanical properties of the extruded 6063 alloy at room temperature were highly sensitive to the ageing temperature and duration. Optimal and stable mechanical properties were achieved with an ageing process of 190 °C for 2.5 hours for the 6063 alloy, and 175 °C for 8 hours for the 6005 alloy. The hardness, tensile strength, and elongation values of the 6063 alloy aged at 190 °C for 2.5 hours were 87.5 HV, 247.6 MPa, and 9%, respectively. Meanwhile, the 6005 alloy aged at 175 °C for 8 hours achieved a hardness of 93.2 HV, a tensile strength of 257.8 MPa, and an elongation of 11.6%.

CRedit author statement

Do Van Quang: Conceptualisation, Methodology, Validation, Formal analysis, Data curation, Visualisation, Supervision, Writing - Reviewing, and Editing; Le Thi Van Anh: Formal analysis; Vu Thi Huong Ly: Formal analysis and Editing; Nguyen Quoc Tuan: Formal analysis.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] K.H. Matucha (1996), *Structure and Properties of Nonferrous Alloys*, Wiley-VCH, 837pp.
- [2] Y. Wenchao, W. Mingpu, S. Xiaofei, et al. (2010), "Study of the aging precipitation and hardening behavior of 6005A alloy sheet for rail traffic vehicle", *Acta Metallurgica Sinica*, **46**(12), pp.1481-1487, DOI: 10.3724/SP.J.1037.2010.00223.
- [3] Y.L. Li (2000), "Properties and processing characteristic of 6005A alloy", *Light Alloy Fabrication Technology*, **28**(6), pp.41-43.
- [4] L. Jingan (2004), "The extrusion of large sized special 6005A aluminum profile", *Light Alloy Fabrication Technology*, **32**(4), pp.36-41.
- [5] Y. Wanming, S. Jian (2006), "On extrusion and heat treatment technique of 6005A alloy for vehicles", *Journal of Chongqing University of Science and Technology: Natural Science Edition*, **8**(1), pp.33-39.
- [6] S. Jian, L. Yanli, Z. Mingfeng (2003), "Effects of press quenching variables on microstructures and mechanical properties of 6005A alloy extrusion sections", *The Chinese Journal of Nonferrous Metals*, **13**(6), pp.1461-1466.
- [7] C.W. Xiao, M.P. Wang, Z.A. Wang, et al. (2003), "Quench sensitivity of 6005A aluminum alloy", *The Chinese Journal of Nonferrous Metals*, **13**(3), pp.635-639.
- [8] H.L. Zi (2001), *Microstructures and Mechanical Properties of AlmgSi Alloys*, Shenyang: Northeastern University.
- [9] Y.W. Chao (2001), *Aged-Hardening Behavior and Microstructural Characterization of Precipitates in AlmgSiCu: 6005A Alloy*, Central South University.
- [10] J.A. Lozano, B.S. Peña, G.F.V. Voort (2014), "Effect of processing steps on the mechanical properties and surface appearance of 6063 aluminium extruded products", *Materials*, **7**(6), pp.4224-4242, DOI: 10.3390/ma7064224.
- [11] J.R. Davis (1993a), *Aluminum and Aluminum Alloys (ASM Specialty Handbook)*, ASM International, 784pp.
- [12] C. Meng (2010), *Effect of Preheating Condition on Strength of AA6060 Aluminium Alloy for Extrusion*, Master's Thesis, School of Engineering, Auckland University of Technology, 136pp.
- [13] C.D. Marioara, S.J. Andersen, T.N. Stene, et al. (2007), "The effect of Cu on precipitation in Al-Mg-Si alloys", *Philosophical Magazine*, **87**(23), pp.3385-3413, DOI: 10.1080/14786430701287377.
- [14] J.R. Davis (1993b), *ASM Specialty Handbook: Aluminum and Aluminum Alloys*, ASM International, DOI: 10.1361/autb2001p351.
- [15] Y.S. Sato, H. Kokawa, M. Enomoto, et al. (1999), "Microstructural evolution of 6063 aluminium during friction-stir welding", *Metallurgical and Materials Transactions A*, **30**, pp.2429-2437, DOI: 10.1007/s11661-999-0251-1.
- [16] P. Mukhopadhyay (2012), "Alloy designation, processing, and use of AA6XXX series aluminium alloys", *ISRN Metallurgy*, DOI: 10.5402/2012/165082.
- [17] X.Y. Liu, Q.L. Pan, L.Y. Zheng, et al. (2013), "Effect of aging temper on the thermal stability of Al-Cu-Mg-Ag heat-resistant alloy", *Materials & Design*, **46**, pp.360-365, DOI: 10.1016/j.matdes.2012.10.039.
- [18] A.J. Ardell (1985), "Precipitation hardening", *Metallurgical Transactions A*, **16**, pp.2131-2165, DOI: 10.1007/BF02670416.
- [19] W. Ming, W.H. Dong (2011), "Study on microstructure and mechanical properties of welded joint of 6005A alloy by double wire MIG welding", *Hot Working Technology*, **40**(9), pp.144-145.
- [20] C.J. Hui, S.G. Jie (2008), "Research on aging process of AlMgSi alloys", *Shanghai Metals*, **30**(4), pp.16-18.
- [21] D.X. San, L.J. An (2004), "Research and development of large 6005A alloy profiles for subway vehicle", *Aluminum Fabrication*, **19**(4), pp.19-26.
- [22] A. Simar, Y. Brechet, B.D. Meester, et al. (2007), "Sequential modeling of local precipitation, strength and strain hardening in friction stir welds of an aluminum alloy 6005A-T6", *Acta Materialia Sinica*, **55**(18), pp.6133-6143, DOI: 10.1016/j.actamat.2007.07.012.
- [23] A. Simar, Y. Brechet, B.D. Meester, et al. (2008), "Microstructure, local and global mechanical properties of friction stir welds in aluminium alloy 6005A-T6", *Materials Science and Engineering A*, **486**(1-2), pp.85-95, DOI: 10.1016/j.msea.2007.08.041.
- [24] S. Zajac, B. Hutchinson, A. Johanson, et al. (1993), "Microstructure control and extrudability of Al-Mg-Si alloys, micro alloyed with manganese", *J. Phys. IV France*, **3**, DOI: 10.1051/jp4:1993740.
- [25] I. Musulin, O.C. Celliers (1990), "Role of manganese in 6063 alloy and effect of quench sensitivity in 6063", *Proceedings of The TMS Annual Meeting on Light Metal*, **119**, pp.951-954.
- [26] O.E. Okorafor (1991), "Effect of heat treatment on the corrosion resistance of 6063 aluminium alloy", *Corrosion Prevention and Control*, **38**(6), pp.141-144.
- [27] D.M. Jiang, B.D. Hong, T.C. Lei, et al. (1990), "Fatigue fracture behavior of an under-aged Al-Si-Mg alloy", *Scripta Metall.*, **24**(4), pp.651-654, DOI: 10.1016/0956-716X(90)90217-5.
- [28] P.S. Veers, J.A.V.D. Avyle (1992), "Fatigue crack growth from narrow-band Gaussian spectrum loading in 6063 aluminium alloy", *Advances in Fatigue Lifetime Predictive Techniques*, DOI: 10.1520/STP24160S.
- [29] R. Kumar, S.B.L. Garg (1989), "Effect of yield stress and stress ratio on fatigue crack closure in 6063-T6 aluminium alloy", *Int. J. Pressure Vessels Piping*, **38**(4), pp.293-307, DOI: 10.1016/0308-0161(89)90079-3.
- [30] N.C.W. Kuijpers, J. Tirel, D.N. Hanlon, et al. (2003), "Characterization of the α -Al(FeMn)Si nuclei on β -AlFeSi intermetallics by laser scanning confocal microscopy", *Journal of Materials Science Letters*, **22**, pp.1385-1387, DOI: 10.1023/A:1025734624469.
- [31] I.J. Polmear (2006), "Light alloys: From traditional alloys to nanocrystals", *Elsevier*, 421pp, DOI: 10.1017/S000192400008670X.
- [32] F. Bakare (2019), *Effect of Extrusion Processing on The Microstructure and Mechanical Properties of AA6082 Aluminium Alloys*, Master Thesis, Macquarie University, DOI: 10.25949/20428254.v1.
- [33] G.I.P.D. Silva, R.A.D. Perera, P.V.S.K. Ranasinghe (2013), "Study of the effects of two step age hardening process on mechanical properties of aluminum 6063-T5 alloy", *National Engineering Conference 2013, 19th Eru Symposium, Faculty of Engineering, University of Moratuwa, Sri Lanka*, pp.110-113.
- [34] G.M. Nowotnik (2010), "Influence of chemical composition variation and heat treatment on microstructure and mechanical properties of 6xxx alloys", *Archives of Materials Science and Engineering*, **46**(2), pp.98-107.
- [35] T. Wu, X.F. Ding, J. Sun, et al. (2013), "Effects of the aging time and temperature on the microstructures and mechanical properties of 6082 aluminum alloy extrusions", *Advanced Materials Research*, **652-654**, pp.1035-1042, DOI: 10.4028/www.scientific.net/AMR.652-654.1035.
- [36] X. Ding, J. Sun, J. Ying, et al. (2012), "Influences of aging temperature and time on microstructure and mechanical properties of 6005A aluminum alloy extrusions", *Trans. Nonferrous Met. Soc. China*, **22**, pp.s14-s20, DOI: 10.1016/S1003-6326(12)61677-X.

Stability of alkyd emulsion resins and anti-corrosion properties of water-based alkyd emulsion paints

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Abstract:

Vegetable oils extracted from nuts are a useful source of raw materials for the paint industry. Alkyd resins were synthesised with a content of 50 wt% soybean oil. The synthesised alkyd resins were evaluated by Fourier transform infrared spectroscopy (FTIR) and gel permeation chromatography (GPC). Particle size was determined using dynamic light scattering (DLS). Soybean oil alkyd resins were emulsified with the anionic surfactants linear alkylbenzene sulfonate (LAS)/sodium lauryl sulphate (SLS) and the nonionic surfactant nonyl phenol ethoxylate (NP9) in an aqueous solvent. The anti-corrosion properties of the alkyd emulsion coatings were determined using the electrochemical method. The results showed that with a content of 13 wt% of the mixture of LAS and NP9 surfactants, the formed alkyd emulsion resins had good stability. Alkyd emulsion paint with a water solvent achieved the physicochemical properties required for steel protection. However, the anti-corrosion properties of the fabricated alkyd emulsion coatings need to be improved. The use of water as a solvent is gradually replacing organic solvents, with many prospects for new applications.

Keywords: alkyd emulsion resin, emulsifiers, soybean oil alkyd resin, surfactants water-based alkyd emulsion paint.

Classification numbers: 2.1, 2.2, 2.3

1. Introduction

The paint industry is developing in an environmentally friendly direction. Vegetable oils extracted from nuts are a useful source of raw materials for the paint industry [1, 2]. Polyester resins modified with vegetable oils form alkyd resins. Thus, alkyd resins are easier to biodegrade and more environmentally friendly than other synthetic polymers [3, 4]. Drying oils and semi-drying oils such as sunflower oil, castor oil, soybean oil, and linseed oil are often used to modify polyester resins for paint [5, 6].

Solvent-based alkyd resins have a high content of volatile organic compounds (VOCs). These compounds pollute the environment. Consequently, paint manufacturers have reduced the VOC content in paints [7, 8].

In one study, the authors prepared water-based acrylic alkyd resins. These resin systems are stable, with a dispersion of 32 compared to a conventional resin of 8 [9]. Many factors affecting the stability of water-based acrylic alkyd resins were examined [10].

The properties of surfactants in water-based acrylic alkyd emulsion resins were also studied [11].

In another study, the authors synthesised alkyd resins from soybean oil and glycerol using a Zirconium Octoate catalyst [12].

In other studies, the authors have also studied the use of aqueous solvents as substitutes for organic solvents in paints [13-15].

The stability of alkyd emulsion resin depends on the emulsifier and emulsification conditions. Water-based alkyd emulsion paints are environmentally friendly. However, previous results showed that the stability of the emulsion system needs to be improved, and efforts must continue to enhance the durability and corrosion resistance of emulsion alkyd paints.

This study presents the effects of the NP9 nonionic emulsifier and the LAS/SLS anionic emulsifier on the stability of alkyd emulsion resins and water-based alkyd emulsion paint.

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2. Materials and methods

2.1. Materials

Glycerol is a liquid with a density at 25°C of 1.261 g/cm³; its boiling point is 290°C; its purity is 99.5% (Duc Giang Chemical, Vietnam). Phthalic anhydride is a white crystal with a density at 25°C of 1.53 g/cm³; a melting point is 131.6°C, a boiling point is 295°C, purity is 99.5% (Shandong Hongxin Chemical, China). Soybean oil is liquid, iodine index (Wijs) is 124-139 (Tuong An, Vietnam). Lead oxide (PbO) is a red crystal with a density of 9.53 g/cm³, a melting point is 888°C, purity is 98% (Jiangsu Tianpeng, China). Xylene (99.5% purity, China), methanol (99.5% purity, China), acetone (99.5% purity, China), and phenolphthalein (99.5% purity, China).

Emulsifiers include NP9 nonionic surfactant. NP9 is a liquid with a density of 1.055 g/cm³ (at 20°C), boiling point is 293-297°C, purity is under 99% (Tergitol, Dow, Indonesia). Anionic surfactant is SLS. SLS is a white solid, melting point is 206°C (99% purity, Disponil SLS 103, China). Anionic surfactant is LAS, density is 1.06 g/cm³, boiling point is 315°C. LAS is a thick liquid with a dark brown colour, soluble in water (99.8% purity, Henan Surface Chemical Industry, China).

Dispersing additive is CFC-690H, (Cheongwoo CFC, Korea). Titanium dioxide powder (Tiona 595, China), talcum powder (Talc 1250, Taiwan), calcium carbonate filler (China). Defoamer is BYK024 (Germany). Octa-Soligen 144 aqua drying agent is a purple viscous liquid with metal content in the mixture: Co²⁺: 0.95-1.05%, Zn²⁺: 3.10-3.30%, Zr²⁺: 7.00-7.40% (Borchers GmbH, Germany). Acrysol thickener RM-825 (Dow, USA).

2.2. Preparation of samples

Alkyd resin synthesis:

Alkyd resin (A0) reaction consists of three stages [16, 17]:

1. Oil triglycerides will react with glycerol at 220-240°C with Pb²⁺ (PbO) catalyst to form monoglycerides.
2. Monoglycerides react with phthalic anhydride at 170°C to form monoester.
3. The monoester condensation reaction continues at a temperature of 220-240°C to form alkyd resins.

Using xylene solvent attracts water to increase the performance of the reaction. The ratio of xylene/water is 4/1 (wt/wt). The stopping time of the reaction is when the acid index (AI) of the mixture is less than 30.

The alkyd resin reaction is presented in Fig. 1.

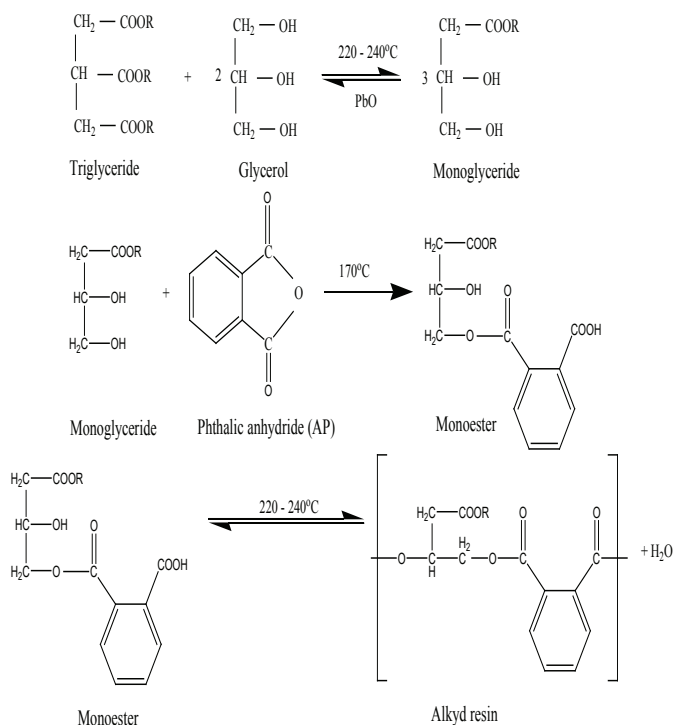


Fig 1. Alkyd resin reaction from glycerol and phthalic anhydride (AP).

Soybean oil (50.00 g) and glycerol (16.02 g) were placed in a 500-ml glass reaction tank. The reaction was heated to 170°C and afterwards, 0.2 g of PbO catalyst was added. The temperature of the reaction was raised to 220°C to continue with the reaction of converting triglycerides into monoglycerides. After a reaction time of 1 h, the solubility of the mixture in ethanol prepared according to an ethanol/mixture ratio of 3/1 (v/v). The reaction temperature was reduced to 170°C and phthalic anhydride (AP) (38.67g) was added to the reaction mixture to conduct a monoester reaction for a period of 1.5 h. Then, the reaction was heated to 220°C to continue the condensation reaction. The xylene solvent (18.81 g) was slowly added to the mixture to separate the water. The reaction ended when the acid index was less than 30 mg KOH/g.

Synthetic method of alkyd emulsion resin:

Emulsification method (phase reversal method): The emulsifiers used are a nonionic mixture of NP9 and the anionic LAS/SLS.

Alkyd resin is placed in a 250 ml glass beaker and heated to 70°C. LAS/SLS and NP9 emulsifiers are added, and the mixture is stirred at 500 rpm for 10 minutes. Water is added slowly at a ratio of 50:50 (v/v) to the emulsifier. The mixture temperature is increased to 90°C, and the stirring speed is increased to 2000 rpm for 45 minutes. Then, the mixture temperature is reduced by 30°C to stabilise and store.

Effect of surfactant content on the stability of emulsion alkyd resins: The surfactant content affects the emulsification ability and properties of the emulsion system. The suitable surfactant content ranges from 10 to 16 wt% [9-12]. After the emulsification process was completed, the samples were left to stabilise at room temperature for 24 h and evaluated for the stability of the alkyd emulsion resin (Table 1).

Table 1. Effect of surfactant content on stability of alkyd emulsion resin.

Specifications	Samples						
	S1	S2	S3	S4	S5	S6	S7
Surfactant content (wt%)	10	11	12	13	14	15	16
Alkyd resins (g)	40.00	39.00	38.00	37.00	36.00	35.00	34.00
SLS anionic surfactant (g)	3.33	3.67	4	4.33	4.67	5.00	5.33
NP9 nonionic surfactant (g)	6.67	7.33	8	8.67	9.33	10.00	10.67
Water (g)	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Parameters for evaluating the stability of the emulsion	- Ability to create an emulsion system - Solid content - pH - Room temperature storage						

Effect of surfactant type on the stability of alkyd emulsion resin: Anionic surfactants provide the ability to emulsify but do not support the storage process of the product. Nonionic surfactants, however, participate in both the emulsification process and the storage. Therefore, choosing the correct surfactant ratio is essential for both the emulsification process and the properties of the emulsion system. The results of the study showed that if the surfactant content was 13 wt%, the alkyd emulsion resin had the best stability (Tables 1 and 5).

The content of the emulsifier is 13 wt%. The ratio of emulsifiers SLS:NP9 is 1:2 (wt/wt) (AE1 emulsion alkyd resin sample) and LAS:NP9 is 1:2 (wt/wt) (AE2 emulsion alkyd resin sample) with the following preparation menu (Table 2).

Table 2. Samples of alkyd emulsion resin.

Ingredients	Weight (g)	
	Alkyd emulsion resin (AE1)	Alkyd emulsion resin (AE2)
Alkyd resin (A0)	37.00	37.00
SLS anionic surfactant	4.33	-
LAS anionic surfactant	-	4.33
NP9 nonionic surfactant	8.67	8.67
Water	50.00	50.00

Water-based alkyd emulsion paints:

Water-based alkyd emulsion paints are presented in Table 3.

Table 3. Water-based alkyd emulsion paints.

Ingredients	White alkyd emulsion paint weight (g)
Water	32.56
CFC-690H dispersing additive	0.72
Tiona 595 titanium dioxide powder	45.66
CaCO ₃ filler	13.50
Talc powder	7.02
BYK024 deformer	0.54
Total of base part	100
Alkyd emulsion resin (AE1)	68.87
Base part	26.67
Soligen 144 Aqua drying agent	1.85
BYK024 deformer	0.75
RM825 thickener	0.75
Water	1.11
Total	100

The composition of water-based alkyd emulsion paint includes alkyd emulsion resins, emulsifier systems, aqueous solvents, pigments, fillers, additives, and drying agents. These ingredients were crushed in a ball machine for 4 h to reach the fineness required for the paint.

2.3. Analytical devices and methods for the analysis

The acid index of the resin is determined according to ASTM D1639-90. The solid content of the resin is measured according to ASTM D2369-98. The molecular mass of the resin is determined using GPC gel chromatography (Cirrus GPC version 3.0). The physicochemical properties of the resin and the mechanical properties of polymer films are assessed according to ASTM and TCVN standards. Functional groups in alkyd resins are identified using FTIR infrared spectroscopy. The stability of the emulsion system is evaluated according to the warm temperature storage method and room temperature according to the JIS K 5400 standard. Particle size and particle size distribution in suspensions are measured by dynamic light scattering (DLS). The anti-corrosion properties of paint films are evaluated electrochemically according to ASTM G5-94, G102-189, and G106-89 (Biologic model VMP3B-5).

3. Results and discussion

3.1. Synthesis of alkyd soybean oil resin

Determination of the acid index of soybean oil alkyd resins:

Alkyd resins are synthesised with a content of 50 wt% soybean oil (sample A0). The results of determining the acid index (AI) of the alkyd resin are presented in Fig. 2.

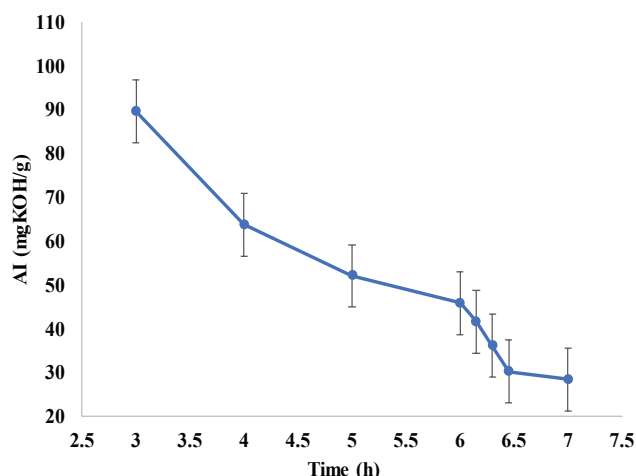


Fig 2. Acid index of soybean oil alkyd resin (sample A0).

From Fig. 2, the acid index rapidly decreased during the reaction phase from 89.6 to 52.1 mg KOH/g after 2 h. After 7 h of reaction, the acid index dropped from 45.9 to 28.46 mg KOH/g. The acid index is the number of mg of KOH used to neutralise all the acid in the sample. A low acid index indicates a low amount of residual acid in the resin, showing that the alkyd resin reaction has occurred with high efficiency and all the acid has reacted. The alkyd resin reaction follows the mechanism of polymerisation, where functional groups react with each other, and the reaction occurs over a long period [1-4].

The results of FTIR analysis of soybean oil-modified alkyd resins (A0 sample) are presented in Fig. 3.

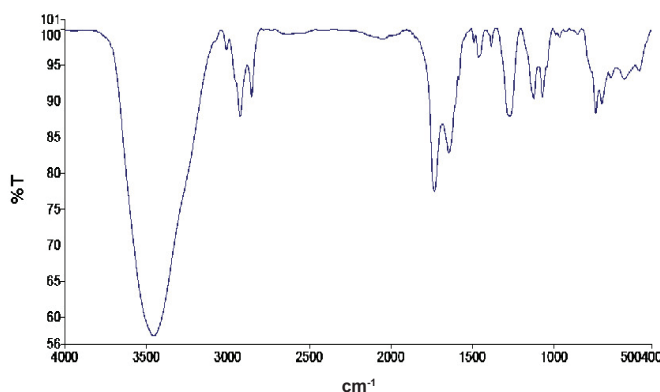


Fig 3. Fourier transform infrared spectrum of soybean oil alkyd resin (sample A0).

The results of infrared analysis from Fig. 3 show that alkyd resins have characteristic peaks. The strong absorption valence oscillation peak at 3455.36 cm^{-1} is characteristic of the free hydroxyl group (-OH group) in the glycerol molecule of unsaturated fatty acids in soybean oil. The absorption peak at 3010.45 cm^{-1} is characteristic of the double bond of the -C=C- group without conjugation of linoleic acid in soybean oil. The oscillation peak at 1732.85 cm^{-1} with strong absorption intensity is characteristic of the C=O group. The oscillation peak at 1122.61 cm^{-1} is characteristic of the -CO group attached to the phenyl radical. The oscillation peak at 1582.56 cm^{-1} is characteristic of the -C=C- group in the aromatic ring of phthalic anhydride. The absorption peak at 1270 cm^{-1} characterises the ester group (-COO-).

Thus, from FTIR spectroscopy, it is shown that the soybean oil alkyd resin (A0 sample) has fully demonstrated characteristic functional groups.

The physical chemistry properties of synthetic soybean oil alkyd resins are presented in Table 4.

Table 4. Properties of soybean oil alkyd resin.

Specifications	Alkyd resin (sample A0)
Solid content (%)	98.2
Viscosity (25°C, seconds)	83
Acid index (mgKOH/g)	28.46
Molecular mass (g/mol)	M_n 1.9144e ³
	M_w 6.2033e ³
Polydispersity (PDI= M_w/M_n)	PDI=3.24

The results from Table 4 showed that the soybean oil alkyd resin (A0 sample) had a low acid index (28.46 mgKOH/g), showing a high efficiency of the reaction and that the reaction occurred completely. If the acid index of alkyd resins is less than 30, then they can be applied to make paint [1, 5, 10]. Alkyd resin A0 has an average molecular mass of $M_n=1.9144.e^3$ g/mol, a molecular weight of $M_w=6.2033.e^3$ g/mol, and a polydispersion index of (PDI= M_w/M_n)=3.24. According to another study [10], the polydispersion of alkyd resins appropriate for making paint is under 8. The polydispersion index indicates the uniform molecular mass level of polymers. Soybean oil alkyd resin A0 is suitable for paint application [2, 5, 7, 9, 10].

3.2. Properties of alkyd emulsion resins

Effect of surfactant content on the stability of alkyd emulsion resins:

The effect of surfactant content on the properties of the emulsion system is presented in Table 5.

Table 5. Effect of surfactant content on the stability of alkyd emulsion resins.

Sample	Surfactant content, wt%	Parameter		
		Solid content, wt%	Pre-storage	Post-storage
S1	10	53.1	pH=3	pH=2 The emulsion system is unstable. The system is separated into resin and water after 1 day
S2	11	51.7	pH=4	pH=3 The emulsion system is unstable. The system is separated into 2 layers: water and emulsion, homogeneous when stirred. The system is separated into resin and water after 1 week
S3	12	52.8	pH=4	pH=4 The emulsion system is unstable. The system is separated into 2 layers: water and emulsion, homogeneous when stirred. The system is separated into resin and water after 2 weeks
S4	13	52.6	pH=4	pH=4 The emulsion system remained stable after 3 weeks
S5	14	49.7	pH=4	The emulsion system is unstable. The system is separated into 2 layers: water and emulsion, homogeneous when stirred. The system is separated into resin and water after 3 weeks
S6	15	48.3	pH=4	pH=4 The emulsion system is unstable. The system is separated into 2 layers: water and emulsion, homogeneous when stirred. The system is separated into resin and water after 2 weeks
S7	16	-	pH=5	pH=3 The emulsion system is unstable. The system is separated into resin and water after 3 days

The results from Table 5 show that all samples were capable of producing alkyd emulsion resins. When stirring ceased, the samples were foamy, the mixture was milky white, and the emulsion alkyd resin could form films. Upon starting storage at room temperature, the samples began to show instability; the system separated the resin from the mixture. Samples S1, S2, and S3 had an unstable emulsion system, with resin separation appearing after 1 day to 2 weeks. Samples S5, S6, and S7 had a storage time of more than 2 weeks but still showed instability. Sample S4 had a storage time of about 3 weeks, and the system remained stable with no separation phenomenon observed.

Therefore, the S4 alkyd emulsion resin sample demonstrated the best stability with a suitable surfactant content of 13 wt%.

The stability of the emulsion alkyd resin based on emulsifiers:

The stability of emulsion alkyd resins based on emulsifiers is presented in Table 6.

Table 6. The stability of alkyd emulsion resins.

Specifications	Alkyd emulsion resin AE1	Alkyd emulsion resin AE2
Solid content	52.6 wt %	48.35 wt%
Stage	The mixture is milky white	The mixture is milky white
Pre-store at room temperature	pH=4	pH=4
After storage at room temperature in 3 weeks	pH=2 After 1 week, the mixture is homogeneous when stirring. After 3 weeks, the mixture is homogeneous when stirring.	pH=3 The mixture is separated into 2 layers of water and emulsion after 1 week. The mixture is homogeneous when stirring. After 2 weeks, the mixture begins to separate the resin and water, settling the resin. After 3 weeks, the mixture completely separates alkyd resin and water.

The results from Table 6 showed that the AE2 alkyd emulsion resin (mixture of LAS and NP9 surfactants) began to show the phenomenon of separation after 2 weeks. After 3 weeks of storage at room temperature, the AE1 sample (mixture of SLS and NP9 surfactants) remained homogeneous and stable in the emulsion system.

Among the anionic surfactants, LAS and SLS, SLS emulsifiers naturally have Na⁺ ions that are well soluble in water, making them more stable with water than LAS. Using SLS, the stability of the emulsion is improved. The separation time of the mixture when using LAS is faster and less stable than when using the SLS emulsifier. Thus, the AE1 sample has better stability than the AE2 sample with the nonionic emulsifier NP9 and anionic SLS.

Stability of emulsion alkyd resin AE1 (SLS and NP9 surfactants mixture):

The stability of the emulsion alkyd resin was investigated by a storage method at room temperature (28±2°C), cold temperature (-5±2°C), and warm temperature (60±2°C). The stability of the alkyd emulsion resin AE1 is presented in Table 7 and Fig. 4.

Table 7. Stability of alkyd emulsion resin AE1.

Specifications	Alkyd emulsion resin AE1
State of the resin mixture	The mixture is liquid, milky white, stable, and homogeneous
Store at room temperature (28±2°C) for 21 days	The mixture is homogeneous and stable
Store at warm temperature (60±2°C) for 2 days	The mixture is homogeneous and stable
Cold storage at cold temperature (-5±2°C) for 01 day	The mixture is separated into 2 phases resin and water
Solid content (wt%)	52.8
Pre-storage pH	4
Post-storage pH	4
Ability to make coating films	Resin has ability to form coating films
Coating films	Coating films are thin, glossy, and smooth
Dried time (hard dried time)	48 h
Particle size at room temperature for 21 days and polydispersion (PI)	3601.1 nm and 0.834
Particle size stored at warm temperatures for 2 days and polydispersion (PI)	2771.4 nm and 0.926
Viscosity (25°C, seconds)	108

The results from Table 7 and Fig. 4 show the stability of alkyd emulsion resin AE1. After storing at room temperature for 21 days, the measured particle size was 3601.1 nm and the polydispersity index (PI) was 0.834 (Fig. 4A). Although the particle size of AE1 resin is quite large, the low dispersion indicates it is still stable [8]. After storage at warm temperatures, the AE1 resin size decreased to 2771.4 nm, and the dispersion increased to 0.926 (Fig. 4B). The mixture separated into two phases, resin and water, after storage at cold temperatures.

The decrease in particle size and increase in dispersion after warm storage can be explained as follows: micelles exist in the alkyd emulsion resin, meaning that alkyd resins are surrounded by two layers of surfactant. During the warm storage test, prolonged elevated temperatures cause the emulsifier to decompose from the surface and dissolve into the dispersed phase, thereby losing the emulsification effect. Consequently, the micelle size decreases when moisture is stored.

Alkyd emulsion resin AE1 remains in a liquid resin state and is suitable for application as water-based alkyd paint.

3.3. The physical chemistry properties and mechanical properties of water-based alkyd emulsion paint

The physicochemical properties and mechanical properties of water-based alkyd emulsion paint are presented in Table 8.

Table 8. Specifications of white alkyd emulsion paint.

Specifications	Standards	White alkyd emulsion paint (AEP)	Alkyd paint according to TCVN 5730-1993 standard
Fineness (μm)	TCVN 2091:2015	20	<23
Viscosity (s)	TCVN 2092:2013	130	>60
Coverage (g/m ²)	TCVN 2095:1993	56.9	<85
Adhesion	TCVN 2097:2015	1	<2
Flexibility (mm)	TCVN 2099:2013	1	<1
Pendulum hardness	TCVN 2098:2007	0.09	>0.15
Dried time (h)	TCVN 2096-3:2015	3.3	<22
Optical gloss (60°)	TCVN 2101:2016	48.1	>75
Impact strength (kg.cm)	TCVN 2100-2:2013	55	>45
Non-volatile content (%)	TCVN 10519:2014	65.5	>50

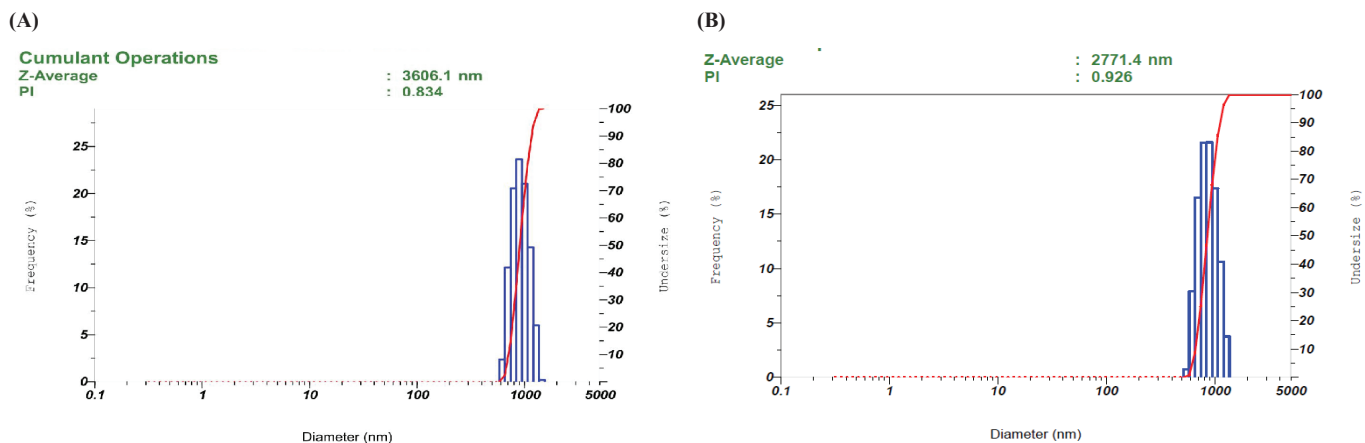


Fig 4. Particle size of emulsion alkyd resin AE1 with room temperature storage (A) and warm storage (B).

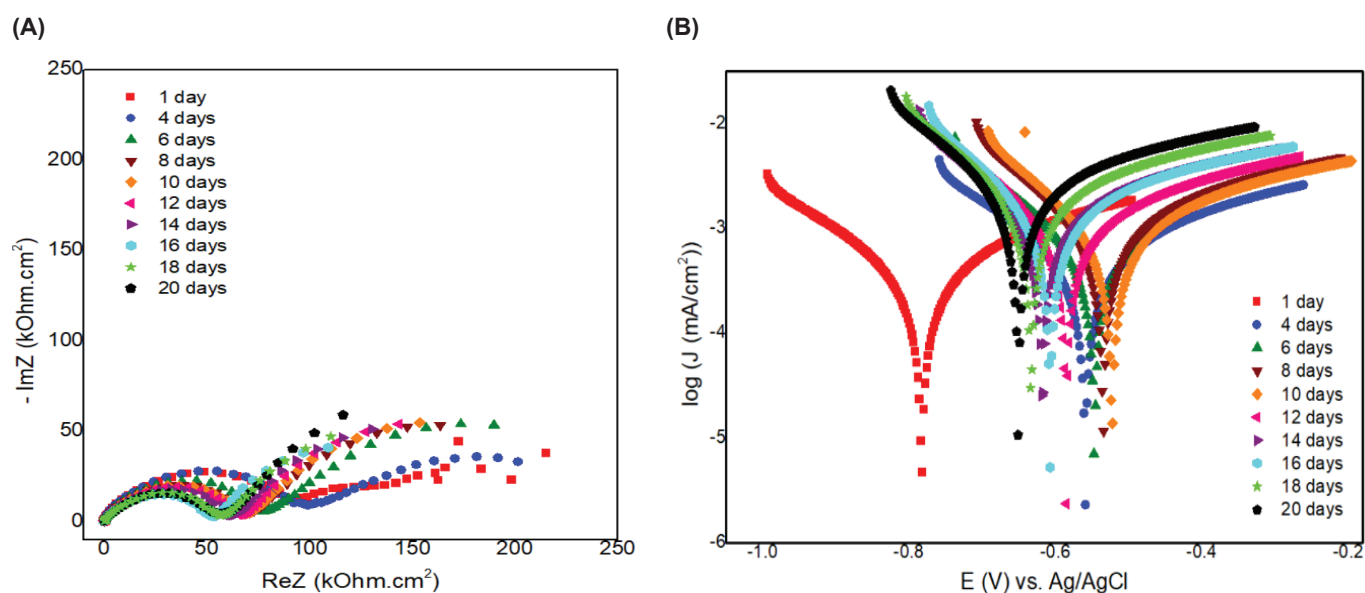


Fig. 5. Nyquist resistance diagram (A) and corrosion current density (B) of white emulsion alkyd paint sample.

The results from Table 6 show that AEP emulsion alkyd paint meets most of the characteristics of the paint in terms of fineness, coverage, viscosity, adhesion, flexibility, and impact strength according to TCVN 5730-1993 for alkyd paints. However, AEP alkyd emulsion paints with a water solvent will reduce the gloss and hardness of the paint film compared to solvent-based alkyd paints. However, using water as a solvent will significantly reduce the content of toxic volatiles.

3.4. Anti-corrosion properties of alkyd emulsion paints

The coating sample on steel was tested for electrochemical corrosion over a period of 28 days in a 3.5% NaCl solution. The results of the determination of Nyquist resistance and corrosion current density of the white emulsion alkyd paint sample are shown in Figs. 5 A and B, respectively.

From Fig. 5A, the Nyquist resistance spectrum of the AEP paint sample appears as a semicircle over time immersion in a 3.5% NaCl solution, which is characteristic of electrolyte immersion and steel corrosion. The coating corrodes rapidly over time. The polarization measurement from Fig. 5B also shows that the coating corrodes rapidly over time. This result is consistent with the Nyquist schema result in Fig. 5A.

AEP coating has good mechanical and physical properties. However, the corrosion resistance of the coating is still limited. Using aqueous solvents is an advantage of the paint, reducing the adverse environmental impact of

paints using organic solvents. However, alkyd emulsion paints need enhanced corrosion inhibitors and additives to increase shielding ability and improve the corrosion resistance of the paint film.

4. Conclusions

Alkyd resin was modified from soybean oil with a content of 50 wt%. The alkyd resin emulsion is stabilised with a mixture of SLS and NP9 with a suitable ratio of SLS:NP9 as 1:2 (wt/wt). The content of the emulsifier is 13 wt%. The ratio of alkyd resin to water is 50:50 (wt/wt), suitable for the alkyd resin emulsion process. Emulsion alkyd paint film has good mechanical properties that meet the standards for paint. However, the corrosion resistance of the paint film is limited and needs to be improved. The use of water solvents, gradually replacing organic solvents, holds great promise for environmentally friendly applications.

CRediT author statement

Le Huy Cuong Huynh: Methodology, Formal analysis, Original draft preparation, Visualisation; Khanh Xuan Phan: Conceptualisation, Data curation, Investigation; Thanh An Truong, Hung Thuy Nguyen: Investigation, Visualisation, Formal analysis.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] I.H. Ifijen, H.D. Odi, M. Maliki, et al. (2020), "Correlative studies on the properties of rubber seed and soybean oil based-alkyd resins and their blends", *Journal of Coatings Technology Research*, **18**(5), pp.459-467, DOI: 10.1007/s11998-020-00416-2.
- [2] N.B. Samarth, P.A. Mahanwar, et al. (2015), "Modified vegetable oil-based additives as a future polymeric material-review", *Open Journal of Organic Polymer Materials*, **5**, pp.1-22, DOI: 10.4236/ojopm.2015.51001.
- [3] G. Lligadas, J.C. Ronda, M. Galia, et al. (2013), "Renewable polymeric materials from vegetable oils: A perspective", *Materials Today*, **16**, pp.337-343, DOI: 10.1016/j.mattod.2013.08.016.
- [4] A. Hofland (2012), "Alkyd resins: From down and out to alive and kicking", *Progress in Organic Coatings*, **73**(4), pp.274-282, DOI: 10.1016/j.porgcoat.2011.01.014.
- [5] M. Islam, M.D.H. Beg, S.S. Jamari, et al. (2014), "Development of vegetable-oil-based polymers", *Journal of Applied Polymer Science*, **131**(18), pp.1-18, DOI: 10.1002/app.40787.
- [6] J. Ho, B. Mudraboyina, C.S. Elder, et al. (2018), "Water-borne coatings that share the mechanism of action of oil-based coatings", *Green Chemistry*, **20**, pp.1899-1905, DOI: 10.1039/C8GC00130H.
- [7] D. Stoye, W. Freitag (1998), "Paints, coatings and solvents", *Wiley*, pp.45-46, DOI: 10.1002/9783527611867.ch2.
- [8] P. Deligny, N. Tuck, P.K.T. Oldring, et al. (2000), "Resins for surface coatings, Alkyds & polyesters", *John Wiley and Sons*, **II**, pp.31-100.
- [9] A. Yousefi, M. Pishvaei, A.A. Yousefi, et al. (2011), "Preparation of water-based alkyd/acrylic hybrid resins", *Progress in Color, Colourants and Coatings*, **4**, pp.15-25, DOI: 10.30509/pccc.2011.75778.
- [10] J. Dziczkowski, M.D. Soucek (2012), "Factors influencing the stability and film properties of acrylic/alkyd water-reducible hybrid systems using a response surface technique", *Progress In Organic Coatings*, **73**, pp.330-343, DOI: 10.1016/j.porgcoat.2011.03.005.
- [11] L. Liang, C. Liu, X. Xiao, et al. (2014), "Optimised synthesis and properties of surfactant-free water-reducible acrylate-alkyd resin emulsion", *Progress in Organic Coatings*, **77**(11), pp.1715-1723, DOI: 10.1016/J.PORGCOAT.2014.05.015.
- [12] E.E. Mohamed, E.M.A. Rehim, R.E. Ashery, et al. (2017), "Synthesis and characterisation of alkyd resin based on soya bean oil and glycerin using zirconium octoate as catalyst", *Int. J. Chem. Technol.*, **2**(1), pp.34-43, DOI: 10.32571/IJCT.347670.
- [13] M. Martinez, E. Gámez, N. Bellotti, et al. (2021), "Alkyd-based water-reducible anticorrosive paints and their antifungal potential", *Progress in Organic Coatings*, **152**, DOI: 10.1016/j.porgcoat.2020.106069.
- [14] Y. Xiaoxing, W. Zhao, X. Qian, et al. (2021), "Effect of water-based emulsion core microcapsules on ageing resistance and self-repairing properties of water-based coatings on linden", *Applied Sciences*, **11**, DOI: 10.3390/app11104662.
- [15] I. Ifijen, M. Maliki, I.J. Odiachi, et al. (2022), "Review on solvents based alkyd resins and water-borne alkyd resins: Impacts of modification on their coating properties", *Chemistry Africa*, **5**, pp.211-225, DOI: 10.1007/s42250-022-00318-3.
- [16] M.D. Soucek, R.R. Salata (2014), *Alkyd Resin Synthesis*, Springer, pp.1-6.
- [17] M. Bora, R. Deka, N. Ahmed, et al. (2014), "Karanja (*Millettia pinata* (L.) Panigrahi) seed oil as a renewable raw material for the synthesis of alkyd resin", *Industrial Crops and Products*, **61**, pp.106-114, DOI: 10.1016/j.indcrop.2014.06.048.

Design approaches proposed for bending components made by 3D concrete printers

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Abstract:

The research and application of 3D printing technology in the construction industry have captured the attention of researchers, businesses, and management agencies. This innovative method of creating structures differs significantly from traditional construction methods and has the potential to revolutionise the field. However, it is crucial to ensure that the printed structures are safe when subjected to loads. This requires careful attention to the materials used, the printing process, and the structural design of the building. As a result, considerable research has been essential to develop standards and guidelines for designing, evaluating, and accepting printed concrete components and structures. This study addresses these issues by (1) providing an overview of the application of structural components made by 3D concrete printers; (2) analysing the behaviour of flexural reinforced concrete components; (3) categorising flexural printed components; and (4) proposing approaches to design printed components according to their category. This study concerns the behaviour of flexural components regarding 3D concrete printing technology. The design approaches for these components will be discussed, focusing on solid and hollow sections.

Keywords: bending components, design approaches, flexural behaviour, hollow cross-section, solid cross-section, 3D concrete printing technology.

Classification numbers: 2.1, 2.3

1. Introduction

Concrete printing, also known as additive manufacturing, constructs 3D models layer by layer [1]. This technology, extensively researched, developed, and applied worldwide, is emerging as a transformative trend in the construction industry. It enables the production of building structures or components of various complexities, offering the advantages of reduced costs and accelerated construction times [2-6]. Moreover, automation and software technology have made 3D printing more effective. This technology is also considered environmentally sustainable because it can utilise various materials, including recycled materials and zero-waste additives, in the construction process [7].

This research begins with a 3D CAD model of the object, saved in “.STL” format. Then, Simplify3D software [8] was used to slice layers of the model and

save it as a “.Gcode” file. Finally, 3D concrete printers controlled by Mach3 software [9] to print components. Fig. 1 illustrates this process.

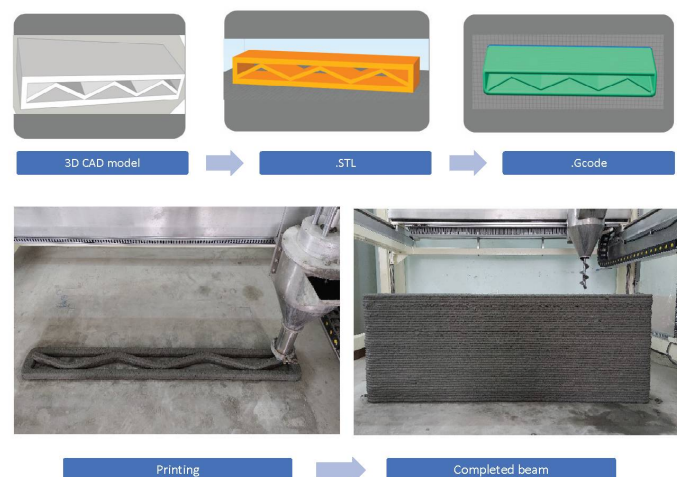


Fig. 1. Steps of the printing process [10].

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(A) Robot system [11].



(B) Gantry system [12].

Fig. 2. Types of concrete printers.

Printing components or entire buildings with 3D printers can be implemented by gantry systems or robot systems, as shown in Fig. 2.

Similarly to traditional constructions, construction works with concrete printers can be done by printing whole buildings or assembling components. An entire project would require a large printer to print everything as per the designed model. However, outdoor conditions pose challenges in controlling the properties of the concrete, transporting and installing printers according to the project's location and dimensions, and other issues [13, 14]. It is worth mentioning that on-site printing of structures provides flexibility in terms of design for projects and leverages the remarkable benefits of 3D concrete printing technology from design to construction and labour [15]. In developed countries, printing the whole building is often preferred over assembling methods [16-18].

Dividing large projects into smaller components for printing is a promising solution for overcoming the limitations of large-sized printers. However, some potential issues arise when using this method as opposed to on-site printing:

- Determining the location of the project's subdivisions.
- Design and construction details of joints.

Nonetheless, printing components and assembling them, known as the inside printing method, also has some significant advantages:

- Printer size does not depend on project size.
- Laboratory construction conditions help better control the properties of the printed concrete.
- Databases of printed concrete component types can be created for projects (sections, joints, working, etc.)

To understand the overall workings of a project, it is crucial to start by examining its parts. In traditional construction projects, the classification of structural systems and the study of their components have been the basis for standards for centuries. The last decade has seen significant research into the performance of components and projects for concrete printing, making it an excellent starting point for understanding their workings.

Numerous projects worldwide have been successfully printed and assembled using 3D technology [19, 20]. One of the most notable examples is the world's tallest tower in the Swiss village of Mulegns, set to be built in spring 2024. The 5-storey tower is primarily constructed of printed white concrete columns, with a total of 124 3D-printed components and 4,000 concrete layers as presented in Fig. 3. This innovative construction approach demonstrates the vast potential of 3D printing technology in building design and construction.

The level of research and application of components for printed concrete projects is necessary but quite complicated due to the diversity of cross-section types and component shapes. To suit the research conditions at Hai Phong University, the research team chose to



(A) Printing components.



(B) Transportation and erection.

Fig. 3. The world's tallest tower in Switzerland [19].

study the bending behaviour of printed concrete beam components. This is also the most essential component in the load-bearing structural system in a building, and the bending problem is considered a fundamental issue in general construction design.

Researching and applying components for printed concrete projects can be complex due to the diversity of cross-section types and component shapes. Based on the research conditions, this study focused on the flexural behaviour of printed concrete components. This is the most fundamental component in a building's load-bearing structural system, and the bending problem is a primary issue in general construction design. By studying this fundamental problem, the results provide valuable insights into the behaviour of printed concrete components in construction projects.

2. Behaviours of flexural reinforced concrete components in general

A flexural member is a structural component subjected to lateral loads, resulting in perpendicular loads to its axis. This causes the internal force components of bending moment and shear force. Consequently, the structure operates under the impact of moment and shear force. Based on the internal force components, the working states can be categorised as follows:

- Pure bending: Internal force components include only one moment.
- Flat bending: Internal force components include both moment and shear force.

In reality, the components in a project typically function in a flat-bending state. Depending on the load application diagram, the pure bending state only appears

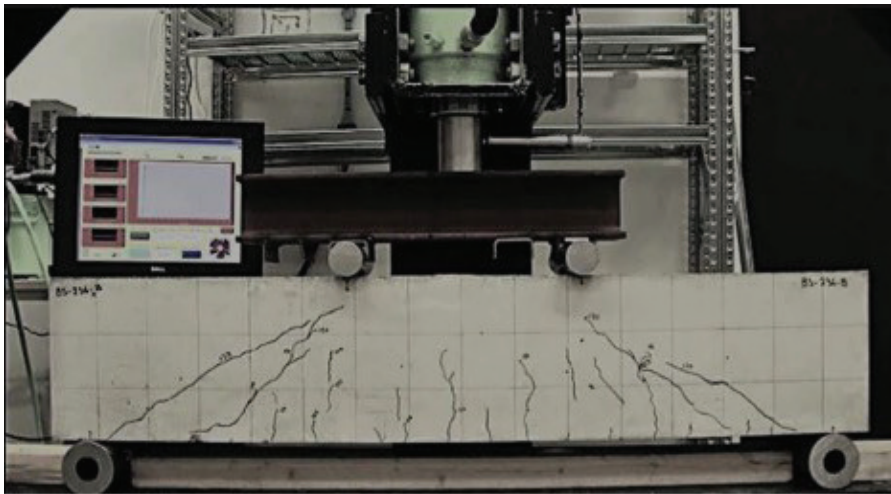


Fig. 4. Typical cracks in a bending test [21].

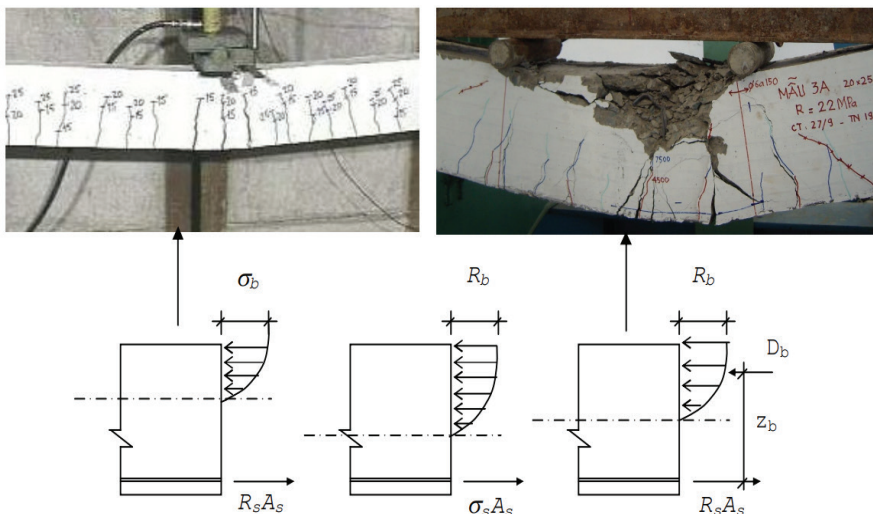


Fig. 5. Development of stress on the perpendicular section.

in specific parts of the structure, resulting in an internal force component of zero shear force. Therefore, studying the behaviour of a bending member's strength involves analysing the simultaneous bending and shearing behaviour of the sections along the axis of the member. To better understand the bending member's behaviour, a simple beam subjected to gradually increasing loads can be tested. When the load is small, the beam is intact with no cracks. As the load increases, cracks appear perpendicular to the beam axis in areas with a significant moment and inclined cracks in the area near the support where there is a sizeable shear force, as shown in Fig. 4. In the final stage, the beam can be damaged in the

section with perpendicular cracks or the section with inclined cracks, depending on the reinforcement content in the beam.

Calculating beams based on strength is crucial to ensure they are not damaged on perpendicular or inclined sections. Strength calculations for perpendicular sections help prevent damage from bending forces, while strength calculations for inclined sections help avoid damage from shear forces. By considering both types of calculations, engineers can ensure that beams are designed to withstand all kinds of forces they may encounter in their intended use. The behaviour of bending members on perpendicular sections is considered under stress-strain development. On a perpendicular section, we can see that the tension cross-sectional area will gradually grow as the load increases, while the compression cross-sectional area will gradually narrow until the cross-section is considered destructive, as presented in Fig. 5.

In beam sections with large shear force, the normal stress due to moment and the tangential stress due to shear force may cause cracks to appear at an angle α to the beam axis, as shown in Fig. 6. Longitudinal and stirrup reinforcement passing through inclined cracks can resist failure along the inclined section. The failure is due to the moment tending to rotate the two parts of the beam around the compression zone while the shear force tends to pull them apart in a direction perpendicular to the beam axis. Despite this, design standards still separate the stirrups and diagonal reinforcement calculation according to shear force from the strength calculation on inclined sections.

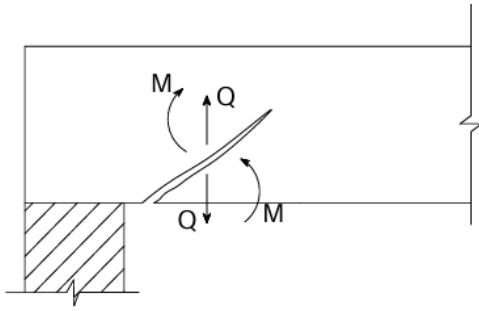


Fig. 6. Failure pattern by shear force.

When considering a bending concrete member as a frame element, moment and shear force control the behaviour of both traditional and printed components. However, the approach to studying printed concrete members will differ from that of conventional reinforced concrete members when examining the behaviour of perpendicular and inclined sections. The degree of difference in performance will depend on the section along the member axis. Therefore, developing new research for design calculations or building upon existing knowledge when working with printed components may be necessary.

3. Flexural components made by 3D concrete printers

Design principles for flexural components made of printed concrete depend on both cross-sectional geometry and reinforced methods. This research will propose a classification based on these principles (Fig. 7).

It is crucial to approach design principles by taking into account the following factors with utmost importance: the bending plane coincides with the printing plane and the bending plane is perpendicular to the printing plane.

It is common for structures with reinforcement, whether prestressed or not, to have an initial structural cross-section similar to traditional structural members with rectangular, I-shaped, or T-shaped shapes. This helps to ensure stability and strength in the overall structure. Design calculation theory used for conventional reinforced concrete bending components can confidently be applied to prestressed reinforcement in printed concrete structures, as the adhesion force is not a significant factor (Figs. 8A, 8B). However, the adhesion factor between the printed and poured concrete must be considered if voids are left when placing the reinforcement. Inheriting the traditional method of

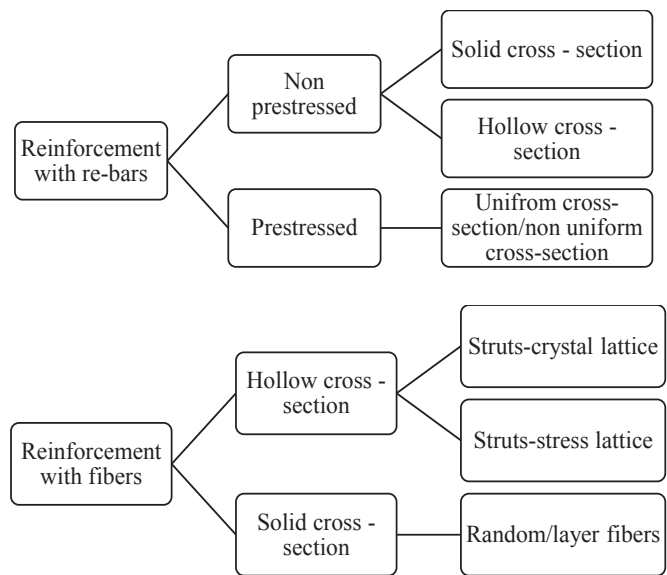
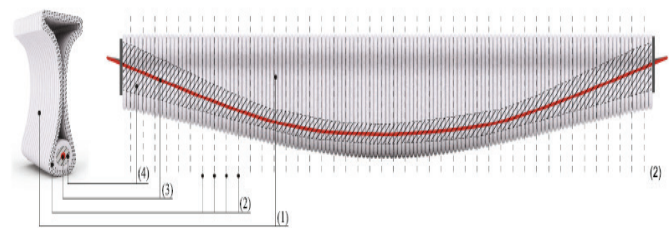
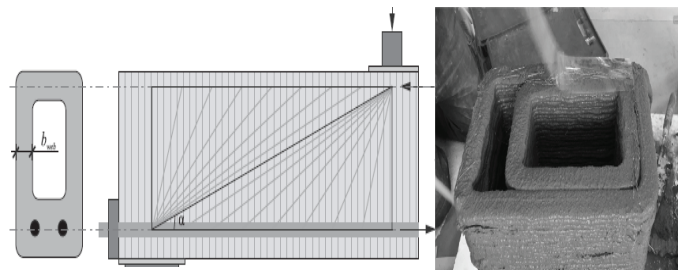


Fig. 7. Proposed categorisation of concrete printed components.



(A) Prestressed with non-uniform cross-section [22].



(B) Prestressed with uniform cross-section [22].



(C) Hollow cross-section [16].

Fig. 8. Printed components with rebar reinforcement.

calculating and designing reinforced concrete structures for printed concrete structures requires the addition of a safety factor or adjusting the section size based on the ratio of the void portion filled with concrete later (Fig. 8C). This approach ensures that the final structure is safe.

Research has shown that both forms of component printing can approach design principles without significantly affecting the results for printed components with reinforcement. This is because the reinforcement is placed in the tensile region of the structure, allowing for more flexibility in the printing process. However, further theoretical and experimental research must provide convincing results and recommendations.

The second method to reinforce materials is to use dispersed fibre. The popular fibres are glass, steel, and polyethylene, thanks to their positive effects on mechanical properties [23-25]. Dispersed fibre reinforcement can be introduced into printed concrete in two forms. The first form involves mixing the dispersed fibres with concrete and randomly dispersing them in the structure with a designed content. The second form strips the fibres into layers according to each layer of printed concrete, as shown in Fig. 9. This second form, as shown in Fig. 10, can be used for solid and hollow cross-sectional structures, but solid cross-sections are preferred. The layer cross-section must be large enough to ensure the fibres spread on the printed layers, usually a few millimetres long and randomly distributed. While the second form has its advantages, the first form is researched and applied more widely due to several outstanding benefits:

- No need to arrange two print heads for the same printer.
- Uniform printed concrete blocks for the entire structure.

- No separation of printed layers because the fibres create separate layers.

In addition to the advantages mentioned earlier, controlling the fibre content and length in dispersed fibre reinforcement is crucial to avoid clogging during printing.

In cases where reinforcement is not arranged in the structure, the printing plane is usually selected to coincide with the bending plane. The reasons are as follows:

- The connection of fibres dispersed in each printed layer will work better in the tensile region.
- The influence of the printed layers' adhesive force on the structure's working results is best limited.

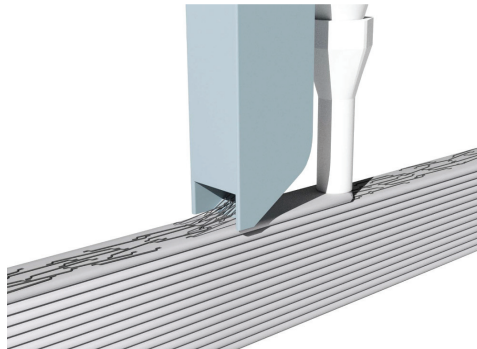


Fig. 9. Printed components reinforced with rebar [22].



(A) Crystal lattice struts [26].



(B) Stress lattice struts [22, 27].

Fig. 10. Hollow cross-section printed components.

When reinforcement is not arranged in a printed concrete structure, the printing plane is often selected to coincide with the bending plane. This is because:

- The connection among fibres dispersed in each printed layer works better in the tensile region.
- The designed model and printing process are more convenient.

4. Proposed design approaches

4.1. Solid cross-sections

For both the case of mixed fibres or fibres spread in layers, the principle of designing flexural members made of printed concrete is proposed according to the following assumptions:

- The material is non-homogeneous and anisotropic.
- Calculation principles designed according to material strength theory.

The non-homogeneous and anisotropic assumption is based on the different strengths of samples when subjected to loads in different directions induced by the layer interface [28-30]. Researchers conducted a series of tests, such as a tensile test, compression test, splitting test, and flexural test [13], which confirmed that 3DPC has apparent anisotropic behaviour compared to ordinary concrete. Its mechanical properties will be affected by the loading direction, printing direction, and printing parameters [31].

Strength conditions:

- Perpendicular section [32]:

$$\sigma_k = \frac{M}{W} \leq [\sigma_k] \quad (1)$$

where σ_k is the maximum tensile stress at the most dangerous section; M is the moment value at the dangerous section; W is the section modulus; $[\sigma_k]$ is the allowable stress corresponding to the failure state of the

component determined from the experiment (sample bending test according to Vietnam Technical Standards (TCVN) 3119:2022 [33]).

- Inclined section [32]:

$$\tau = \frac{|Q_y| \cdot S_x^c}{I_x \cdot b^c} \leq [\tau] \quad (2)$$

where Q_y is the shear force acting on the bar; b^c is the actual width of the surface area at the point calculated by the tangential stress distribution; S_x^c is the static moment of the section limited by the actual width; I_x is the moment of inertia of the section; $[\tau]$ is the allowable shear stress.

Deformation condition:

$$f \leq [f] \quad (3)$$

where f is the member's deflection due to the load, calculated depending on the member's working diagram using methods in material strength, structural mechanics, or structural analysis applications such as SAP or ETABS; $[f]$ is the allowable deflection of bending members according to (TCVN) 5575:2012 [34] or other national codes.

4.2. Hollow cross-sections

Bending components with hollow cross-sections are becoming a popular design trend in concrete printing (Fig. 11). Digital manufacturing technology makes it technically and economically feasible to create structurally optimised components designed according to the principle of "shape contributed with stress contribution", also known as stress-based design [35]. This approach allows for the creation of structures that save materials, reduce the weight of components, and ultimately lower the weight of the entire building. Designing components with customised shapes, such as bending structures with hollow cross-sections, can be complex, and specific but complex-shaped structures can suffer from severe stress and strain localisation problems beyond the scope of current structural design

standards [36]. While many components and structures have been printed, the commercial application of 3D printing in construction has been slow due to its insufficient structural safety [37]. Moreover, modelling such components requires technical and analytical skills from the designer and software supporting structural analysis. However, with increasing computing power, there has been a significant increase in the number of numerical models available to predict and improve the structural performance of concrete structures. Despite the challenges involved, this has made it easier to calculate and design components with complex shapes.

Depending on the shape, printers can make two hollow cross-section components: crystal lattice and stress lattice, according to the author's suggestion. Within the scope of the study, the crystal lattice is understood to have representative units that are repeated according to rules. In studying printed concrete structures, a crystal lattice is a repeating pattern of representative units that follow specific rules. These units can come in various shapes and sizes, such as cubes, stars, hexagons, diamonds, etc. By combining these shapes, designers can create specific

lattice structures for their systems. The size of the lattice unit is determined by adjusting the thickness and length of the components and connecting nodes. Higher repetition density leads to smaller and more complex crystal lattices. While larger cells can be easily printed, the geometrical uniformity across any component cross-section will be lower.

To design crystal lattice structures, it is essential to follow a preliminary approach proposed through the process presented in Fig. 12.

Analysing the numerical model of the behaviour of printed concrete components is an important and complex step (Steps 3 and 4). The design principle for perpendicular or inclined sections for traditional solid or hollow members will be challenging because the material distribution on perpendicular or inclined sections varies with location. The design of crystalline printed concrete components will depend on stress analysis of each cross-section at each different material distribution area to check the conditions in Step 5. Equations (1), (2), and (3) give the conditions of strength and deformation. The

difference is that these checking conditions apply to each crystal lattice member. By following this approach, you can design crystal lattice structures that are visually appealing and structurally sound.

A component printed in the form of a stress lattice is a structure printed according to the tensile and compressive stress fields distributed in the structure when subjected to load. The lattices forming the components are printed according to the stress isostatic

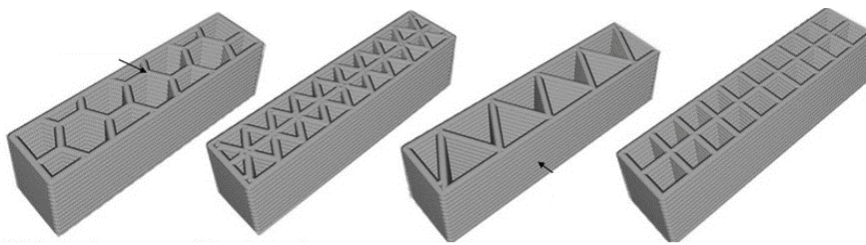


Fig. 11. Bending components with crystal lattice struts [38].

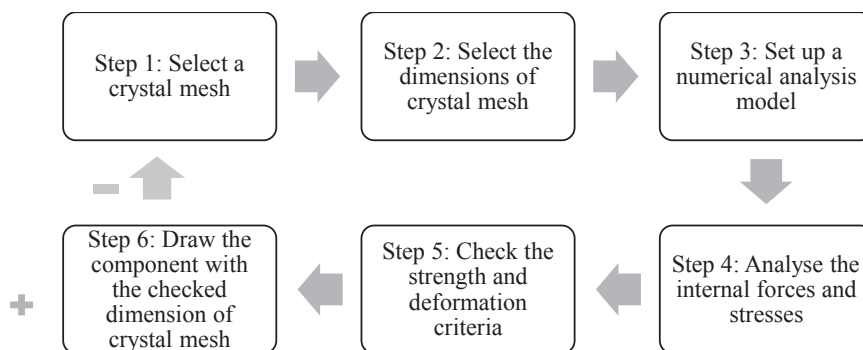


Fig. 12. Design process proposed for crystal lattice structures.

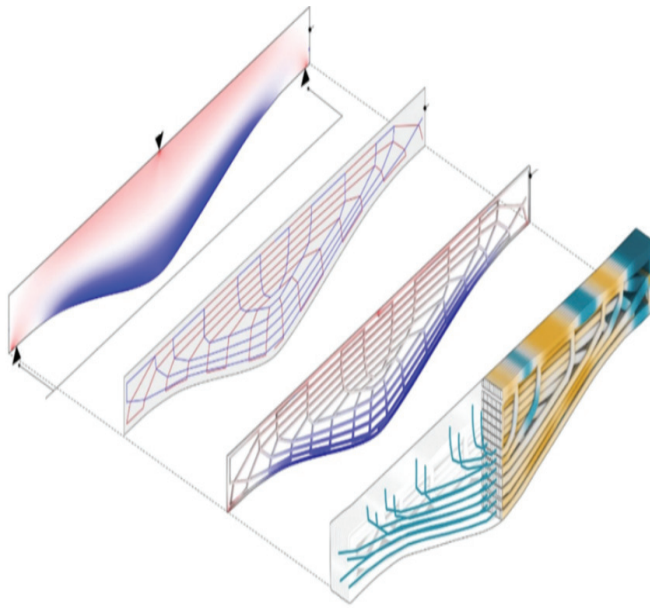
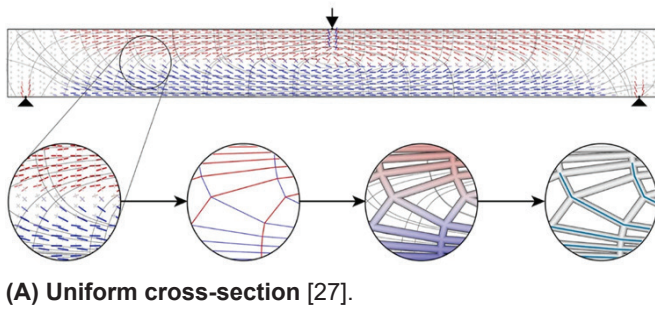


Fig. 13. Stress distribution inside the bending components.

curves, which are the orthogonal pairs of curves representing the trajectory of internal forces in each load impact diagram. These curves indicate the directions in space where the stress is purely axial, and sections oriented along the principal stress direction are not subjected to any shear or bending stresses. This printing method is used to reduce the weight of structural elements and increase structural efficiency. Fig. 13 illustrates the number of flexural members printed according to the stress lattices.

The analysis of structural behaviour and the process of designing and manufacturing stress lattice components is a highly elaborate and complex task that requires advanced techniques. This process is more intricate

than the problem of designing crystal components, as it involves analysing the distribution of tensile and compressive stresses in the structure when subjected to force. The design and manufacturing process of stress lattice components involves printing lattice lines according to the stress isostatic curves, which requires high precision and expertise. This is necessary to ensure the resulting structure is structurally sound and can withstand potential stress. To design stress lattice structures, a preliminary approach can be proposed in the following process, as shown in Fig. 14.

For such structures, numerical models will be analysed two times. The first time, the distribution of stress of the behaviour of printed concrete components with solid cross-sections will be found (Step 2). Then, the filament details of the longitudinal section will be designed using experience. A numerical model of stress lattice components will be carried out (Step 3) to get the results of stress and internal forces (Step 4). Equations (1), (2), and (3) also give the conditions of strength and deformation. The difference is that these checking conditions apply to each stress lattice filament.

In addition to the previously mentioned approaches to designing flexural printed concrete members, the study utilises an experimental design approach known as “Design by testing” [40, 41]. This approach is becoming increasingly popular for printed concrete structures as design calculation theory is still forming. The author has conducted experimental investigations, as shown in Fig. 15, on the flexural behaviours with triangle lattice structures [10, 26].

The failure pattern and cracked surfaces of all printed triangle lattice components indicate that the adhesion force between the printed layers ensures the overall working of the structure. The results prove good capacity by considering the flexural strength, bending stiffness, ductility, and compressive strength of concrete reinforced

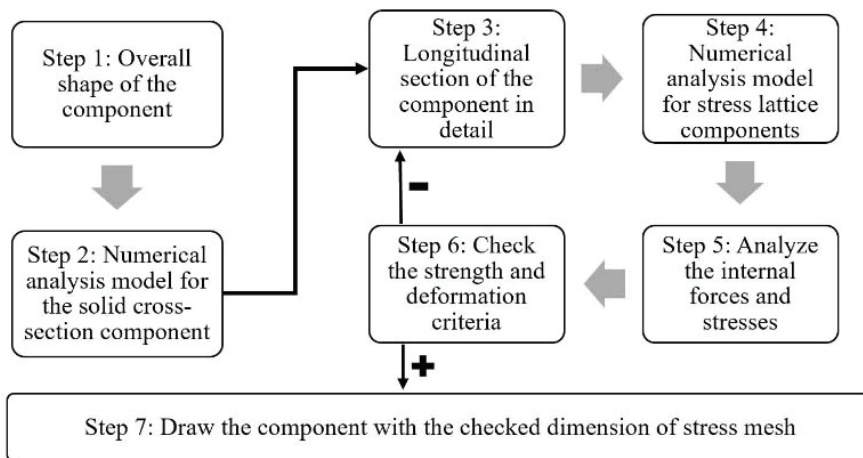


Fig. 14. Design process proposed for stress lattice structures.



Fig. 15. Flexural test of triangle lattice model.

with fibres. By conducting experiments and analysing the resulting data, researchers can gain valuable insights into the behaviour of printed concrete components and use this information to refine and improve the design methods.

5. Conclusions

After analysing the behaviour of bending members in general, as well as printed members, it can be concluded that 3D concrete printing is a promising technology for the production of complex concrete structures. This technology has the potential to reduce the use of formwork, increase the accuracy of concrete placement, and enable the fabrication of structures with intricate geometries. However, further research is needed to fully understand

the behaviour of 3D-printed concrete components under different loading conditions and to optimise the printing process for various applications. Overall, 3D concrete printing has the potential to revolutionise the construction industry and offer new opportunities for architects, engineers, and designers.

Research on the structural behaviour of 3D-printed components is crucial for their successful application in building structures. In particular, the category of bending printed components can be chosen for efficient designing methods. The printing plane is often selected to coincide with the bending plane to take advantage of the connection among fibres dispersed in each printed layer, the convenient printing process, and the designed model. When designing the solid cross-section of a bending component, material strength theory can be used based on

homogeneous and anisotropic assumptions. The design process for struts crystal lattice components can be followed by the approach proposed, which consists of four steps. Similarly, the design process for struts stress lattice components can be followed by the approach proposed, which consists of five steps. In the formation stage, the design approach known as “Design by testing” is popular. This approach involves testing the printed components and adjusting the design based on the results to ensure that the final structure meets the required safety standards. Overall, research on the structural behaviour of 3D-printed components is vital for their successful application in building structures, and careful design and testing are necessary to ensure their safety and efficiency.

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COMPETING INTERESTS

The author declares that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] R. Kawalkar, H.K. Dubey, S.P. Lokhande (2022), "A review for advancements in standardization for additive manufacturing", *Mater. Today Proc.*, **50(5)**, pp.1983-1990, DOI: 10.1016/j.matpr.2021.09.333.
- [2] L. Stinson (2019), "World's largest 3D-printed building completed in Dubai", *Curbed*, <https://archive.curbed.com/2019/12/30/21035765/world-largest-3d-printed-building-dubai-apis-cor>, accessed 10 January 2024.
- [3] L. Alter (2018), "3D printed house displayed at Milan Design Week", *Treehugger*, <https://www.treehugger.com/green-architecture/3d-printed-house-displayed-milan-design-week.html>, accessed 10 January 2024.
- [4] 3dsourced (2024), "ICON & new story 3D printed house in Austin, Texas USA", https://3dsourced.com/guides/3d-printed-house-2/#4_ICON, accessed 25 February 2024.
- [5] S. Saunders (2022), "Robotic 3D printed Yhnova house officially inaugurated, tenants to move in soon", *3DR Holding*, <https://3dprint.com/207936/3d-printed-yhnova-house-done/>, accessed 24 January 2024.
- [6] A. Frearson (2014), "Chinese company 3D prints 10 buildings in a day using construction waste", *Dezeen*, <https://www.dezeen.com/2014/04/24/chinese-company-3d-prints-buildings-construction-waste/>, accessed 24 January 2024.
- [7] J. Nilimaa (2023), "Smart materials and technologies for sustainable concrete construction", *Dev. Built Environ.*, **15**, DOI: 10.1016/j.dibe.2023.100177.
- [8] Simplify3D (2024), "Simplify3D desktop software", <https://www.simplify3d.com/>, accessed 24 January 2024.
- [9] Goodwin University (2024), "Mach3 CNC control software", <https://www.goodwin.edu/glossary/mach-3-cnc-control-software>, accessed 24 January 2024.
- [10] T.L. Pham, D.T. Trinh, T.H.T. Nguyen, et al. (2023), "Study on flexural behaviour of printed concrete wide beams using polypropylene fibres", *Vietnam Journal of Science, Technology and Engineering - MOST*, **65(4)**, pp.48-53, DOI: 10.31276/vjste.65(4).48-53.
- [11] L. Grozdanic (2017), "Giant robots are building a futuristic house in Switzerland", *Inhabitat*, <https://inhabitat.com/giant-robots-and-3d-printers-are-building-a-futuristic-house-in-switzerland/>, accessed 24 January 2024.
- [12] Cobod (2024), "Cobod products", <https://cobod.com/>, accessed 24 January 2024.
- [13] P.S. Ambily, S.K. Kaliyavaradhan, N. Rajendran (2024), "Top challenges to widespread 3D concrete printing (3DCP) adoption - A review", *Eur. J. Environ. Civ. Eng.*, **28(2)**, pp.300-328, DOI: 10.1080/19648189.2023.2213294.
- [14] A.S. Rao, M. Radanovic, Y. Liu, et al. (2022), "Real-time monitoring of construction sites: Sensors, methods, and applications", *Autom. Constr.*, **136**, DOI: 10.1016/j.autcon.2021.104099.
- [15] H. Tu, Z. Wei, A. Bahrami, et al. (2023), "Recent advancements and future trends in 3D concrete printing using waste materials", *Dev. Built Environ.*, **16**, DOI: 10.1016/j.dibe.2023.100187.
- [16] M. Alawneh, M. Matarneh, S. El-Ashri (2018), "The world's first 3D-printed office building in Dubai", *PCI/NBC*, https://www.pci.org/PCI_Docs/Papers/2018/32_Final_Paper.pdf, accessed 24 January 2024.
- [17] Cobod (2022a), "The largest 3D printed building project in the US", <https://cobod.com/the-us-largest-3d-printed-building-project/>, accessed 24 January 2024.
- [18] Cobod (2022b), "The tallest 3D printed building in the world is now in Saudi Arabia", <https://cobod.com/the-worlds-tallest-3d-printed-building-is-now-in-saudia-arabia/>, accessed 24 January 2024.
- [19] Swisstech (2024), "A tower from a 3D printer", <https://www.swiss.tech/news/tower-3d-printer>, accessed 18 March 2024.
- [20] R. Wolfs, D. Bos, T. Salet (2023), "Lessons learned of project Milestone: The first 3D printed concrete house in the Netherlands", *Mater. Today Proc.*, DOI: 10.1016/j.matpr.2023.06.183.
- [21] D.W. Harris (2018), "Steel fibres and concrete flexural strength", *Inforce*, <https://www.inforceglobal.com/steel-fibres-and-concrete-flexural-strength/>, accessed 18 March 2024.

- [22] L. Gebhard, J.M. Falcón, A. Anton, et al. (2021), “Structural behaviour of 3D printed concrete beams with various reinforcement strategies”, *Eng. Struct.*, **240**, DOI: 10.1016/j.engstruct.2021.112380.
- [23] A.A. Jhatial, S. Sohu, N.K. Bhatti, et al. (2018), “Effect of steel fibres on the compressive and flexural strength of concrete”, *Int. J. Adv. Appl. Sci.*, **5(10)**, pp.16-21, DOI: 10.21833/ijaas.2018.10.003.
- [24] D. Sinha, A. Verma (2017), “Investigation on the effect of varying dosages of steel fibre on the strength and workability properties of high strength concrete”, *ICRISET2017. International Conference on Research and Innovations in Science, Engineering and Technology. Selected Papers in Civil Engineering*, pp.352-356, DOI: 10.29007/rjbd.
- [25] I. Hussain, B. Ali, T. Akhtar, et al. (2020), “Comparison of mechanical properties of concrete and design thickness of pavement with different types of fiber-reinforcements (steel, glass, and polypropylene)”, *Case Stud. Constr. Mater.*, **13**, DOI: 10.1016/j.cscm.2020.e00429.
- [26] L.T. Pham, T.D. Trinh, Q.T. Do, et al. (2023), “Flexural behavior of printed concrete wide beams with dispersed fibers reinforced”, *Res. Eng. Struct. Mater.*, **10(2)**, pp.727-742, DOI: 10.17515/resm2023.19me0925rs.
- [27] L. Breseghello, R. Naboni (2022), “Toolpath-based design for 3D concrete printing of carbon-efficient architectural structures”, *Addit. Manuf.*, **56**, DOI: 10.1016/j.addma.2022.102872.
- [28] B. Panda, S.C. Paul, M.J. Tan (2017), “Anisotropic mechanical performance of 3D printed fiber reinforced sustainable construction material”, *Mater. Lett.*, **209**, pp.146-149, DOI: 10.1016/j.matlet.2017.07.123.
- [29] G. Ma, Z. Li, L. Wang, et al. (2019), “Mechanical anisotropy of aligned fiber reinforced composite for extrusion-based 3D printing”, *Constr. Build. Mater.*, **202**, pp.770-783, DOI: 10.1016/j.conbuildmat.2019.01.008.
- [30] T. Ding, J. Xiao, S. Zou, et al. (2020), “Anisotropic behavior in bending of 3D printed concrete reinforced with fibers”, *Compos. Struct.*, **254**, DOI: 10.1016/j.compstruct.2020.112808.
- [31] P. Zarean, P. Malgaroli, P. Zarean, et al. (2023), “Effect of printing parameters on mechanical performance of material-extrusion 3D-printed PEEK specimens at the point-of-care”, *Appl. Sci.*, **13(3)**, DOI: 10.3390/app13031230.
- [32] G. Mendes, B. Lago (2009), *Strength of Materials*, Nova Science Publishers, 272pp.
- [33] Vietnam Ministry of Science and Technology (2022), *TCVN 3119:2022 on Hardened Concrete - Test Method for Flexural Tensile Strength* (in Vietnamese).
- [34] Vietnam Institute for Building Science and Technology (2018), *TCVN 5574:2018 on Design of Concrete and Reinforced Concrete Structure*, Construction Publishing House, 198pp (in Vietnamese).
- [35] J.H. Vargas (2023), *Design for 3D Concrete Printing: Optimisation Through Integrated Workflows*.
- [36] T.Y.P. Yuen, T.H. Wen, C.C. Hung, et al. (2022), “An eigendecomposition-based and mesh-sensitivity reduced constitutive model for nonlinear analysis of concrete structures under non-proportional cyclic loading”, *J. Build. Eng.*, **47**, DOI: 10.1016/j.jobbe.2021.103875.
- [37] K. Liu, K. Takasu, J. Jiang, et al. (2023), “Mechanical properties of 3D printed concrete components: A review”, *Dev. Built Environ.*, **16**, DOI: 10.1016/j.dibe.2023.100292.
- [38] D. Liu, Z. Zhang, X. Zhang, et al. (2023), “3D printing concrete structures: State of the art, challenges, and opportunities”, *Constr. Build. Mater.*, **405**, DOI: 10.1016/j.conbuildmat.2023.133364.
- [39] L. Breseghello, H. Hajikarimian, H.B. Jørgensen, et al. (2023), “3DLightBeam+. Design, simulation, and testing of carbon-efficient reinforced 3D concrete printed beams”, *Eng. Struct.*, **292**, DOI: 10.1016/j.engstruct.2023.116511.
- [40] G. Monti, S. Alessandri, S. Santini (2009), “Design by testing: A procedure for the statistical determination of capacity models”, *Constr. Build. Mater.*, **23(4)**, pp.1487-1494, DOI: 10.1016/j.conbuildmat.2008.07.016.
- [41] G. Sedlacek, C. Müller (2008), “Eurocode - EN 1990 basis of structural design”, *Design*, pp.18-20.

Numerical investigation of the collision of a vortex ring with a wavy sphere

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Abstract:

The numerical investigation of vortex dynamics resulting from the collision of a vortex ring with a wavy sphere of various wavenumbers (n) at a Reynolds number of 1000 was conducted using a developed remeshed vortex particle method. The influence of the wall's wavenumber on vortex dynamic mechanisms, such as the primary vortex ring's deformation, the boundary layer's separation, and the formation, interaction, and reconnection of secondary and tertiary vortices, was analysed. When the primary ring collides with a smooth sphere ($n=0$), secondary and tertiary rings are formed, and their interaction with the primary ring is similar to that observed with a flat plate. In the case of the wavy sphere, the boundary layer separates from the wall's hills more rapidly than from the valleys, leading to the boundary layer rolling up into the secondary vortex in a wavy pattern. The segments of the secondary vortex originating from the hills move around the primary ring, while those from the valleys convect upwards, forming incomplete vortex loops at $n=5$. However, at $n=9$, the reconnection of segments from the valleys results in the formation of a chain of small-scale rings. Furthermore, tertiary vortices are generated from the valleys due to the additional separation of the wall boundary layer induced by the secondary vortex segments in the valleys. These vortices dissipate rapidly at $n=5$, whereas reconnection occurs to form additional vortex loops at $n=9$.

Keywords: fluid dynamics, vortex dynamics, vortex interactions.

Classification numbers: 2.1, 2.3

1. Introduction

Vortex-wall interaction is widely observed in engineering applications as well as natural processes, such as wingtip vortices near the ground in aerospace engineering, the conning tower of submarines in naval engineering, computer chips mounted on electrical circuit boards in the electronics industry, and flow around buildings in civil engineering [1]. A comprehensive understanding of this flow phenomenon is essential for improving the design and control of related devices. Consequently, this has attracted the interest of many numerical and experimental researchers over several decades. The vortex ring has frequently been employed to examine the characteristics of vortex-wall interaction because it has a simple prototype structure and is relatively easy to produce. However, it can cause various flow phenomena when colliding with a wall. When a vortex ring moves towards a wall, it induces a boundary layer on the wall. The wall geometry strongly influences this boundary layer's

distribution, intensity, and separation. These characteristics lead to different phenomena of vortex dynamics (formation, deformation, merging, breakdown, and reconnection) when a distinctive wall geometry is applied.

The wall geometries often employed to investigate vortex flow, vortex instability, and vortex interaction include flat plates [2, 3], V-walls [4], round cylinders [5], and spheres [6, 7]. When a vortex ring collides with a flat wall at a low Reynolds number (Re), the ring dissipates without generating a secondary vortex ring. At a moderate Re and some low angles of incidence of the wall, the collision produces a secondary vortex ring, or even a tertiary one [2]. This secondary ring develops azimuthal instability and may even form hairpin-like small vortices. As the angle of incidence increases beyond a critical value, depending on the Reynolds number, the primary vortex ring generates only the secondary ring, which then evolves into a helical structure [3]. When a vortex ring collides

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with a V-wall, leapfrogging of the secondary and tertiary rings past the primary ring occurs. The upstream separated boundary layers interact with these rings to produce small daisy-chained vortex dipoles [4]. T.H. New, et al. (2017) [5] reported that the behaviour of the collision of a vortex ring on a round cylinder differs from that on a flat plate, in which the disconnection and reconnection of the primary and secondary vortex rings produce small-scale vortex ringlets. These vortex ringlets may even collide to form vortex dipoles further. These vortex ringlets even collide to form vortex dipoles further. V.L Nguyen, et al. (2019) [6] pointed out that when a vortex ring collides coaxially with a sphere, the deformation of the primary ring and its interaction with the secondary and tertiary rings are similar to the flat plate, and the collision is two-dimensional. However, the non-coaxial collision of a vortex ring with a sphere forms a three-dimensional vortex without the formation of a secondary vortex ring. T.H. New, et al. (2024) [7] further explained the recurring formation of the tertiary vortex ring due to the slowdown of the primary vortex ring. This slowdown leads to the entrainment of the tertiary ring into the primary ring and renders it incoherent. The primary ring then continues to induce another tertiary ring. The primary ring then continues to induce another tertiary ring.

Some specific wall geometries have also been explored due to their potential engineering applications and to extend the fundamental knowledge of vortex dynamics. T. Ahmed, et al. (2023) [8] investigated the collision of a vortex ring with a hemispherical cavity and found loop-like instabilities in the secondary vortex ring. Moreover, another ring is formed on the lip/edge of the cavity, and this ring disrupts the separation of the wall-bounded boundary layer, which forms the secondary ring. H. Ren, et al. (2014) [9] employed a large-eddy simulation to study the collision of a vortex ring with a bump at a Reynolds number of 4×10^4 . They discovered that the bump height greatly influences the induced vortex shapes and turbulent flow. They also revealed that wrapping and hairpin vortices are vital in the flow's transition from laminar to turbulent. Wrapping vortices are related to azimuthal instability in the vortex rings, whereas the primary vortex ring causes the hairpin vortex formation. These hairpin vortices impinge on the bump's surface, breaking into smaller ones. H. Ren, et al. (2015) [10] discovered that when a thin or thick vortex ring collides with a flat wall, the large-scale wrapping and hairpin vortices break down into smaller ones. This breakdown obeys the $-5/3$ power law, suggesting a fully developed turbulent flow. Moreover, they also found that thin and thick vortex rings suffer from instabilities with modes of 11 and 16, respectively. M. Cheng, et al. (2014) [11] numerically investigated the impact of a three-dimensional vortex ring on a permeable wall for a range of physical and flow parameters, including wall open-area ratio, structural dimension, wall thickness, and Reynolds

number. They found that these parameters affect flow characteristics upstream and downstream of a permeable wall. Increasing the wall open-area ratio or Reynolds number improves vorticity transport over the porous wall, resulting in a regenerated vortex ring. Conversely, increasing wall thickness hinders vorticity transport and the development of regenerated vortex rings. A. Pirnia, et al. (2017) [12] investigated the vortex dynamics and flow-induced vibrations of a flexible plate arising from a vortex ring passing tangentially over it. When the centreline of the vortex ring is positioned greater than approximately twice the vortex ring radii away from the plate, it can be considered as the far field, and the resulting vibrations are well predicted through the potential flow model. As the offset distance of the vortex ring decreases, the diffusion of induced vorticity on the plate into the flow field significantly alters the fluid dynamics, pressure loading, and resultant plate dynamics, dramatically increasing the strain energy compared to the potential flow model.

Previous research has provided fundamental knowledge on vortex dynamics resulting from the collision of a vortex ring with a solid wall. However, a comprehensive understanding of vortex dynamics has not yet been elucidated, and expanding the scope of knowledge on flow dynamics remains of interest. It is clear that vortex dynamics depend strongly on the geometry of the solid wall, and several solid wall models with potential applications in heat transfer engineering have yet to be examined. In this study, we will focus on clarifying the characteristics of the vortex dynamics caused by the collision of a vortex ring with a wavy sphere of various wavenumbers. To our knowledge, prior researchers have not yet conducted flow dynamics investigations with this type of wall. We will demonstrate that the mechanisms of vortex dynamics, such as vortex formation, interaction, and reconnection, induced by the collision of a vortex ring with a solid wavy sphere, strongly depend on the wall's wavenumber. The remainder of this article is organised as follows: the numerical method and its validation are discussed in Section 2, the simulation results are analysed in Section 3, and the conclusions are presented in Section 4.

2. Numerical method and its validation

The mass and momentum Navier-Stokes equations for the flow of an incompressible viscous fluid around a solid body are written in the velocity-pressure form as:

$$\nabla \cdot \mathbf{u} = 0 \quad (1)$$

$$\frac{\partial \mathbf{u}}{\partial t} + (\mathbf{u} \cdot \nabla) \mathbf{u} = -\frac{1}{\rho} \nabla p + \nu \nabla^2 \mathbf{u} + \mathbf{g} + \lambda (\mathbf{u} - \mathbf{u}_s) \quad (2)$$

where ∇ is the gradient operator; $\frac{\partial \mathbf{u}}{\partial t}$ is the derivative of $\mathbf{u}$ with respect to t , t is the time; $\mathbf{u}$ is flow velocity; p is pressure; ρ is the fluid density; ν is the kinematic viscosity of the fluid;

∇^2 is the Laplace operator; $\mathbf{g}$ is gravitational acceleration; $\mathbf{u}_s$ is solid velocity; and λ is penalisation parameter. The last term in Eq. (2) expresses the appearance of the solid body in fluid flow, where the no-slip condition of flow is imposed on this body surface using the penalisation technique. When taking the curl operation on both sides of Eq. (2), the momentum equation is expressed in the velocity - vorticity form as

$$\frac{\partial \boldsymbol{\omega}}{\partial t} + (\mathbf{u} \cdot \nabla) \boldsymbol{\omega} = (\boldsymbol{\omega} \cdot \nabla) \mathbf{u} + \nu \nabla^2 \boldsymbol{\omega} + \nabla \times [\lambda (\mathbf{u} - \mathbf{u}_s)] \quad (3)$$

where the vorticity is defined as $\boldsymbol{\omega} = \nabla \times \mathbf{u}$. The fluid is discretized into vortex particles p at position $\mathbf{x}_p$ carrying the vorticity field $\boldsymbol{\omega}(\mathbf{x}_p)$ with the flow velocity $\mathbf{u}(\mathbf{x}_p)$. Therefore, the transport momentum equation, Eq. (2), can be rewritten in the Lagrangian reference frame of vortex particles p as

$$\frac{d\mathbf{x}_p}{dt} = \mathbf{u}(\mathbf{x}_p) \quad (4)$$

$$\frac{d\boldsymbol{\omega}(\mathbf{x}_p)}{dt} = [\boldsymbol{\omega}(\mathbf{x}_p) \cdot \nabla] \mathbf{u}(\mathbf{x}_p) + \nu \nabla^2 \boldsymbol{\omega}(\mathbf{x}_p) + \nabla \times [\lambda (\mathbf{u}(\mathbf{x}_p) - \mathbf{u}_s(\mathbf{x}_p))] \quad (5)$$

The vorticity field of the vortex particles is updated on the grid using Eq. (5). These particles move at their velocity $\mathbf{u}(\mathbf{x}_p)$ using Eq. (4). Their velocity on the grid is calculated using the stream function ψ as $\mathbf{u} = \nabla \times \psi$, while ψ is computed using the Poisson equation, $\nabla^2 \psi = -\boldsymbol{\omega}$. The numerical method described above is known as a remeshed-vortex particle method. A further explanation of the current numerical method is found in the previous research by V.L. Nguyen, et al. (2021) [13].

The current numerical method is verified using the benchmark problem of the collision of a vortex ring upon a flat solid wall at the Reynolds number of $Re_r = 1743$, where the ring at the outset is modelled using a Gaussian distribution function as

$$(\omega_r, \omega_\theta, \omega_z) = \left(0, \frac{\Gamma}{\pi \sigma^2} e^{-\frac{r^2(x,y,z)}{\sigma^2}}, 0 \right) \quad (6)$$

where $r^2(x, y, z) = \left(r_0 - \sqrt{(x - x_0)^2 + (y - y_0)^2} \right)^2 + (z - z_0)^2$,

$(x_0, y_0, z_0) = (0, 0, 3r_0)$ is the initial position of the vortex ring, $r_0 = 1$ is the ring radius, and $\sigma = 0.2r_0$ is the initial core radius. The Reynolds number of the vortex ring is defined as $Re_r = \Gamma/\nu$, where $\Gamma = 1$ is the initial ring circulation. The distance between the ring and the plane surface at the outset is set as $3r_0$. The computational domain of $(-4r_0, 4r_0) \times (-4r_0, 4r_0) \times (-2r_0, 6r_0)$ is discretized into 320^3 cubic grid cells, and the time step ($\Delta t^* = \Delta t \Gamma / r_0^2$) is set as 0.002. Using the r_0 and Γ , the flow quantities are rewritten in the nondimensional form as follows: $\mathbf{x}^* = \mathbf{x}/r_0$, $t^* = t/\Gamma r_0^2$, $\mathbf{u}^* = \mathbf{u} r_0 / \Gamma$, $\boldsymbol{\omega}^* = \boldsymbol{\omega} r_0^2 / \Gamma$, $\omega_y^* = \omega_y r_0^2 / \Gamma$. The total enstrophy and kinetic energy are respectively defined as

$$Ens(t^*) = \frac{1}{2} \int_V \boldsymbol{\omega}^{*2}(\mathbf{x}^*, t^*) dV \quad (7)$$

$$Ek(t^*) = \frac{1}{2} \int_V \mathbf{u}^{*2}(\mathbf{x}^*, t^*) dV \quad (8)$$

where V indicates the whole computational domain.

Figure 1 shows the time evolution of the collision of a vortex ring with a flat plane at $Re_r = 1743$. The creation of the boundary layer and the secondary ring, their interaction with the primary ring, and the primary ring's rebound phenomenon are visible. From the figure, the present simulation results agree well with the experimental ones provided by C.C. Chu, et al. (1993) [2]. Fig. 2 describes the time variation of the primary vortex ring center. The primary ring's rebound phenomenon develops due to the secondary ring pushing it upward. The current simulation results also agree with the experimental data [2]. Thus, the present numerical method adequately captures the vortex dynamics induced by the collision of a vortex with a solid wall.

Figure 3 shows the time variation of the total enstrophy and kinetic energy of the flow caused by the collision of a vortex ring with a flat plate at $Re_r = 1743$ using four grid resolutions. The total enstrophy of the flow slightly reduces from the outset to $t^* = 8$ due to vortex diffusion effects. This diffusion spreads the vorticity field to neighbouring locations, but it reduces the overall intensity of vorticity. The total enstrophy dramatically increases from $t^* = 8$ to 16 because of the formation of the boundary layer as the primary ring approaches the wall. The enstrophy is maximal at $t^* = 16$ when the secondary vortex ring is formed. During the final stage of the flow, the total enstrophy strongly decreases because the diffusion of existing vortices plays a vital role in the flow. The total kinetic energy of the flow slightly reduces in the early stage of the flow from the beginning to $t^* = 12$ before a drastic decrease for the remaining period. This is explained by dissipation happening throughout the flow period, and its rate is faster after the secondary vortex ring is formed. From the figure, we observe that the results of total enstrophy are convergent as the grid resolution increases, and the difference in enstrophy between two resolutions using 280^3 and 320^3 grid cells is insignificant. The results of the total kinetic energy of the flow almost overlap as the grid resolution varies. Therefore, we will use the grid resolution of 320^3 grid cells for later simulations.

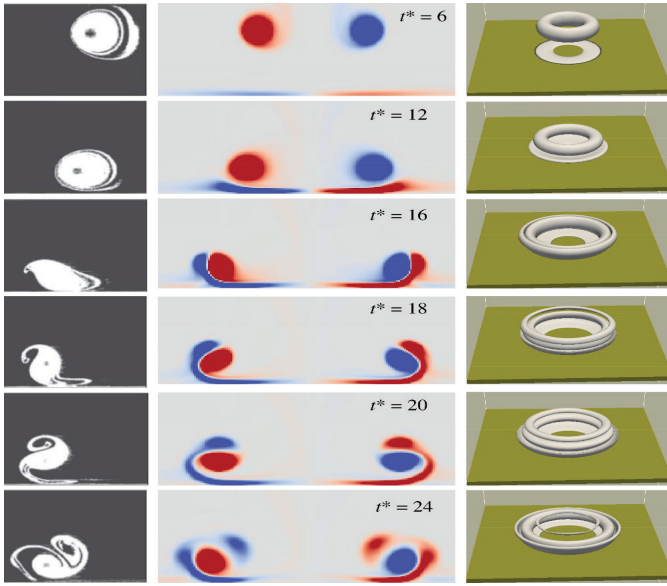


Fig. 1. Time evolution of the vortex structures induced by the collision of a vortex ring upon a flat plane at $Re_\tau = 1743$. The first column is experimental data given by C.C. Chu, et al. (1993) [2]. The second and third columns present simulation results plotted using the vorticity contour of ω_y^* on the x-z plane and the vorticity magnitude $|\omega^*|=1$.

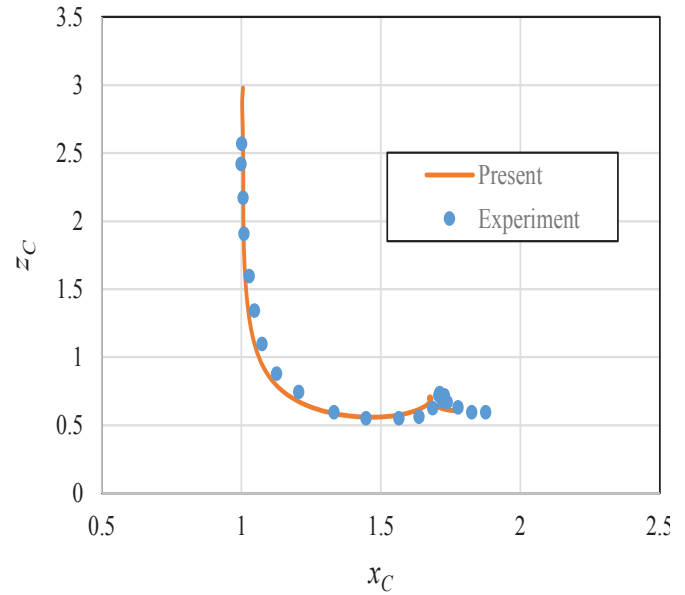


Fig. 2. Time variation of the primary vortex ring centre (x_c, z_c) . The current simulation results are compared with the experimental results given by C.C. Chu, et al. (1993) [2].

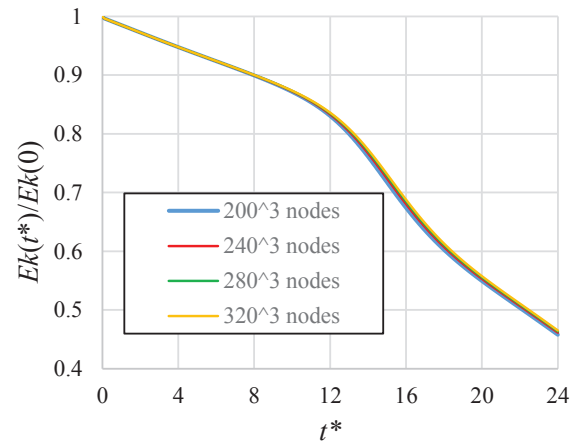
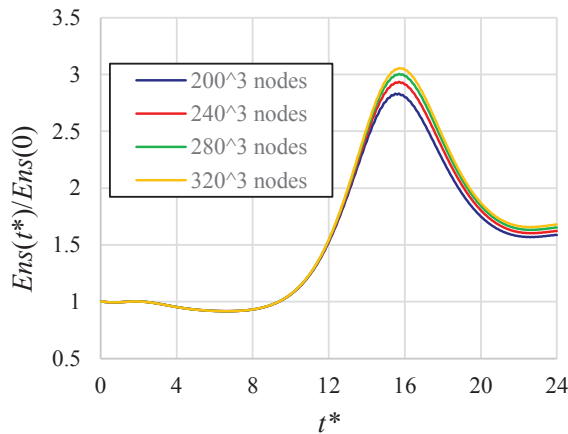


Fig. 3. Time evolution of the total enstrophy (Ens) and total kinetic energy (Ek) of the flow induced by the collision of a vortex ring with a flat plate at $Re_\tau = 1743$.

3. Results and discussion

The collision of a vortex ring with a wavy sphere is numerically investigated at $Re_\tau = 1000$, where the vortex ring at the outset is modelled using the Gaussian distribution function, as described above. The vortex ring has a radius of $r_0 = 1$, core radius of $\sigma = 0.21r_0$ at the position of $(0, 0, 4r_0)$. The wavy sphere surface is modelled as

$$\frac{x^2 + y^2}{(R + a \sin(n\theta))^2} + \frac{z^2}{R^2} = 1 \quad (9)$$

where $R = 2r_0$; $n = 0, 5, 9$ is the wavenumber; $a = 0.05r_0$ is the wave amplitude; and $\theta = \sin^{-1}\left(\frac{y}{\sqrt{x^2 + y^2}}\right)$. The computational domain is set as $(-4r_0, 4r_0) \times (-4r_0, 4r_0) \times (-4r_0, 6r_0)$. The grid resolution and the time step are the same as those used for the above simulation of the collision of a vortex ring on a flat plane.

Figures 4 and 5 show the time evolution of the collision of a vortex ring upon a sphere ($n=0$) at $Re_\tau=1000$, where the vortex structures are expressed using plots of the vorticity contour and the iso-surface of Q-criteria, with the colour range from blue to red representing the enstrophy values from -1 to 1. The boundary layer is generated on the sphere surface as the primary vortex ring moves toward the sphere at $t^*=2$ and 4. This boundary layer separates from the sphere surface at $t^*=6$ and rolls up to form the secondary vortex ring at $t^*=8$. This vortex moves around the primary vortex ring while the boundary layer separates from the sphere at $t^*=10$. The shear layer rolls up to create the tertiary vortex ring at $t^*=12$. During the last stage of the flow, the secondary and tertiary vortex rings gradually decay with time, and they disappear at $t^*=20$. The behaviour of the primary ring, the formation of the secondary ring, and their interaction are similar to those observed in the case of a flat wall.

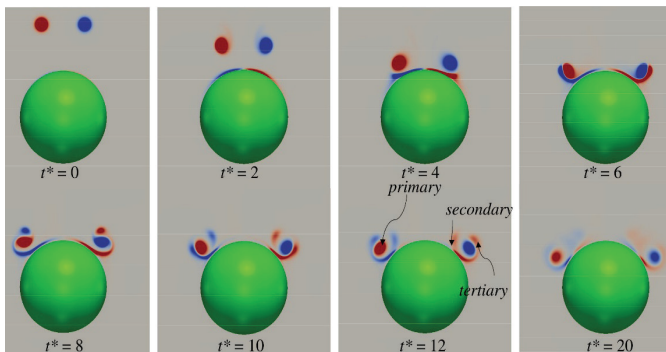


Fig. 4. Time evolution of the collision of a vortex ring upon a sphere with $n=0$ at $Re_\tau=1000$, in which the vortex structures are represented using the vorticity contour of ω_y^* on the x - z plane.

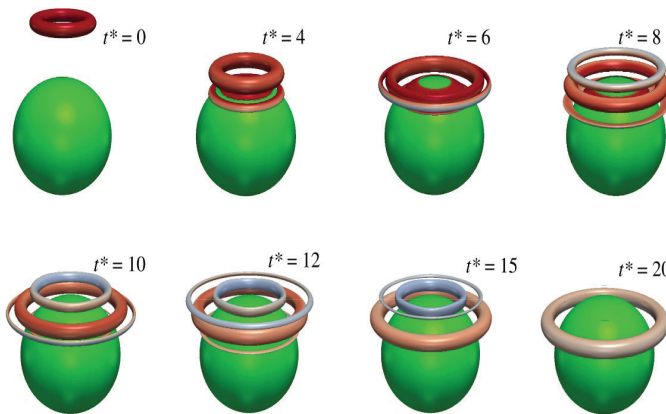


Fig. 5. Time evolution of the collision of a vortex ring upon a sphere with $n=0$ at $Re_\tau=1000$, where the vortex structures are represented using the iso-surface of Q-criteria and the

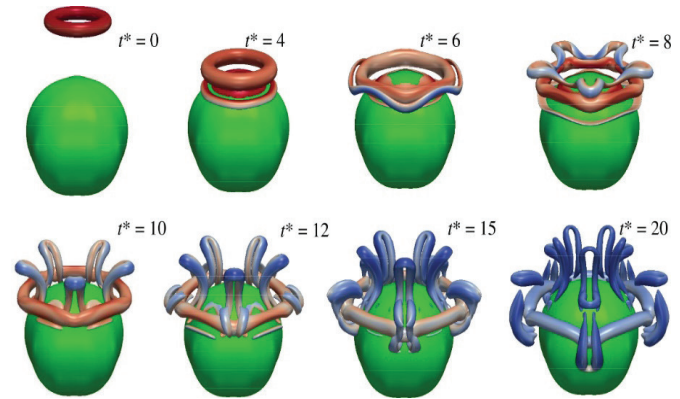


Fig. 6. Time evolution of the collision of a vortex ring upon a wavy sphere with $n=5$ at $Re_\tau=1000$, where the vortex structures are represented using the iso-surface of Q-criteria and the enstrophy.

Figure 6 describes the time variation of the collision of a vortex ring with a wavy sphere with $n=5$ at $Re_\tau=1000$, in which the vortex structures are represented using the iso-surface Q-criteria with the colour range representing the enstrophy. The boundary layer is also formed as the vortex ring advances closer to the wall, as seen at $t^*=4$. The boundary layer on the hills (the peaks of the wave) first separates from the wall, followed by that at the valleys (the bottoms of the wave), and they roll up to produce the secondary vortex at $t^*=6$. The portion of the secondary vortex near the hills moves around the primary vortex at $t^*=8$, while the rest of the secondary vortex ring remains behind. At this moment, the boundary layer continues to separate from the wall. During the last stage of the flow, the secondary vortex near the hills decays, while that near the valleys rises upward into incomplete vortex loops. Furthermore, the tertiary vortices generated at the valleys move around the primary vortex, as shown at $t^*=15$ and 20. The primary vortex is significantly deformed due to its interaction with the secondary and tertiary vortices. The reconnection of the secondary and tertiary vortices is incomplete before they dissipate over time.

Figure 7 illustrates the time evolution of the vortex structures induced by the collision of a vortex ring with a wavy sphere at $n=9$ and $Re_\tau=1000$. We observe that the boundary layer first separates at the hills and subsequently at the valleys, leading to the formation of the secondary vortex at $t^*=6$. The portion of this vortex originating from the wall hills rapidly moves around the primary vortex, while the part from the valleys ascends at $t^*=8$. At this stage, the boundary

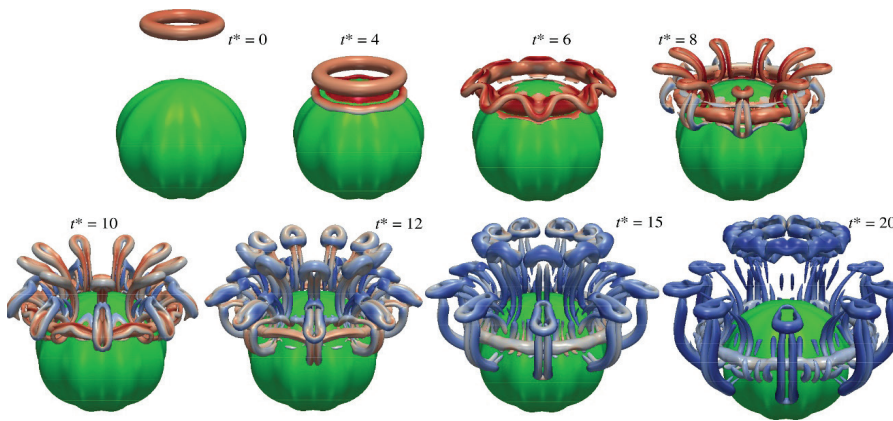


Fig. 7. Time evolution of the collision of a vortex ring upon a wavy sphere with $n=9$ at $Re_r = 1000$, where the vortex structures are represented using the iso-surface of Q-criteria and the enstrophy.

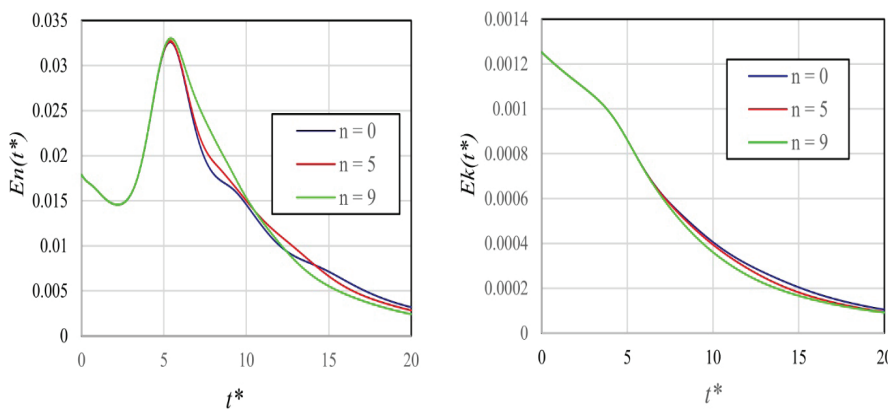


Fig. 8. Time evolution of the total enstrophy ($En(t^*)$) and kinetic energy ($Ek(t^*)$) of the flow induced by the collision of a vortex ring with a wavy sphere at $Re_r = 1000$, where n stands for number of wavelengths.

layer separates from the wall, forming tertiary vortex loops. By $t^*=10$, the part of the secondary vortex detached from the hills deforms the primary vortex significantly, while the remainder from the valleys reconnects, forming small-scale vortex rings that ascend. A portion of the secondary vortex continues to move around the primary vortex at $t^*=12$ before decaying at $t^*=15$ and 20. In contrast to the scenario with $n=5$, the reconnection of the secondary vortex that separated from the valleys is more complete, resulting in a chain of smaller vortex rings. Additionally, the tertiary vortex from the valleys successfully reconnects, producing vortex loops. These findings indicate that the reconnection of the secondary and tertiary vortices occurs over a greater number of wavelengths.

Figure 8 illustrates the temporal evolution of the total enstrophy and kinetic energy of the flow induced by the collision of a vortex ring with a wavy sphere at various wavelengths. The total enstrophy gradually decreases during the initial stages of the flow as the vortex ring diffuses. Enstrophy then increases significantly between $t^*=2.5$ due to the formation of a boundary layer on the wall as the ring approaches, which generates a vorticity field near the wall. After $t^*=5$, enstrophy decreases sharply as the boundary layer formation completes, and the diffusion of the primary and secondary vortices becomes prominent during the final stages of the flow. The peaks of the wave cause a higher enstrophy throughout the interval $t^*=5$. There is a slight change in enstrophy at $t^*=10$ as the boundary layer separates, leading to the formation of tertiary vortices. In the concluding phase of the flow, from $t^*=14$, the enstrophy for the wavy wall with a higher number of peaks is reduced, which is attributed to the wavy wall dissipating vortices at a greater rate compared to a smooth wall.

4. Conclusions

Numerical simulations of the collision of a vortex ring with a wavy sphere at various wavenumbers (n) and $Re_r = 1000$ are performed for the first time using the vortex particle method. The numerical method is validated against the benchmark simulation of a vortex ring impinging on a flat plate at $Re_r = 1743$. The simulation results indicate that the current numerical method successfully captures the vortex dynamics induced by the collision of a vortex with a solid wall. The key findings for the collision of a vortex ring with a wavy sphere are summarised as follows:

When a vortex ring collides with a smooth sphere ($n=0$), secondary and tertiary vortex rings are formed due to the separation and rolling up of the boundary layer on the wall. Their interaction with the primary vortex ring is similar to

that observed in the flat plate case, where they move around the primary ring, resulting in the rebound phenomenon, before dissipating over time. The difference here is the tendency for the boundary layer to separate more easily from the sphere than from the flat plate.

For the collision of a vortex ring with a wavy wall, the behaviour of the secondary and tertiary vortices is highly dependent on the wavenumber (n) of the wall. The boundary layer on the hills (wave crests) separates first, followed by the valleys (wave troughs), leading to the formation of a secondary vortex ring in a wavy pattern. This vortex near the hills moves around the primary vortex ring, while the remainder near the valleys ascends. At $n=9$, these parts reconnect to form small-scale vortex rings, whereas at $n=5$, the reconnection does not occur, resulting in incomplete vortex loops. Tertiary vortices are generated in the valleys as the secondary vortex ring ascends. These vortices also reconnect to form vortex loops at $n=9$, while reconnection does not take place at $n=5$.

CRediT author statement

Nguyen Van Luc: Algorithm, Simulation, Original draft preparation, Reviewing, Editing; Le Tuan Phuong Nam, Phan Toai Tuyn: Simulation, Writing, Reviewing, and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] T.L. Doligalski, C.R. Smith, J.D.A. Walker (1994), "Vortex interactions with walls", *Annual Review of Fluid Mechanics*, **26**(1), pp.573-616, DOI: 10.1146/annurev.fl.26.010194.003041.
- [2] C.C. Chu, C.T. Wang, C.S. Hsieh (1993), "An experimental investigation of vortex motions near surfaces", *Physics of Fluids A*, **5**(3), pp.662-676, DOI: 10.1063/1.858650.
- [3] M. Cheng, J. Lou, L.S. Luo (2010), "Numerical study of a vortex ring impacting a flat wall", *J. Fluid Mech.*, **660**, pp.430-455, DOI: 10.1017/S0022112010002727.
- [4] T.H. New, J. Long, B. Zang, et al. (2020), "Collision of vortex rings upon V-walls", *Journal of Fluid Mechanics*, **899**, DOI: 10.1017/jfm.2020.425.
- [5] T.H. New, B. Zang (2017), "Head-on collisions of vortex rings upon round cylinders", *Journal of Fluid Mechanics*, **833**, pp.648-676, DOI: 10.1017/jfm.2017.599.
- [6] V.L. Nguyen, K. Takamure, T. Uchiyama (2019), "Deformation of a vortex ring caused by its impingement on a sphere", *Physics of Fluids*, **31**(10), DOI: 10.1063/1.5122260.
- [7] T.H. New, B. Xu, S. Shi (2024), "Collisions of vortex rings with hemispheres", *Journal of Fluid Mechanics*, **980**, DOI: 10.1017/jfm.2024.13.
- [8] T. Ahmed, B.D. Erath (2023), "Experimental study of vortex ring impingement on concave hemispherical cavities", *Journal of Fluid Mechanics*, **967**, DOI: 10.1017/jfm.2023.501.
- [9] H. Ren, C. Xu (2014), "Three-dimensional numerical simulation of a vortex ring impacting a bump", *Theoretical and Applied Mechanics Letters*, **4**(3), DOI: 10.1063/2.1403204.
- [10] H. Ren, X.Y. Lu (2015), "Dynamics and instability of a vortex ring impinging on a wall", *Communications in Computational Physics*, **18**(4), pp.1122-1146, DOI: 10.4208/cicp.150115.210715s.
- [11] M. Cheng, J. Lou, T.T. Lim (2014), "A numerical study of a vortex ring impacting a permeable wall", *Physics of Fluids*, **26**(10), DOI: 10.1063/1.4897519.
- [12] A. Pirnia, J. Hu, S.D. Peterson, et al. (2017), "Vortex dynamics and flow-induced vibrations arising from a vortex ring passing tangentially over a flexible plate", *Journal of Applied Physics*, **122**(16), DOI: 10.1063/1.5009068.
- [13] V.L. Nguyen, H.L. Duong, N.T.P. Le (2021), "Oblique collision and reconnection of a vortex ring with a vortex tube", *Physics of Fluids*, **33**(12), DOI: 10.1063/5.0073126.

A geometrical optimisation of a coplanar micro-electrode structure for microfluidic flow cytometry

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Abstract:

Single-cell analysis provides a more information-rich approach to disease diagnosis than traditional methods. At the cellular level, electrical properties have been established as reliable disease markers, capable of revealing variations between individual cells. This study focuses on optimising the geometry of a coplanar micro-electrode structure for detecting human lung adenocarcinoma cells (A549) within a fluid channel using impedance flow cytometry. A549 cells were chosen due to their frequent occurrence in cancer cases and the extensive documentation of their electrical properties and size. To further investigate the electric field and optimise the electrode design for single-cell detection, a numerical 3D model based on the finite element method (FEM) was developed and implemented. The functionality of the sensing structure was validated using COMSOL Multiphysics, with the Electric Current module defining scalar electric potential within the 3D model. Simulations explored various parameters, including electrode dimensions, frequency range, object sizes, and electrical conductivity, to fine-tune the sensor's performance. Additionally, the study elucidates the impact of cell position within the channel structure and cell size on impedance measurements. This numerical investigation provides insights into the acquired impedance signals, contributing to the optimisation and standardisation of the device. The proposed sensor system holds significant potential across various applications in biomedicine and chemistry, particularly in point-of-care scenarios, where the sensor chip can be conveniently configured for measurements and discarded after use.

Keywords: cell detection, impedance flow cytometry, microfluidic system, reactance, resistance.

Classification numbers: 2.1, 2.3, 3.2

1. Introduction

Cancer remains the leading cause of mortality in both developed and developing nations [1]. Among the primary causes of cancer-related deaths is lung cancer [2]. Currently, lung cancer diagnosis predominantly relies on a combination of medical imaging and invasive biopsies for histopathological confirmation [3]. However, these methods possess several drawbacks, such as requiring highly trained personnel, confirmation times of approximately 48 hours per sample, and a lack of ongoing monitoring [4]. Additionally, these modalities often have limited capability to assess phenotypic changes associated with chemoresistance and biomarkers. Cancer progression is closely linked to changes in the number of cancer cells present in the blood, particularly in cases of metastasis, which necessitates the development of mobile cancer cell counting systems that enable rapid, low-cost testing, requiring minimal sample sizes and analysis time.

In recent decades, impedance flow cytometry (IFC) has emerged as an efficient method for single-cell analysis [5-8]. This technique also provides additional cell information through non-invasive, label-free impedance measurements, offering applications ranging from single-cell counting to point-of-care diagnosis [9-13]. However, one of its main limitations is the variability of the signal impedance with the cell's position in the sensing region. This method has also been applied to analyse single cells in controlled oxygen microenvironments to study cellular responses to hypoxia [14-16]. The position-dependent impedance signal poses a challenge to the accuracy of IFC. Furthermore, the electrode configuration influences both the electric field distribution and impedance, considering shape and size. Variations in impedance signals as cells pass through the sensing area allow for the extraction of data about the size, shape, and dielectric properties of these cells [17]. The dimensions of the channel and micro-electrodes are typically selected

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based on experimental correlation with the micro-object of interest. Several previous studies have employed impedance analysis to investigate parameter influence and optimise channel structure [18-23]. However, the analysis of both resistance and reactance components has not been widely explored recently. Additionally, most studies of single-cell analysis using the IFC method have focused on single-frequency analyses, neglecting the reactance component and excitation frequency optimisation.

A previous study introduced a novel impedance analysis technique, which includes the assessment of both resistance and reactance [24]. This study presents an effective methodology for the detection and quantification of human lung cells (A549) using complex impedance analysis within an impedance-based microfluidic (IFC) device. This approach provides additional insights into the electrical characteristics of cells within the microfluidic channel, facilitating the differentiation of various cell types in the fluidic system. The detection and quantification capabilities were simulated using COMSOL Multiphysics. This model is optimised to identify the most effective structure and stimulation frequency for biological cell sizes. The numerical analysis enhances the accuracy, improving the specificity and sensitivity of conventional impedance sensing structures. This innovative approach lays the groundwork for developing Lab-on-a-Chip (LoC) devices, poised for future biomedical applications. By harnessing the capabilities of complex impedance analysis in microfluidic systems, these devices hold the potential to revolutionise diagnostics, research, and biomedical applications by offering advanced tools for detecting and analysing specific cell types.

2. Materials and methods

2.1. Sensor design

Figure 1 presents the proposed complex impedance-based biosensor structure. The microfluidic chip consists of a symmetrical channel structure with a length of 1,000 μm , a width of 50 μm , a height of 50 μm , and a sensing electrode area of 50 μm in length and 30 μm in width.

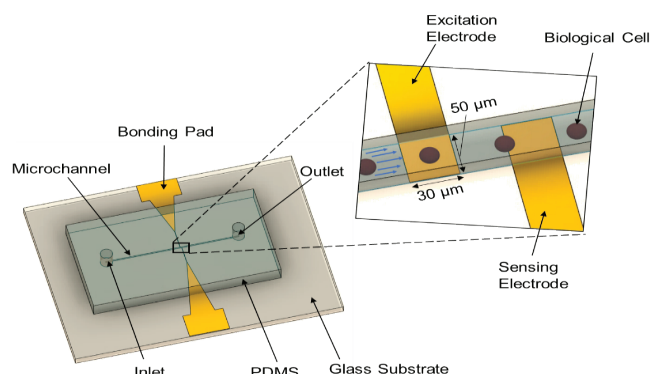


Fig. 1. 3D visualisation of the proposed complex impedance analysis sensor structure.

The selection of the microfluidic chip size is critical, as clogging may occur if the channel is too small, while a significant decrease in sensitivity could result if the channel is too large. The dimensions chosen in this manuscript are based on commonly used sizes in recent experimental studies within the biomicrofluidics field, and are deemed suitable to meet these requirements. Similar channel dimensions have been utilised in previous studies for cancer cell detection experiments [25, 26].

2.2. Single sphere modelling

For cell impedance evaluation, a frequency-dependent voltage is applied to the system containing the cell in suspension, and the electrical current response of the system is measured. The electrical impedance of the system $\tilde{Z}(j\omega)$ is defined as the ratio of the voltage $\tilde{U}(j\omega)$ and current response $\tilde{I}(j\omega)$.

$$\tilde{Z}(j\omega) = \frac{\tilde{U}(j\omega)}{\tilde{I}(j\omega)} = R + jX \quad (1)$$

where $j = \sqrt{-1}$ and ω is the frequency. The biological impedance is a complex number with resistance (R) as the real part and reactance (X) as the imaginary part, both expressed in Ω , respectively.

Impedance signals indicate which cells are collected when an A549 cell passes through the electrode sensing area. In this simulation, an alternating current signal is applied, and a single-sphere spherical suspension cell model is used for cell modelling to collect detailed information about the cell. The modelled A549 cell was assumed as a single sphere of 7.5- μm radius, with dielectric permittivity (ϵ_r) of $60\epsilon_0$ and electrical conductivity (σ_r) of 1.05 S/m [27, 28], and was suspended in a sucrose solution medium ($\epsilon_r=78$; $\sigma=1.76 \times 10^{-3}$ S/m). Sucrose plays a vital role in maintaining the osmotic potential and preserving water within cells. Furthermore, it has been utilized as a buffer solution in microchannels, with some studies employing an 8.6% sucrose solution for this purpose [29].

Regarding the influence of cell membranes, certain studies have demonstrated that the cell membrane acts as a strong electrical insulator, with cells behaving like insulating particles at low frequencies [30-32]. However, research findings indicate that minor membrane conductivities exert little influence on impedance data, while larger conductivities lead to the dominance of the cell's dielectric properties by internal factors, notably those linked to the nucleus [33]. Consequently, this study employs a single-sphere model for analysis. For a simplified system analysis, an equivalent circuit model is often utilised, with such an approach developed by K.R. Foster, et al. (1989) [34].

3. Numerical simulation

To explore the electric field and determine the conductance of specific biological entities, a three-dimensional model employing the finite element method (FEM) was developed. This model was designed and analysed using COMSOL Multiphysics 6.0 software from COMSOL Inc., headquartered in Stockholm, Sweden. The software excels in simulating electrical components, including conductivity and permittivity, as well as devices used in electrostatic, magnetic static, and electromagnetic quasistatic applications. Special attention is given to capturing the influence of various physical characteristics. In this study, the biological cell, assumed to have no deformation, is represented as a single-sphere model within the simulation [35].

Biological cells in the fluid stream are identified based on changes in impedance between two sensing electrodes. Impedance values for the stimulating and sensing electrodes were assessed according to the specific position of the moving cell within the microchannel, and the differences between these values were evaluated. In this simulation, the stimulating electrode is consistently maintained at a voltage of 1 V, while the sensing electrode remains at 0 V. Consequently, the relative impedance of the electrode structure can be modulated by the current flowing between the electrode pairs.

In the steady state, the electrostatic current in the conductive medium is regulated by the static continuity equation as follows:

$$\nabla \cdot \mathbf{J} = Q_{(j,v)} \quad (2)$$

In a static scenario, the potential relationship V is established as follows:

$$\mathbf{E} = -\nabla V \text{ (V/m)} \quad (3)$$

Here, E represents the gradient of the electric potential V , analogous to the classical gravitational field. The electric isolation node serves as the default boundary condition, implying that no current enters the boundary.

$$\mathbf{n} \cdot \mathbf{J} = 0 \quad (4)$$

At this boundary, the absence of current flow is enforced. This condition is particularly relevant at symmetric boundaries where the potential is recognised to exhibit symmetry about the boundary. Importantly, the default insulation condition is not imposed on the internal boundary.

The outside environment was filled with air. Table 1 lists the material properties used in the numerical calculation [36]. Fig. 2 shows the mesh of the proposed complex impedance sensing structure, which is denser in the important elements, including cells and electrodes while remaining less dense in other areas to optimize the resources.

Table 1. Parameters of materials used in simulations [36].

Material	Permittivity	Conductivity (S/m)
Air	1	10^{-15}
Polydimethylsiloxane (PDMS)	2.75	4×10^{-15}
Glass	5.5	10^{-15}
A549 cell	60	1.05
8.6% sucrose solution	78	1.76×10^{-3}

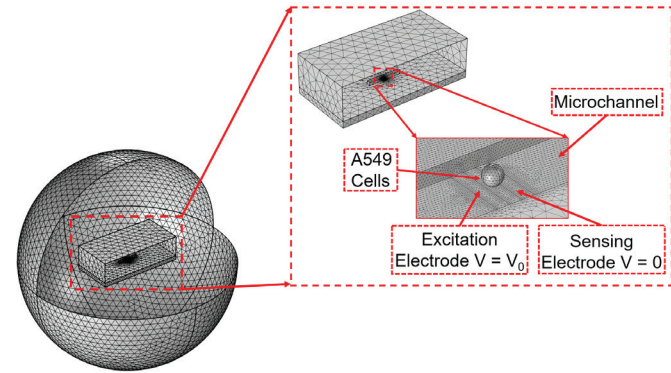


Fig. 2. Simulated A549 cells.

4. Results and discussion

4.1. The electric field distribution of the sensor

Figure 3 illustrates the electric field distribution within the sensor structure, depicting the electric field intensity that extends beyond the air, encompassing the space between the stimulating and sensing electrodes.

The visualisation demonstrates the reach and coverage of the electric field, highlighting its presence and influence over the specified region within the sensor structure. This comprehensive depiction provides insights into the spatial characteristics and extent of the electric field, offering a

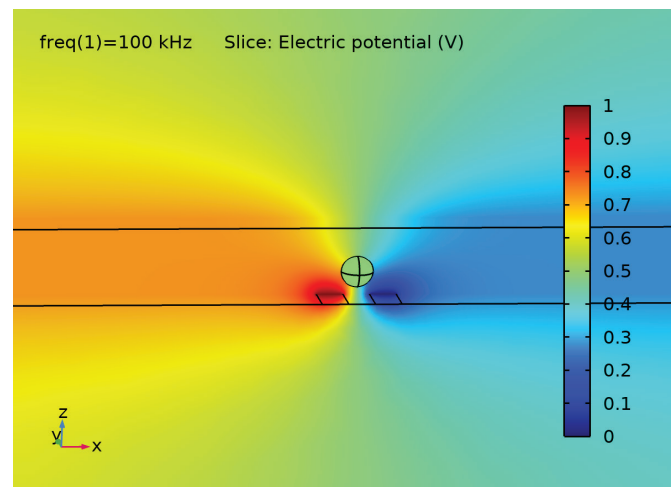


Fig. 3. Electric potential distribution.

better understanding of how it interacts with the surrounding environment and potential targets, such as biological cells within the fluid stream.

4.2. Detection of A549 cells inside the microfluidic channel

The impedance change ΔZ is given by the difference between the impedance Z_A received when the A549 cell passes through the sensing structure in the sucrose 8.6% solution and the impedance Z_B obtained in the absence of the A549 cell in the Sucrose 8.6% solution channel:

$$|\Delta Z| = |Z_A - Z_B| \quad (5)$$

The impedance change due to the passage of the A549 cell through the sensing area in the real part is ΔR , also known as resistance change, while the change in the imaginary part ΔX is known as reactance change. The simulation results illustrate the correlation between the impedance change and the position of the A549 cell within the sucrose 8.6% solution channel (Fig. 4). Notably, the impedance signal shows distinct peaks as the A549 cell enters the region between the two electrodes.

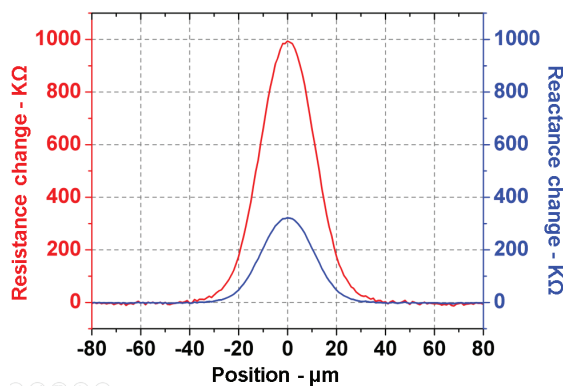


Fig. 4. Simulation results: A typical signal with a 7.5 μm radius A549 cell moves through the sensing area in the 8.6% Sucrose solution channel.

In this analysis, the frequency domain of the electric current module in a simulation model was employed to scrutinise the complex impedance of the sensor under an excitation signal frequency of 100 kHz. As depicted in Fig. 4, the simulation results reveal maximum changes in resistance and reactance of approximately 1,000 k Ω and 325 k Ω , respectively. These variations are observed as the simulated A549 cell, with a radius of 7.5 μm , traverses the sensing electrode structure within the 8.6% sucrose solution channel.

This simulation provides valuable insights into the dynamic response of the sensor under specific frequency conditions, demonstrating substantial changes in both resistance and reactance parameters. Understanding these impedance variations, particularly in response to

cell movement, contributes to a more comprehensive understanding of the sensor's behaviour and aids in refining the design for optimal performance. The observed changes underscore the sensor's sensitivity to the presence of cells, further emphasising the potential utility of this technology for precise cell detection applications within microfluidic environments.

4.3. Impact of A549 cell size on impedance signal

Figure 5 illustrates the relationship between impedance change and the radius of cells passing through the sensing area. In order to examine impedance changes concerning A549 cells of different radii moving through the sensor channel, simulations were conducted with cell radii ranging from 2 to 10 μm . Notably, the impedance change becomes more pronounced as larger A549 cells pass through the sensing region. At the 0 mm position, located between the two electrodes, the most significant impedance change corresponds to a cell radius of 7.5 μm , is evident. These results were obtained by varying the excitation frequency from 10 kHz to 10 MHz. Numerical calculations reveal a proportional relationship between the change in impedance and the increase in cell radius.

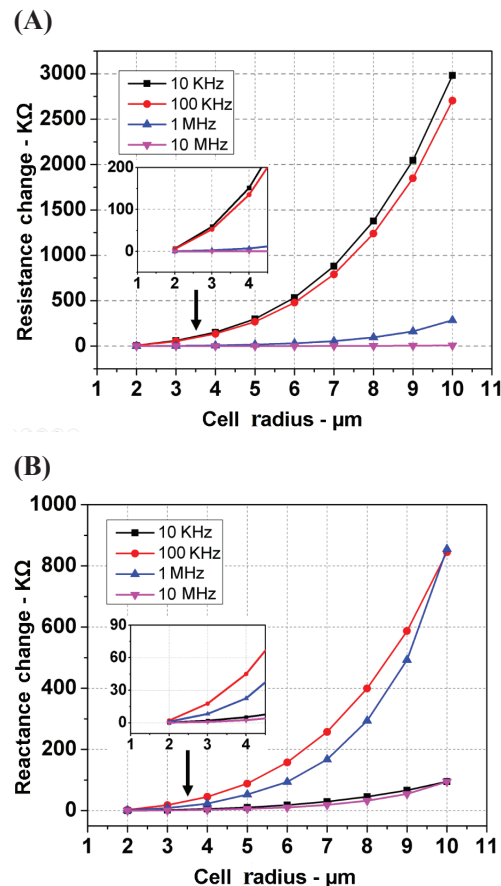


Fig. 5. The dependence of (A) resistance change and (B) reactance change on A549 cell size in fluidic channel structure.

Moreover, only marginal impedance changes are observed as the cell size decreases. The inset demonstrates the simulation results for cell radii ranging from 2 to 4 μm to explore impedance variations in instances where the cell size is small. The results show that the change in resistance varies up to approximately 150 $\text{K}\Omega$ while the reactance exhibits a maximum change of about 22.6 $\text{K}\Omega$. Additionally, the simulation results show that the change in impedance and reactance due to cell size is negligible at low frequencies. However, significant changes are observed at higher frequencies (10 MHz). This marked variation may be associated with changes in the cell membrane and electrical double layer, which behave like a capacitor. As a result, the capacitance of C_{mem} and C_{dl} change depending on the applied AC frequency (ω).

In the lower frequency range ($\omega < 1 \text{ MHz}$), the electrical double layer primarily influences the system impedance, resulting in low sensitivity for cell detection. As the frequency increases ($1 < \omega < 10 \text{ MHz}$), the capacitance of the electrical double layer gradually diminishes, and the system impedance is determined by the size of the cell. T. Sun, et al. (2010) [27] introduced a comprehensive equivalent circuit model for a cell, incorporating membrane resistance and cytoplasmic capacitance. Their previous research [27] extensively discussed the methodology for calculating the values of R_p , C_p , R_{mem} , and C_{mem} in this model.

Hence, the results demonstrate that the impedance undergoes a notable change in both the resistance and reactance components when large A549 cells pass through. Moreover, variations in cell sizes and electrical characteristics across different cell types make it possible to distinguish between them by analysing changes in impedance, encompassing both resistance and reactance.

However, Fig. 5 in this study depicts the relationship between changes in resistance and reactance relative to the cell's radius, rather than focusing on impedance variation. This research specifically examined the relationship between impedance changes and cell radius, as shown in Fig. 6. The results indicate that the sensitivity of electrical impedance to particle size tends to be more pronounced at lower frequencies compared to higher frequencies, consistent with theoretical expectations. Moreover, the relationship between frequency and changes in resistance and impedance. Importantly, the results highlight that reactance alterations do not follow the same trend as resistance. This approach offers a more comprehensive understanding of the electrical properties unique to each cell.

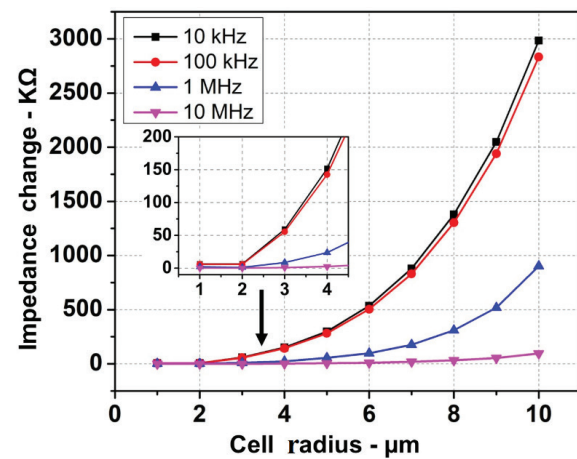


Fig. 6. The dependence of impedance change on A549 cell size in fluidic channel structure.

4.4. Influence of cell vertical position on impedance signal

In recent years, various impedance flow cytometry methods have been developed for biomedical applications, ranging from blood cell quantification for disease diagnosis to microbiological studies and monitoring cell phenotype changes.

However, a significant challenge to the accuracy of impedance sensing technology is its dependency on the position of objects within the channel. As a result, acquired impedance signals can vary, affecting the sensor's sensitivity when objects pass through the sensor area along different trajectories. Numerous technical advancements have been introduced to improve the precision of impedance-based tests. These include approaches such as controlling cell positions in microfluidic channels [37], signal processing [38], and designing specific electrode configurations [39].

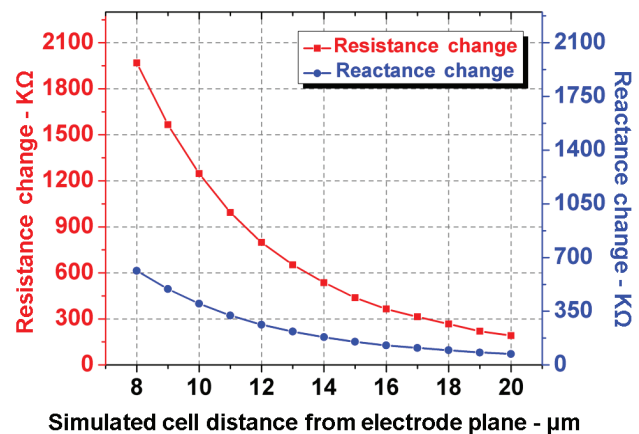


Fig. 7. Simulated impedance changes according to the vertical position.

To further investigate these phenomena, this study conducted an analysis of impedance signals by varying the position of the A549 cell within the channel, ranging from 8 to 20 μm away from the electrode plane. The survey frequency was held constant at 100 kHz, and an 8.6% sucrose solution was used. Fig. 7 visually illustrates the dependency of impedance changes on the cell's location in the sensing region. The resistance exhibited an approximate change of 2,000 $\text{K}\Omega$, while the reactance shifted by around 610 $\text{K}\Omega$. Importantly, the impedance changes in impedance sensing demonstrate an inverse relationship with the distance between the simulated cell and the electrode plane. Therefore, to enhance the sensitivity of the microsensor, reducing the cell-to-electrode surface height to match typical cell sizes becomes crucial.

4.5. Influence of electrode size, the distance between two electrodes on impedance signal

This study examines the impact of coplanar electrode dimensions to enhance the sensitivity of impedance flow cytometry. The standard setup includes two coplanar electrodes with a width equal to the spacing between them.

In this research, numerical calculations were performed using electrodes of uniform size, with distances ranging from 5 to 50 μm . The cell-to-surface distance was consistently set at 11 μm , with the cell positioned between two electrodes. Fig. 8 depicts the impedance values obtained as a simulated cell traversed between the excitation and sensing electrodes in an 8.6% sucrose solution. The results indicate that the most significant impedance change occurs when the electrode size and spacing are within the 10 to 15 μm range, resulting in a resistance change of 1,000 $\text{K}\Omega$ and a reactance change of 340 $\text{K}\Omega$.

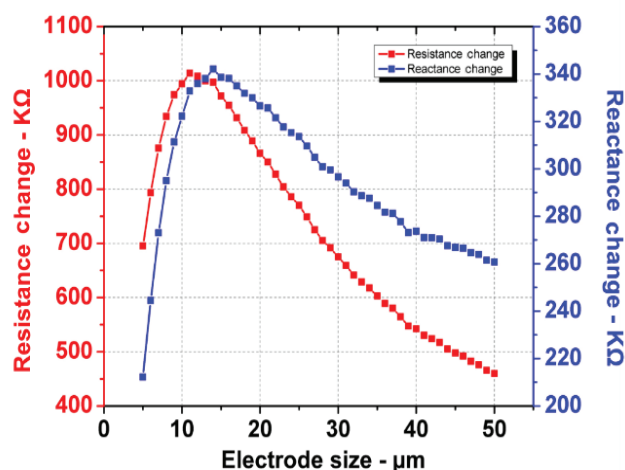


Fig. 8. The impedance variation of the simulated cell depends on the electrode size.

These findings underscore the importance of optimizing electrode size and the spacing between electrodes, suggesting that within the 10 to 15 μm range, the impedance changes are most pronounced. Optimising these parameters can potentially enhance the sensitivity of impedance flow cytometry measurements. Further exploration of these optimised parameters holds promise for advancing studies in impedance-based cell detection and characterisation in microfluidic environments, contributing to the development of more effective methodologies for future research.

4.6. The influence of the frequency of excitation on the impedance signal

The frequency of excitation directly affects the complex impedance between electrodes. This study investigated the influence of excitation frequency on the performance of the proposed method. Under varying frequency conditions, both the resistive and reactive components of impedance were computed using the FEM model.

Building on the investigation of impedance variation in the proposed sensor structure, including both resistance and reactance, a detailed analysis was conducted across a frequency spectrum ranging from 1 kHz to 10 MHz. The Fig. 9 revealed substantial variations in resistance at distinct frequencies, with a significant reduction in resistance change as the frequency exceeded 90 kHz. Additionally, a marked alteration in reactance was observed, peaking between 500 and 600 kHz, followed by a gradual decrease beyond this range.

Based on the simulation findings, it is recommended to select an excitation frequency within the 500 to 600 kHz range for experimental purposes. This frequency range shows significant variations in both resistance and reactance components, providing an optimal balance between

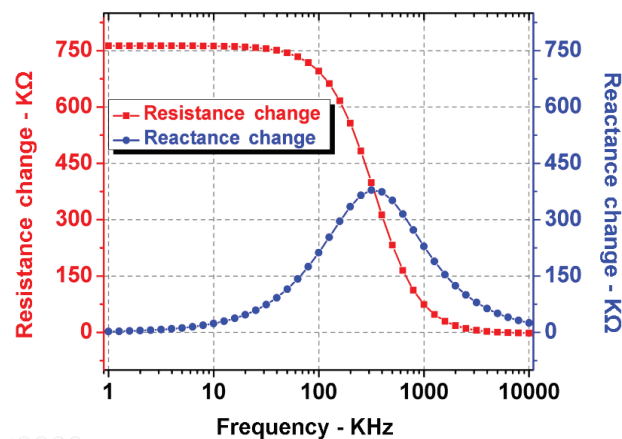


Fig. 9. The impact of frequency on the variations in resistance and reactance of the proposed sensing structure for detecting A549 cells.

sensitivity and precision. Moreover, the choice of excitation frequency should consider the performance of the integrated circuit, with careful attention to cost implications, especially at higher frequencies, in future experiments.

Beyond the technical aspects, this frequency range is ideal for cell detection in microchannels, extending to low-cost, portable devices used in biomedical settings. The optimised characteristics within this range enhance the microsensor's performance, making it suitable for practical and resource-efficient applications in biomedical fields. This highlights the multifaceted advantages of the proposed sensor structure, not only in its technical capabilities but also in its potential real-world applications.

5. Conclusions

This study investigated and structurally optimised a microfluidic chip featuring embedded coplanar electrodes to detect cells within a microfluidic channel using a complex impedance approach. A novel impedance analysis technique, which includes assessments of both resistance and reactance, was applied. The functionality of the sensing structure was validated through the FEM using COMSOL Multiphysics, with the Electric Current module defining scalar electric potential in a 3D model. The analysis results indicate that the sensitivity of the coplanar electrode design for detecting A549 cells is optimised when the electrode structure size falls within the 10 to 15 μm range. The simulation further explored the impact of cell size and position within the channel on impedance changes. This study not only validates the efficiency and feasibility of the proposed analysis method but also establishes a solid foundation for future experimental endeavours focused on detecting and enumerating biological cells for biomedical applications. The integration of coplanar electrodes in a microfluidic chip, as examined in this work, holds promise for enhanced cell detection sensitivity and sets the stage for advancing research in microfluidic-based cell analysis.

CRedit author statement

Van Phu Nguyen: Conceptualisation, Methodology, Data collection and analysis, Interpretation of results, Original draft preparation; Van-Anh Bui: Methodology, Design, Data collection, Interpretation of results; Thu Hang Nguyen: Data analysis, Interpretation of results, Revision; Trung Kien Do: Data collection and analysis; Van Thanh Pham: Interpretation of results, Original draft preparation; Thi Thanh Thuy Dang: Data analysis, Revision; Quang Loc Do: Conceptualisation, Methodology, Revision.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] World Health Organization (2004), *The Global Burden of Disease: 2004 Update*, 146pp.
- [2] D.R. Shopland, H.J. Eyre, T.F. Peachacek (1991), "Smoking-attributable cancer mortality in 1991: Is lung cancer now the leading cause of death among smokers in the United States?", *J. Natl. Cancer Inst.*, **83**(16), pp.1142-1148, DOI: 10.1093/jnci/83.16.1142.
- [3] J. Krüger, K. Singh, A. O'Neill, et al. (2002), "Development of a microfluidic device for fluorescence activated cell sorting", *J. Micromechanics Microengineering*, **12**(4), DOI: 10.1088/0960-1317/12/4/324.
- [4] World Health Organization (2009), *Guidelines for HIV Diagnosis and Monitoring of Antiretroviral Therapy*, <https://apps.who.int/iris/bitstream/handle/10665/205162/B4375.pdf?sequence=1&isAllowed=y>, accessed 15 October 2023.
- [5] S. Zhu, X. Zhang, Z. Zhou, et al. (2021), "Microfluidic impedance cytometry for single-cell sensing: Review on electrode configurations", *Talanta*, **233**, DOI: 10.1016/j.talanta.2021.122571.
- [6] C. Honrado, P. Bisegna, N.S. Swami, et al. (2021), "Single-cell microfluidic impedance cytometry: From raw signals to cell phenotypes using data analytics", *Lab Chip*, **21**(1), pp.22-54, DOI: 10.1039/d0lc00840k.
- [7] D.N. Hernández, J.R. Tosina, M. Min (2019), "Fundamentals, recent advances, and future challenges in bioimpedance devices for healthcare applications", *Journal of Sensors*, **2019**, DOI: 10.1155/2019/9210258.
- [8] B.W. Dulken, M.T. Buckley, P.N. Negredo, et al. (2019), "Single-cell analysis reveals T cell infiltration in old neurogenic niches", *Nature*, **571**(7764), pp.205-210, DOI: 10.1038/s41586-019-1362-5.
- [9] H. Cho, J. Kim, H. Song, et al. (2018), "Microfluidic technologies for circulating tumor cell isolation", *Analyst*, **143**(13), pp.2936-2970, DOI: 10.1039/C7AN01979C.
- [10] R. Nasiri, A. Shamloo, J. Akbari, "Design of a hybrid inertial and magnetophoretic microfluidic device for CTCs separation from blood", *Micromachines*, **12**(8), DOI: 10.3390/mi12080877.
- [11] J. Chen, C.Y. Liu, X. Wang, et al. (2020), "3D printed microfluidic devices for circulating tumor cells (CTCs) isolation", *Biosens. Bioelectron.*, **150**, DOI: 10.1016/j.bios.2019.111900.
- [12] N.D. Hai, V.Q. Tuan, D.Q. Loc, et al. (2016), "Differential C⁴D sensor for conductive and non-conductive fluidic channel", *Microsyst. Technol.*, **22**(10), pp.2511-2520, DOI: 10.1007/s00542-015-2586-4.
- [13] L.Q. Do, T.T. Bui, H.T.T. Tran, et al. (2018), "Fluidic platform with embedded differential capacitively coupled contactless conductivity detector for micro-object sensing", *Int. J. Nanotechnol.*, **15**(1-3), pp.24-38, DOI: 10.1504/IJNT.2018.089543.

- [14] Y. Qiang, D. Dieujuste, J. Liu, et al. (2023), "Rapid electrical impedance detection of sickle cell vaso-occlusion in microfluidic device", *Biomed. Microdevices*, **25**(3), DOI: 10.1007/s10544-023-00663-1.
- [15] D. Dieujuste, Y. Qiang, E. Du (2021), "A portable impedance microflow cytometer for measuring cellular response to hypoxia", *Biotechnol. Bioeng.*, **118**(10), pp.4041-4051, DOI: 10.1002/bit.27879.
- [16] J. Liu, Y. Qiang, O. Alvarez, et al. (2018), "Electrical impedance microflow cytometry with oxygen control for detection of sickle cells", *Sensors Actuators B Chem.*, **255**, Pt. 2, pp.2392-2398, DOI: 10.1016/j.snb.2017.08.163.
- [17] J. Chen, C. Xue, Y. Zhao, et al. (2015), "Microfluidic impedance flow cytometry enabling high-throughput single-cell electrical property characterization", *Int. J. Mol. Sci.*, **16**(5), pp.9804-9830, DOI: 10.3390/ijms16059804.
- [18] F. Caselli, P. Bisegna (2017), "Simulation and performance analysis of a novel high-accuracy sheathless microfluidic impedance cytometer with coplanar electrode layout", *Med. Eng. Phys.*, **48**, pp.81-89, DOI: 10.1016/j.medengphy.2017.04.005.
- [19] J. Cottet, A. Kehren, H.V. Lintel, et al. (2019), "How to improve the sensitivity of coplanar electrodes and micro channel design in electrical impedance flow cytometry: A study", *Microfluid. Nanofluidics*, **23**(1), DOI: 10.1007/s10404-018-2178-6.
- [20] A. Farooq, N.Z. Butt, U. Hassan (2021), "Biochip with multi-planar electrodes geometry for differentiation of non-spherical bioparticles in a microchannel", *Sci. Rep.*, **11**(1), DOI: 10.1038/s41598-021-91109-2.
- [21] M.A. Warren, A. Shakouri, V.P. Peña, et al. (2023), "Development of a novel design of microfluidic impedance cytometry for improved sensitivity and cell identification", *ACS Omega*, **8**(21), pp.18882-18890, DOI: 10.1021/acsomega.3c00797.
- [22] F. Caselli, M. Shaker, L. Colella, et al. (2014), "Modeling, simulation, and performance evaluation of a novel microfluidic impedance cytometer for morphology-based cell discrimination", *J. Microelectromechanical Syst.*, **23**(4), pp.785-794, DOI: 10.1109/JMEMS.2014.2325979.
- [23] T. Tang, X. Liu, R. Kiya, et al. (2021), "Microscopic impedance cytometry for quantifying single cell shape", *Biosens. Bioelectron.*, **193**, DOI: 10.1016/j.bios.2021.113521.
- [24] P.N. Van, A.B. Hoang, H.T. Thanh, et al. (2023), "Numerical calculation and analysis of a novel complex impedance sensing approach for in-flow droplet detection utilizing the C⁴D technique", *Model. Simul. Mater. Sci. Eng.*, **31**(7), DOI: 10.1088/1361-651X/acf513.
- [25] H. Song, Y. Wang, J.M. Rosano, et al. (2013), "A microfluidic impedance flow cytometer for identification of differentiation state of stem cells", *Lab Chip*, **13**(12), DOI: 10.1039/c3lc41321g.
- [26] M. Kumar, S. Yadav, A. Kumar, et al. (2019), "MEMS impedance flow cytometry designs for effective manipulation of micro entities in health care applications", *Biosens. Bioelectron.*, **142**, DOI: 10.1016/j.bios.2019.111526.
- [27] T. Sun, H. Morgan (2010), "Single-cell microfluidic impedance cytometry: A review", *Microfluid. Nanofluidics*, **8**(4), pp.423-443, DOI: 10.1007/s10404-010-0580-9.
- [28] K. Wang, Y. Zhao, D. Chen, et al. (2017), "The instrumentation of a microfluidic analyzer enabling the characterization of the specific membrane capacitance, cytoplasm conductivity, and instantaneous Young's modulus of single cells", *Int. J. Mol. Sci.*, **18**(6), DOI: 10.3390/ijms18061158.
- [29] M.E.G.D. Araújo, L.A. Huber, T. Stasyk (2008), "Isolation of endocytic organelles by density gradient centrifugation", *Methods Mol. Biol.*, **424**, pp.317-331, DOI: 10.1007/978-1-60327-064-9_25.
- [30] U. Lei, P.H. Sun, R. Pethig (2011), "Refinement of the theory for extracting cell dielectric properties from dielectrophoresis and electrorotation experiments", *Biomechanics*, **5**(4), DOI: 10.1063/1.3659282.
- [31] A.D. Biasio, C. Cametti (2011), "On the dielectric relaxation of biological cell suspensions: The effect of the membrane electrical conductivity", *Colloids Surfaces B Biointerfaces*, **84**(2), pp.433-441, DOI: 10.1016/j.colsurfb.2011.01.038.
- [32] K. Asami, Y. Takahashi, S. Takashima (1990), "Frequency domain analysis of membrane capacitance of cultured cells (HeLa and myeloma) using the micropipette technique", *Biophys. J.*, **58**(1), pp.143-148, DOI: 10.1016/S0006-3495(90)82360-2.
- [33] D. Spencer, V. Hollis, H. Morgan (2014), "Microfluidic impedance cytometry of tumour cells in blood", *Biomechanics*, **8**(6), DOI: 10.1063/1.4904405.
- [34] K.R. Foster, H.P. Schwan (1989), "Dielectric properties of tissues and biological materials: A critical review", *Crit. Rev. Biomed. Eng.*, **17**(1), pp.25-104.
- [35] Y. Zheng (2014), *Microfluidic Systems for High-Throughput Biophysical Characterization of Single Cells*, University of Toronto, 161pp.
- [36] L.D. Quang, T.T. Bui, A.B. Hoang, et al. (2019), "Development of a passive capacitively coupled contactless conductivity detection (PC4D) sensor system for fluidic channel analysis toward point-of-care applications", *IEEE Sens. J.*, **19**(15), pp.6371-6380, DOI: 10.1109/JSEN.2019.2908179.
- [37] G. Mernier, E. Duqi, P. Renaud (2012), "Characterisation of a novel impedance cytometer design and its integration with lateral focusing by dielectrophoresis", *Lab Chip*, **12**(21), DOI: 10.1039/c2lc40551b.
- [38] F. Caselli, A.D. Ninno, R. Reale, et al. (2018), "A novel wiring scheme for standard chips enabling high-accuracy impedance cytometry", *Sensors Actuators B Chem.*, **256**, pp.580-589, DOI: 10.1016/j.snb.2017.10.113.
- [39] J.W. Wang, M.H. Wang, L.S. Jang (2010), "Effects of electrode geometry and cell location on single-cell impedance measurement", *Biosens. Bioelectron.*, **25**(6), pp.1271-1276, DOI: 10.1016/j.bios.2009.10.015.

Study on chemical composition and biological activities of essential oil of *Limnophila aromatica* (Lamk.) Merr.

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Abstract:

Limnophila aromatica (Lamk.) Merr. is a widely used spice and medicinal herb in many countries and Vietnam is one of them. However, there are relatively few studies on this plant, especially within Vietnam. This research focuses on the chemical composition analysed via Gas Chromatography-Mass Spectrometry (GC-MS), antioxidant activity (measured by the DPPH method), and antimicrobial properties (determined using the paper plate method) of the essential oil derived from *Limnophila aromatica* (Lamk.) Merr. The results show that the primary component of the essential oil is limonene (46.86%), followed by β -cis-ocimene (24.1%), α -pinene (5.69%), and 1,2-epoxy-5,9-cyclododecadiene (4.57%). The antioxidant activity was quantified with an IC₅₀ value of 266.6 $\pm$ 0.56 μ g/ml. The antimicrobial assays demonstrated that the essential oil was effective against six of the eight tested microorganisms, including *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Listeria innocua* ATCC 33090, *Bacillus cereus* ATCC 14579, and *Aspergillus niger* ATCC 6275. These findings suggest that *Limnophila aromatica* has potential applications in functional foods, cosmetics, and antibiotics.

Keywords: essential oil, hydrodistillation, *Limnophila aromatica*, steam distillation.

Classification numbers: 2.2, 3.4

1. Introduction

Limnophila aromatica (Lamk.) Merr., commonly known as rice paddy herb, is a tropical plant from the *Scrophulariaceae* family, belonging to the genus *Limnophila*, and can be taxonomically classified as follows [1]:

Kingdom	Plantae
Sub-kingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Sub-class	Asteridae
Order	Scrophulariales
Family	Scrophulariaceae
Genus	<i>Limnophila</i>
Species	<i>L. aromatica</i>

Binomial name *Limnophila aromatica* (Lamk.) Merr. [1]

This plant originates from Southeast Asia and was introduced to North America in the 1970s [1]. It thrives in aquatic or semi-aquatic environments such as rivers, lakes, ponds, and marshy areas [2, 3]. The plant can be harvested approximately 40 days after planting [3]. *Limnophila*

aromatica (Lamk.) Merr. is a staple vegetable in Laotian, Thai, Cambodian, and Vietnamese cuisines, valued for the fragrance it adds to sour soups, noodle soups, and sauces [1-3].

In traditional medicine, *Limnophila aromatica* (Lamk.) Merr. is used for its antispasmodic, antiseptic, anthelmintic, anti-inflammatory, and diuretic properties. Its leaves are also used in treatments for fever and kidney stones [1-3]. The plant's beneficial effects are thought to be due to its essential oil (EO), flavonoids, and phenolic compounds [3]. There have been several studies on the EO of *Limnophila aromatica* cultivated in Thailand, Malaysia, and Bangladesh [2-5]. However, limited research has been conducted on the EO of *Limnophila aromatica* (Lamk.) Merr. in Vietnam.

This study aims to investigate the chemical composition, antioxidant activity, and antimicrobial properties of the EO of *Limnophila aromatica* (Lamk.) Merr. in Vietnam, contributing to the scientific understanding of the plant's potential applications in the economic, pharmaceutical, and culinary fields.

2. Materials and methods

2.1. Distillation of essential oil

The *Limnophila aromatica* (Lamk.) Merr. plant was harvested in the urban area of Can Tho city, Vietnam. After

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harvesting, the plant (without roots) was washed, drained, and stored at 5°C. The fresh samples (approximately 90% moisture) underwent EO extraction via hydrodistillation using a Clevenger apparatus. Several parameters influencing EO yield were investigated, including material size (5 and 0.5 mm), the ratio of material to water (1:2 to 1:4, g/ml), and distillation time (1.5-3.5 hours). After the distillation process, the essential oil was dehydrated using anhydrous sodium sulphate and subsequently analysed for physicochemical properties, chemical composition, and biological activities.

The EO yield was calculated as shown below:

$$\text{Essential oil yield (\%)} = \frac{\text{weight of essential oil (g)}}{\text{weight of material (g)}} \times 1000 \quad (1)$$

2.2. Physicochemical index analysis

Physicochemical indices were analysed according to the International Organization for Standardization (ISO) methods: ISO 279:1998: density at 26°C of EO [6], ISO 1242:1999: acid index analysis of EO [7].

2.3. Analysis of essential oil

The chemical composition of *Limnophila aromatica* (Lamk.) Merr. EO was determined using GC-MS with a Thermo Fisher Scientific system equipped with a TG SQC column (15 m x 0.25 mm x 0.25 µm). The following conditions were applied: injection volume (1 µl), carrier gas (helium at 1 ml/min), oven temperature programme (50 to 80°C at 5°C/min, 80 to 200°C at 10°C/min, and 200 to 250°C at 10°C/min), ion source temperature (200°C), and transfer line temperature (275°C). The mass spectrometer was operated in EI mode at 70 eV with a mass scan range of 35-450 amu. Compounds were identified using the National Institute of Standards and Technology (NIST MS) spectral library [8, 9].

2.4. Biological activities

2.4.1. Antioxidant activity: The antioxidant activity was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. A solution of 180 µl DPPH (78 µg/ml in methanol) was mixed with 20 µl of essential oil (1% v/v in ethanol). The reaction mixture was left at room temperature for 30 minutes, after which the absorbance was measured at 515 nm to determine the percentage of inhibition (Q%) [10].

$$Q (\%) = \frac{A_0 - A_c}{A_0} \times 100 \quad (2)$$

where A_0 is the absorbance of DPPH (control) and A_c is the absorbance of the sample.

2.4.2. Antimicrobial activity: The antimicrobial activity was assessed using the paper plate method against 7 microorganisms: *Bacillus subtilis* ATCC 6633,

Staphylococcus aureus ATCC 25923TM, *Escherichia coli* ATCC 25922TM, *Bacillus cereus* ATCC 10876TM, *Listeria innocua* ATCC 33090TM, *Enterococcus faecalis* ATCC 29212TM, and *Aspergillus niger* ATCC 6275.

A clean tube containing 4 ml of Luria-Bertani (LB) liquid medium was inoculated with the tested microorganisms and shaken at 200 rpm overnight. Subsequently, 200 µl of the inoculum (concentration of approximately $4\text{-}5 \times 10^8$ CFU/ml) was spread onto the surface of petri dishes containing LB solid medium. A sterile paper plate impregnated with 10 µl of diluted EO (dimethyl sulfoxide, concentration of original EO- 10^{-2}) was then placed on the petri dish, and the plate was incubated at 37°C for 18-20 hours. After incubation, the diameter of the inhibition zone was measured. A sterile ring with a diameter (D) > 6 mm indicated antimicrobial activity; D = 6 mm indicated no inhibition [11].

2.5. Statistical analysis

Data were expressed as the average of duplicate experiments. A two-sample T-test assuming equal variance was performed using Excel software (2019) to evaluate significant and insignificant differences in the investigated parameters.

3. Results and discussion

3.1. Investigation of influencing parameters affecting essential oil yield

3.1.1. Effects of material size on essential oil yield: A 2000 ml flask containing 200 g of *Limnophila aromatica* leaves (either 5 mm or 0.5 mm in size) and 400 ml of deionised water (DIW) was used for distillation. The yield of EO for the two material sizes is shown in Fig. 1. The highest yield was obtained with the larger material size (5 mm), as smaller materials (0.5 mm) tended to float on the surface of the distillation flask, limiting the evaporation of water. This resulted in a lower EO yield compared to the larger material size. Hence, a material size of 5 mm was chosen for subsequent experiments.

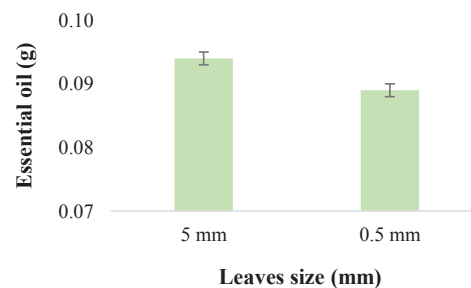


Fig. 1. Effect of material sizes on essential oil yield. Conditions: 200 g of sample, 400 ml deionised water and 2 hours of distillation.

3.1.2. Effect of time on EO yield: A 2000 ml flask containing 200 g of *Limnophila aromatica* leaves (5 mm) and 400 ml of DIW was used to study the effect of distillation time on EO yield. Distillation times of 1.5, 2.0, 2.5, 3.0, and 3.5 hours were investigated, as shown in Fig. 2.

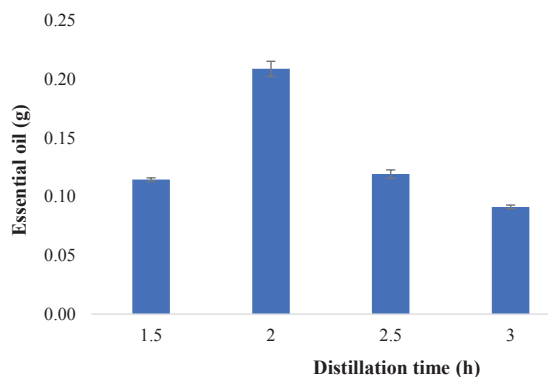


Fig. 2. Effect of distillation time on essential oil yield. Conditions: 200 g of sample, 400 ml deionised water, and material size of 5 mm.

The EO yield as seen in Fig. 2 increased from 0.11 to 0.21 g as the distillation time increased from 1.5 to 2 hours. However, EO yield decreased after 2 hours, similar to the observations of V.D. Zheljaskov, et al. (2013) [12], who reported that EO yields for lavender peaked at 60 minutes and declined thereafter. A shorter distillation time (<2 hours) does not fully extract the EO, whereas a distillation time of 2 hours allows the essential oil sacs to rupture and release the maximum amount of oil. Prolonged distillation times, however, lead to a decrease in EO yield due to the dissolution of polar components in water. Thus, a distillation time of 2 hours was considered optimal.

3.1.3. Effect of water volume on essential oil yield: A total of 200 g of *Limnophila aromatica* leaves was placed in a 2000 ml flask and distilled with leaf-to-water ratios of 1:1, 1:2, 1:3, and 1:4 (g/ml) for 2 hours. The effect of the water amount on essential oil yield is illustrated in Fig. 3.

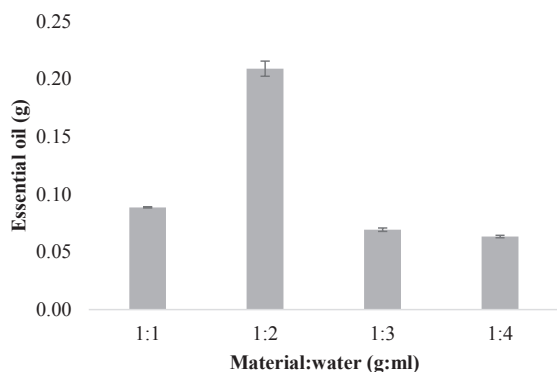


Fig. 3. The effect of water volume on essential oil yield. Conditions: material size of 5 mm and 2 hours of distillation time.

As shown in Fig. 3, a low water amount (1:1) is insufficient to allow water to penetrate the material, resulting in a lower EO yield. Conversely, an excessive amount of water (e.g., 1:4) reduces the amount of EO obtained, as the distillation process requires more time with additional water. Therefore, a leaf-to-water ratio of 1:2 was selected as optimal for the experiments.

In summary, the optimal parameters for the distillation process of *Limnophila aromatica* EO were found to be: material size (5 mm), distillation time (2 hours), and a material-to-water ratio of 1:2 (g/ml). Under these conditions, the highest oil yield was 0.21 g per 200 g of material, corresponding to a distillation yield of 1.05% (w/w).

3.2. Physicochemical index analysis

The obtained essential oil from *Limnophila aromatica* had a characteristic fragrance, a light yellow colour, and was transparent.

Table 1. Physicochemical indices.

Index	This study	References
Density (26°C) (g/cm ³)	0.88±0.04	0.87 [13]
Acid	2.79±0.17	5.61 [14]

As shown in Table 1, the density of the obtained essential oil is less than 1, which is consistent with the reference [13]. However, there is a noticeable difference in the acid value compared with the reference [14], which could be attributed to geographical variation. A lower acid value indicates a high-quality essential oil with less degradation or rancidity [15].

3.3. Chemical composition

The essential oil of *Limnophila aromatica* contains 18 components, with limonene (46.86%) being the most abundant. Limonene is widely used in the cosmetics, food, and industrial cleaning industries [16]. According to J.U. Chowdhury, et al. (2011) [5], limonene is also combustible and has potential applications as a biofuel. Interestingly, the limonene content in *Limnophila aromatica* cultivated in Bangladesh was reported to be significantly lower (3.75%), while Z-ocimene content was higher (39.21%) [5]. These variations suggest that the chemical composition of the EO is influenced by geographical origin [17, 18].

The second most prevalent compound is β -cis-ocimene (24.1%), one of the most common monoterpenes found in nature. In medicinal applications, β -cis-ocimene exhibits anticonvulsant, antifungal, antitumour, and pest-resistant properties [19]. Other notable constituents of *Limnophila*

Table 2. Chemical composition of the essential oil.

No	Compounds	(%)
1	<i>α</i> -Pinene	5.69
2	<i>β</i> -Myrcene	2.39
3	Limonene	46.86
4	<i>β</i> -cis-Ocimene	24.10
5	Carane, 4,5-epoxy-, trans	2.16
6	1,2-Epoxy-5,9-cyclododecadiene	4.57
7	Perilla aldehyde	0.81
8	<i>β</i> -Elemen	0.51
9	Cis-Myrtanyl acetate	1.05
10	Germacrene	0.86
11	<i>α</i> -Caryophyllene	1.57
12	Germacrene D	2.60
13	<i>α</i> -Amorphene	0.44
14	<i>γ</i> -Muurolene	0.63
15	<i>δ</i> -Cadinene	1.17
16	Ledol	0.44
17	<i>δ</i> -Cadinol	2.02
18	<i>α</i> -Cadinol	2.13

aromatica EO include *α*-pinene (5.69%), *β*-myrcene (2.39%), and germacrene D (2.6%) (Table 2).

3.4. Biological activities

3.4.1. Antioxidant activity: The antioxidant activity of the EO is shown in Fig. 4. The free radical scavenging efficiency (Q%) of the oil increases in direct proportion to its concentration. As the concentration of the EO increases from 50 to 300 µg/ml, the scavenging efficiency increases from 26.64±1.00% to 54.65±0.08%. The IC₅₀ value of the EO was determined to be 266.6±0.56 µg/ml, as shown in Fig. 4.

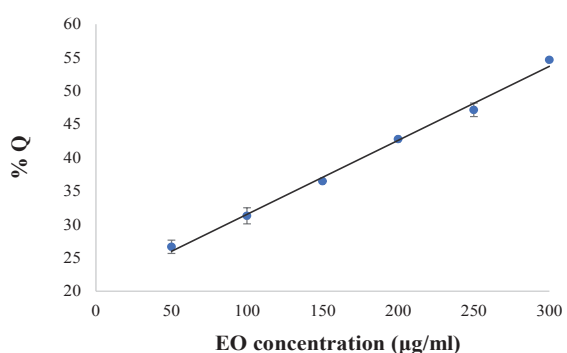


Fig. 4. Antioxidant capacity of *Limnophila aromatica* essential oil.

In comparison, the IC₅₀ value of ascorbic acid (used as a positive control) was found to be 9.54±1.22 µg/ml, based on concentration ranges from 5.0 to 25 µg/ml. Thus, the antioxidant activity of the EO is approximately 27.9 times weaker than that of ascorbic acid. No published data on the IC₅₀ of *Limnophila aromatica* EO could be found for comparison; however, relative to other plants [20, 21], the IC₅₀ value of *Limnophila aromatica* EO (266.6±0.56 µg/ml) is lower than that of *Ammodaucus leucotrichus* (IC₅₀=3326.7 µg/ml) [20], *Thymus vulgaris* (IC₅₀=861.5 µg/ml), *Cymbopogon citratus* (IC₅₀=601.60 µg/ml), *Origanum marjorana* (IC₅₀=524.00 µg/ml), and *Rosmarinus officinalis* (IC₅₀=444.30 µg/ml), but higher than that of *Zingiber officinalis* (IC₅₀=129.4 µg/ml), *Salvia officinalis* (IC₅₀=59.07 µg/ml), and *Petroselinum crispum* (IC₅₀=15.51 µg/ml) [21]. This indicates that the antioxidant activity of *Limnophila aromatica* EO is weaker than that of ascorbic acid, *Zingiber officinalis*, *Salvia officinalis*, and *Petroselinum crispum*, but stronger than that of *Ammodaucus leucotrichus*, *Thymus vulgaris*, *Cymbopogon citratus*, *Origanum marjorana*, and *Rosmarinus officinalis*.

3.4.2. Antimicrobial activity: The antimicrobial activity of the EO was tested against eight microorganisms, as reported in Table 3.

Table 3. Antimicrobial activity of *Limnophila aromatica* essential oil based on sterile ring diameter (D).

Strains	Dilution concentration (C): µl/sterile ring diameter (D): mm			
	C ₀ (Original EO)	C ₁ =5 ⁻¹	C ₂ =10 ⁻¹	C ₃ =10 ⁻²
<i>Escherichia coli</i> ATCC 25922	D=13±0.20	D=10±0.04	D=6±0.00	D=6±0.00
<i>Pseudomonas aeruginosa</i> ATCC 27853	D=6±0.00	D=6±0.00	D=6±0.00	D=6±0.00
<i>Enterococcus faecalis</i> ATCC 29212 TM	D=6±0.00	D=6±0.00	D=6±0.00	D=6±0.00
<i>Staphylococcus aureus</i> ATCC 25923	D=12±0.08	D=6±0.00	D=6±0.00	D=6±0.00
<i>Bacillus subtilis</i> ATCC 6633	D=16±0.10	D=10±0.05	D=6±0.00	D=6±0.00
<i>Listeria innocua</i> ATCC 33090	D=14±0.09	D=8±0.09	D=7±0.10	D=6±0.00
<i>Bacillus cereus</i> ATCC 14579	D=10±0.10	D=6±0.00	D=6±0.00	D=6±0.00
<i>Aspergillus niger</i> ATCC 6275	D=25±1.20	D=6±0.00	D=6±0.00	D=6±0.00

D>6 mm: antimicrobial activity; D=6 mm: no-inhibition.

Table 3 illustrates that the pure EO (C₀) exhibited antimicrobial activity against six bacterial strains, including *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Listeria innocua* ATCC 33090, *Bacillus cereus* ATCC 14579, and the fungus *Aspergillus niger* ATCC 6275. This activity may be attributed to the bactericidal properties of limonene, which is known to significantly inhibit both Gram-negative and Gram-positive bacteria as well as fungi [22].

Research by A. Gupta, et al. (2021) [23] demonstrated that limonene effectively disrupts cell membranes, causing leakage and cell death in *Escherichia coli*. Similarly, Y. Han, et al. (2021) [24] observed that limonene inhibited bacterial growth by damaging the cell morphology and wall integrity of *Staphylococcus aureus*, as confirmed through scanning electron microscopy (SEM) and fluorescence microscopy. As shown in Table 2, limonene constitutes 46.86% of the EO composition, likely contributing to its antimicrobial properties. Additionally, the presence of other compounds, such as β -cis-Ocimene, α -Pinene, and others, which are known for their antibacterial effects, may further enhance the oil's activity [25, 26].

At a 5^{-1} dilution (C_1), the EO remained effective against three bacterial strains: *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, and *Listeria innocua* ATCC 33090. However, the fungus *Aspergillus niger* ATCC 6275 did not show any inhibition at this concentration. When the EO was diluted 10 times (C_2), inhibition was only observed for *Listeria innocua* ATCC 33090. No antimicrobial activity was detected when the oil was diluted 100 times (C_3).

Comparatively, the EO of *Mentha arvensis* L. var. *javanica* (Blume) Hook. f. [27] exhibited antimicrobial activity against *Listeria innocua* ATCC 33090 ($D=9.0\pm0.2$ mm), *Bacillus cereus* ATCC 10876 ($D=8.0\pm0.03$ mm), and *Bacillus subtilis* ATCC 6633 ($D=7.0\pm0.05$ mm) at a 10^{-1} dilution. These results demonstrate the strong antimicrobial potential of herbs like *Limnophila aromatica* EO on the tested bacterial strains.

4. Conclusions

The optimal parameters for the distillation of *Limnophila aromatica* EO were identified as follows: material size (5 mm), distillation time (2 hours), and a material-to-water ratio of 1:2 (g/ml). Under these conditions, the highest oil yield was 0.21 g per 200 g of material, corresponding to a distillation yield of 1.05% (w/w). Limonene, the primary component of the EO (46.86%), played a significant role in its biological activities. The EO exhibited antioxidant capacity with an IC_{50} value of 266.6 μ g/ml and demonstrated antimicrobial activity against six bacterial strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Listeria innocua* ATCC 33090, *Bacillus cereus* ATCC 14579, and *Aspergillus niger* ATCC 6275. These results highlight the potential applications of *Limnophila aromatica* in pharmaceutical and cosmetic industries.

CRediT author statement

Nguyen Thi Bich Thuyen: Methodology, Data analysis, Writing - Reviewing and Editing; Tran Thanh Men, Ho Quoc Phong, Huynh Lien Huong: Methodology, Data analysis.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] D. Gorai, S.K. Jash, R.K. Singh, et al. (2014), "Chemical and pharmacological aspects of *Limnophila aromatica* (Scrophulariaceae): An overview", *American Journal of Phytomedicine and Clinical Therapeutic*, **2**(3), pp.348-356.
- [2] C.S. Vairappan, T. Nagappan (2014), "Major volatile hydrocarbons of rice paddy herb, *Limnophila aromatica* Lam. Merr as possible chemotaxonomic marker", *Journal of Tropical Biology and Conservation*, **11**, pp.41-48, DOI: 10.51200/jtbc.v11i.261.
- [3] B. Yingngama, A. Brantnerb, M. Treichlera, et al. (2021), "Optimization of the eco-friendly solvent-free microwave extraction of *Limnophila aromatica* essential oil", *Industrial Crops & Products*, **165**, DOI: 10.1016/j.indcrop.2021.113443.
- [4] P. Thanatuskitti, V. Siripornpanich, W. Sayorwan, et al. (2020), "The effects of inhaled *Limnophila aromatica* essential oil on brain wave activities and emotional states in healthy volunteers: A randomized crossover study", *Research Journal of Pharmacognosy*, **7**(4), pp.1-9, DOI: 10.22127/rjp.2020.230400.1586.
- [5] J.U. Chowdhurya, M.N.I. Bhuiyanb, J. Begum (2011), "Constituents of volatile oils from *limnophila aromatica* and *adenosma capitatum*", *Bangladesh Journal of Scientific and Industrial Research*, **46**(3), pp.385-388, DOI: 10.3329/bjsir.v46i3.9048.
- [6] International Organization for Standardization (1998), *ISO 279:1998: Essential Oils - Determination of Relative Density at 20°C - Reference Method*, <https://www.iso.org/standard/25308.html>, accessed 10 August 2023.
- [7] International Organization for Standardization (1999), *ISO 1242:1999: Essential Oils - Determination of Acid Value*, <https://www.iso.org/standard/25308.html>, accessed 10 August 2023.
- [8] I. Zribi, J. Bleton, F. Moussa, et al. (2019), "GC-MS analysis of the volatile profile and the essential oil compositions of Tunisian *Borago Officinalis* L.: Regional locality and organ dependency", *Industrial Crops and Products*, **129**, pp.290-298, DOI: 10.1016/j.indcrop.2018.12.021.
- [9] M. Hassanabadia, M. Ebrahimia, M. Farajpour, et al. (2019), "Variation in essential oil components among Iranian *Ferula assa-foetida* L. accessions", *Industrial Crops and Products*, **140**, DOI: 10.1016/j.indcrop.2019.111598.

- [10] T. Baj, A. Baryluk, E. Sieniawska (2018), "Application of mixture design for optimum antioxidant activity of mixtures of essential oils from *Ocimum basilicum* L., *Origanum majorana* L. and *Rosmarinus officinalis* L.", *Industrial Crops and Products*, **115**, pp.52-61, DOI: 10.1016/j.indcrop.2018.02.006.
- [11] A.A. Dobre, V. Gagui, N. Pertu (2011), "Antimicrobial activity of essential oils against food-borne bacteria evaluated by two preliminary methods", *Romanian Biotechnological Letters*, **16(6)**, pp.119-125.
- [12] V.D. Zheljazkov, C.L. Cantrell, T. Astatkie, et al. (2013), "Distillation time effect on Lavender essential oil yield and composition", *Journal of Oleo Science*, **62(4)**, pp.195-199, DOI: 10.5650/jos.62.195.
- [13] N. Brugger (2021), *Plant-part Anatomy Related Composition of The Volatile Oils in Limnophila aromatica (Lam.) Merr. (Plantaginaceae), An Important Herb used for Anti-flatulence*, Master Thesis University of Graz, Austria.
- [14] D.Q. Diem, N.T.T. Suong (2020), "Effect of water content and extraction time on essential oil recovery efficiency from *Limnophila aromatica*", *Journal of Science and Technology*, **44(2)**, pp.15-22, DOI: 10.46242/jst-juh.v44i02.566 (in Vietnamese).
- [15] Y. Uddin, N.M. Khan, F. Ali, et al. (2021), "Estimation of various physicochemical properties of walnut oil from different areas of Northern Kpk, Pakistan", *J. Mex. Chem. Soc.*, **65(4)**, pp.572-581, DOI: 10.29356/jmcs.v65i4.1512.
- [16] J.I. García, J.A. Dobado, F.G.C. Flores, et al. (2015), "Chapter 7: Basic operation experiments", *Experimental Organic Chemistry: Laboratory Manual*, Amsterdam: Academic Press, pp.207-239.
- [17] E. Derwich, Z. Benziane, A. Boukir, et al. (2009), "GC-MS analysis of the leaf essential oil of *Mentha rotundifolia*, a traditional herbal medicine in Morocco", *Chem. Bull. "Politehnica" University of Timisoara, Romania*, **54(68)**, pp.85-88.
- [18] A. Şanlı, T. Karadoğan (2017), "Geographical impact on essential oil composition of endemic *Kundmannia anatolica* Hub.-Mor. (apiaceae)", *Afr. J. Tradit. Complement Altern. Med.*, **14(1)**, pp.131-137, DOI: 10.21010/ajtcam.v14i1.14.
- [19] E.B. Russo, J. Marcu (2017), "Chapter three - Cannabis pharmacology: The usual suspects and a few promising leads", *Advances in Pharmacology*, **80**, pp.67-134, DOI: 10.1016/bs.apha.2017.03.004.
- [20] M. Manssouri, M. Znini, L. Majidi (2020), "Studies on the antioxidant activity of essential oil and various extracts of *Ammodaucus leucotrichus* Coss. & Dur. fruits from Morocco", *Journal of Taibah University for Science*, **14(1)**, pp.124-130, DOI: 10.1080/16583655.2019.1710394.
- [21] T.M. Mapeka, M. Sandasi, A.M. Viljoen, et al. (2022), "Optimization of antioxidant synergy in a polyherbal combination by experimental design", *Molecules*, **27(13)**, DOI: 10.3390/molecules27134196.
- [22] Y. Han, Z. Sun, W. Chen (2020), "Antimicrobial susceptibility and antibacterial mechanism of Limonene against *Listeria monocytogenes*", *Molecules*, **25(1)**, DOI: 10.3390/molecules25010033.
- [23] A. Gupta, E. Jeyakumar, R. Lawrence (2021), "Strategic approach of multifaceted antibacterial mechanism of limonene traced in *Escherichia coli*", *Scientific Reports*, **11(1)**, DOI: 10.1038/s41598-021-92843-3.
- [24] Y. Han, W. Chen, Z. Sun (2021), "Antimicrobial activity and mechanism of limonene against *Staphylococcus aureus*", *Journal of Food Safety*, **41(5)**, DOI: 10.1111/jfs.12918.
- [25] A.G. Pirbalouti, A. Izadi, F.M. Poor, et al. (2016), "Chemical composition, antioxidant and antibacterial activities of essential oils from *Ferulago angulata*", *Pharmaceutical Biology*, **54(11)**, pp.2515-2520, DOI: 10.3109/13880209.2016.1162816.
- [26] L.P.A. Reddy, K.V. Ratnam, B.M. Lepakshi, et al. (2015), "Investigation of chemical and pharmacological properties of essential oils from two *Syzygium* species of Andhra Pradesh, India", *Int. J. Pharm. and Pharm. Sci.*, **7(9)**, pp.375-380.
- [27] N.T.B. Thuyen, H.Q. Phong, C.L.N. Hanh, et al. (2023), "Study on morphology, distillation process, chemical compositions, and biological activities of *Mentha arvensis* L. var. *javanica* (Blume) Hook. f essential oil", *Journal of The Taiwan Institute of Chemical Engineers*, **153**, DOI: 10.1016/j.jtice.2023.105209.

Farming of genetically improved strains of rainbow trout in Ganderbal district - Biological and economic perspective

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Abstract:

Rainbow trout (*Oncorhynchus mykiss*) is a species of freshwater fish belonging to the salmonid family. They are native to North America, particularly the Pacific Coast, and have been introduced to various parts of the world due to their popularity in recreational fishing and aquaculture. A comparative study on the biological and economical improvement of trout farmers in district Ganderbal was carried out by batching the farmers for raising the normal strain (NS) and the genetically improved strain (GIS) of rainbow trout for a period of 240 days (one cycle). The objective of the research was to ascertain the difference between NS and GIS, analyse biological parameters. The GIS farming reported the highest net weight gain (347.7 ± 5.55), average daily weight gain (1.44 ± 0.02), specific growth rate (1.75 ± 0.001), and the lowest conversion ratio (1.22 ± 0.007). The farming of GIS recorded a survival rate of 95.12 ± 0.03 , compared to the NS (89.0 ± 0.38). The benefit-cost ratio was highest in GIS (2.26 ± 0.02), compared to the NS (2.15 ± 0.007), which justifies that GIS is a better performer than NS.

Keywords: benefit-cost ratio, economics, genetically improved strain, growth, rainbow trout.

Classification numbers: 3.1, 3.4

1. Introduction

Rainbow trout (*Oncorhynchus mykiss*) is a species of freshwater fish belonging to the salmonid family. They are native to North America, particularly the Pacific Coast, and have been introduced to various parts of the world due to their popularity in recreational fishing and aquaculture. These fish have a streamlined torpedo-shaped body, adorned with a distinctive pinkish-red stripe along their sides, reminiscent of a rainbow. Depending on their habitat and food availability, rainbow trout can vary in size, typically ranging from 16 to 24 inches in length and weighing between 0.9 to 3.6 kg. Rainbow trout are opportunistic feeders and are known for their diverse diet. They primarily consume aquatic insects, crustaceans, small fish, and fish eggs. In some habitats, they can also feed on terrestrial insects that fall into the water. Globally, rainbow trout are one of the most commonly farmed fish species, prized for their culinary appeal. They thrive in freshwater hatcheries and can be raised in ponds or raceways until they attain market size.

Rainbow trout was introduced to Kashmir waters approximately 100 years ago, but it was not until 2014 that trout cultivation was extended to the private sector. Trout is

a prized fish, which was bred and reared by the Department of Fisheries only, but with the aim to enhance production and create employment opportunities for unemployed youth, trout culture was emphasised in the private sector with a subsidy component under various centrally sponsored schemes from time to time. The Department of Fisheries, Government of Jammu and Kashmir, is a well-established department undertaking the culture and breeding of exotic cold-water fishes (brown and rainbow trout), and conservation of indigenous cold water fish species, including a broad variety of *Schizothoracines*. With the privatisation of trout, many entrepreneurs have come forward, who have established their trout units with financial support under various centrally sponsored schemes. Although there has been a boom in overall production, certain constraints restrict the growers to meet the challenges.

One of the grave challenges was the poor performance of rainbow trout in the private as well as government sectors in terms of growth, disease resistance, food conversion efficiency, and poor breeding performance. The reason was the inbreeding depression, which was due to continuous crossbreeding of the same stock, leading to homozygosity, due to which the morphometric, meristic, physiological,

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and biological parameters were gravely distorted, resulting in a loss of interest by the farmers due to low economic returns. The Department of Fisheries in 2018-2019 planned to import an all-female stock of genetically improved eyed ova of rainbow trout fish for raising as pure-line brooders for crossbreeding with the existing stock to increase heterozygosity among the livestock. The reason for investigating the impact of GIS is to establish a need to revive the NS and improve the heterozygosity by crossbreeding between GIS and NS. With an attempt to investigate the impact of crossbreeding the normal and genetically improved stock, the current research was carried out to assess the biological improvement in livestock, leading to economic improvement in district Ganderbal.

2. Materials and methods

During the present research work, the Mammar hatchery in district Ganderbal was selected as the sole source of rainbow trout fingerlings for the fish farmers of the district. The Mammar hatchery has a spring source, and 1.5-2.0 lakh fingerlings of rainbow trout are produced every year and supplied to various private as well as government-run units. The all-female stock of genetically improved trout was raised in the Mammar trout hatchery to keep records of growth performance and mortality, and a pure line was maintained for two years. The genetically modified brooders (all female) were crossbred with the existing male stock, and the progeny was reared in separate raceways.

The existing normal stock was bred as usual in November 2021, along with the genetically improved stock, which matures prior to the normal stock. The F1 generation of the genetically improved stock was raised in separate raceways and monitored regularly on all biological aspects. Forty farmers (in replicas of two) were selected, and the water was analysed for physiochemical characteristics. Twenty farmers were stocked with 2000 fingerlings (5 g each) of NS, and twenty farmers were stocked with genetically improved strains. The stocking was done in April 2022. The culture custom size ranged between 250-350 g. The feed was adjusted as a 2% ratio of the total biomass calculated on a fortnightly basis. Regular monitoring for disease occurrence, comorbidities, and physiochemical water parameters was evaluated regularly.

Water temperature was monitored daily at the inlet and inside the raceway. Dissolved oxygen (DO) and pH were monitored on a weekly basis at 10:00 AM using a portable data logger pH/ORP, DO, CD/TDS Meter, Lutron, Model No. WA-2015, Bench Type, RS23/USB.

Fish feed and feeding: Extruded pelleted non-sinking feed from a fish feed mill in Manasbal containing 33-35% crude protein (CP) was fed to all the stock at all locations during this study. Feeding rate was a maximum of 2% of body weight, which is within the recommended range (2-5%) of feeding rate in cages as suggested by F. Wynne (2015) [1]. Fish were fed to satiation during lower water temperature (10-13°C) conditions.

Various growth parameters were used to assess the changes in biological phenomena of the normal and genetically improved strains of rainbow trout.

Net weight gain (NWG) was calculated by the following formula:

$$\text{NWG} = \text{Final weight (g)} - \text{Initial weight (g)}.$$

Average daily weight gain (ADWG) was calculated by using the formula:

$$\frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Days}}$$

Average daily length gain (ADLG) was calculated using the formula:

$$\frac{\text{Final length (cm)} - \text{Initial length (cm)}}{\text{Days}}$$

Specific growth rate (SGR) was calculated using the formula:

$$\frac{\ln \text{final weight (g)} - \ln \text{initial weight (g)}}{\text{Days}} \times 100$$

Food conversion ratio (FCR) was calculated using the formula of I. Okumus, et al. (1999) [2]:

$$\frac{\text{Total feed (g)}}{\text{Weight gain (g)}}$$

Food conversion efficiency (FCE) was calculated using the formula:

$$\frac{\text{Biomass (g)}}{\text{Total feed intake (g)}}$$

Condition factor: The coefficient of condition K was calculated by using the Fulton equation [3]: $K = W/L^3 \times 100$, where W = weight in grams, L = length in mm, and 100 is a factor to bring the value of K near unity. CF was calculated using the following formula:

$$\frac{\text{Final weight (g)}}{(\text{Final length})^3 \text{ cm}} \times 100$$

Survival (%): Survival of fish was considered as the remaining fish after deducting the number of died, missing, or escaped. Survival was calculated using the following formula:

$$\frac{\text{Total number of live animals present}}{\text{Total number of animals stocked}} \times 100$$

The benefit-cost ratio was calculated using the following formula:

$$\frac{\text{Total income}}{\text{Total cost}}$$

3. Results

The fingerlings (average weight = 5 g) from NS and GIS were transported with full quarantine to the selected raceways and stocked with full care after proper acclimatisation. The water quality parameters were almost the same at all the farm sites, the water source being the spring source, with a good gradient, the oxygen concentration fluctuated between 8-9 ppm. All the raceways (50 m³) had more or less the same flow rate. The sampling for enumeration of growth parameters was done on a weekly basis, and the ration was decided on a 2% basis of the total biomass.

The growth parameters measured during the present research work are summarised in Fig 1. Results revealed that the net weight gain was on the higher side in the GIS group with an average of 347.7±5.55, compared to the NS group which recorded a net weight gain of 232.8±4.28, with a variance of 4422.041 (Fig. 2). Similar patterns of changes were reported in average daily weight gain, with a value of 1.44±0.02 in the GIS group and a lower value of 0.96±0.01 in the NS group with a variance of 0.076 (Fig. 3). The fish length aspects showed a similar pattern of changes, with not much difference in the total length because of the increase in the overall girth of the fishes of the GIS group, which eventually led to an increase in weight.

The total length gain was comparatively higher (21.7±0.56) in the GIS group than NS (17.45±0.63) with a variance of 6.26. There was no significant difference in the length gain because of the muscle weight in the GIS group, which resulted in a higher body girth ratio compared to the NS group. Average daily length gain showed a similar pattern with higher values (0.08±0.002) in the GIS group than the NS group (0.07±0.02), with a variance of 0.0001. Specific growth rate is one of the fundamental growth parameters to assess the changes in a particular test set. During the present experimental period, specific growth rate values were higher (1.75±0.001) in the GIS group compared to the NS group (1.59±0.01) with a variance of 0.008 (Fig. 4). Specific growth rate indicated better growth performance of the genetically improved strains of rainbow trout in flow-through systems.

Length variation in NS and GIS groups

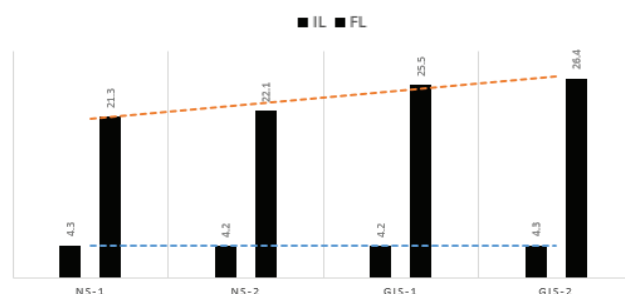


Fig. 1. Length variations in normal strain and genetically improved strains of Rainbow trout.

Weight variation in NS and GIS groups

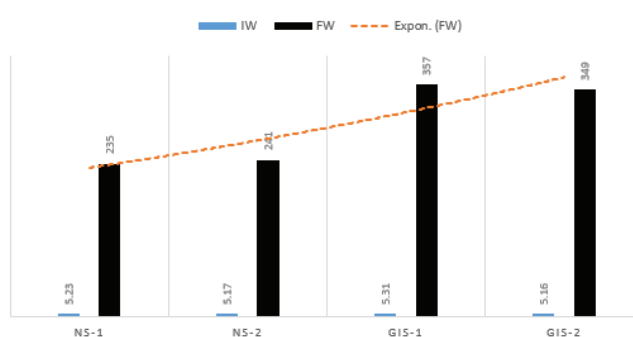


Fig 2. Weight variations in normal strain and genetically improved strains of Rainbow trout.

Average daily weight gain

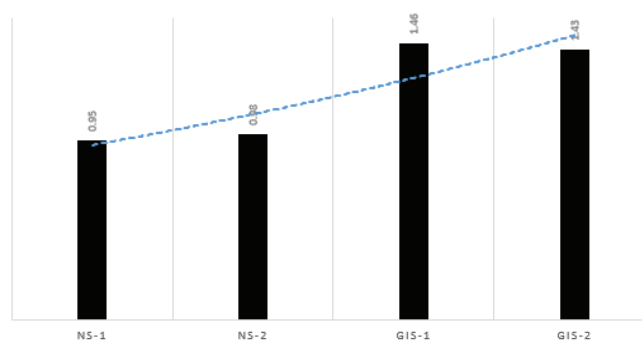


Fig 3. Average daily weight gain in normal strain and genetically improved strains of Rainbow trout.

Specific growth rate

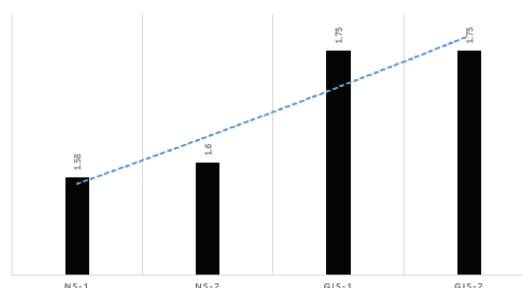


Fig 4. Specific growth rate in normal strain and genetically improved strains of Rainbow trout.

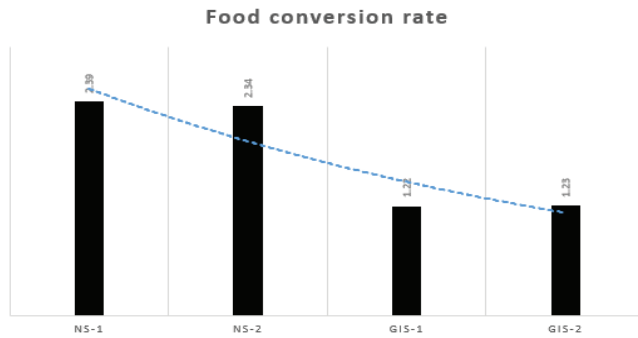


Fig 5. Food conversion rate in normal strain and genetically improved strains of Rainbow trout.

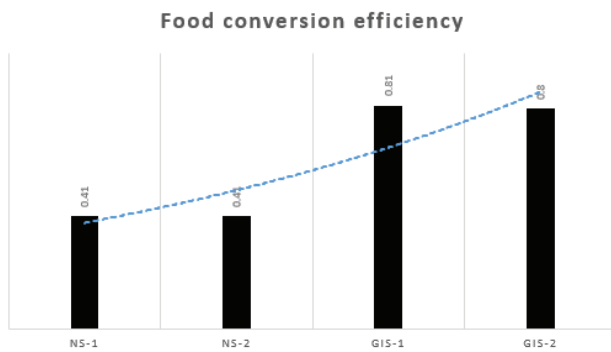


Fig 6. Food conversion efficiency in normal strain and genetically improved strains of Rainbow trout.

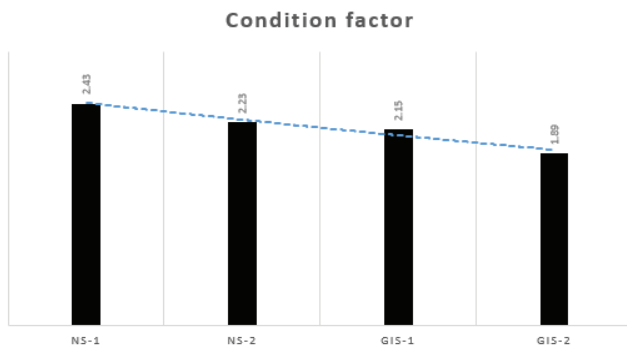


Fig 7. Condition factor in normal strain and genetically improved strains of Rainbow trout.

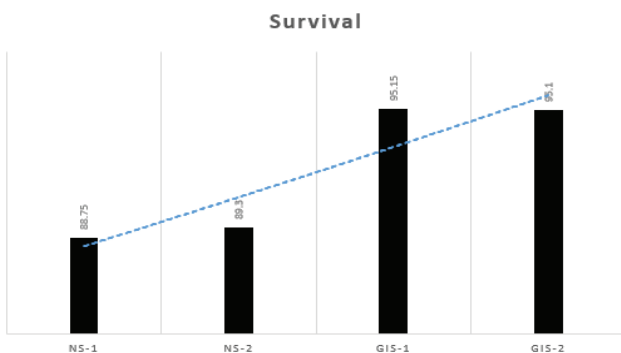


Fig 8. Survival in normal strain and genetically improved strains of Rainbow trout.

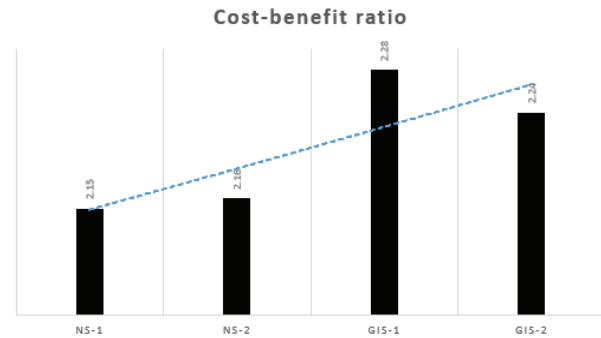


Fig 9. Cost-benefit ratio in normal strain and genetically improved strains of Rainbow trout.

Food conversion ratio is an important parameter that describes the efficiency of the fish to convert and assimilate the maximum of the feed contents for overall development. A lower conversion ratio is an indicator of the profitability of fish farming. During the present research period, the lowest conversion ratio (1.22 ± 0.007) was reported in the GIS group compared to the NS group (2.36 ± 0.03), with a variance of 0.43 (Fig. 5), depicting the efficiency of the GIS group. Food conversion efficiency is, in contrast, the efficiency of the fish to convert the assimilated food into the muscle mass, which was reported to be higher (0.80 ± 0.007) in the GIS group compared to the NS group (0.41 ± 0.0), with a variance of 0.05 (Fig. 6).

Condition factor is a numerical value given to a trout or salmon that reflects its condition. This value is arrived at using a mathematical formula that takes into account both the weight and length of the fish. A well-conditioned fish has a high condition factor, while one in poor condition has a low factor. A higher CF value was reported in the GIS group (2.33 ± 0.14) compared to the NS group (2.02 ± 0.18), with a variance of 0.049 (Fig. 7). No wider differences in the CF value were reported in the two groups because of the same diet plan, same feed ingredients, and the same water quality. The survival (%) was as high as 95 ± 0.03 in the GIS group compared to the NS group (89.0 ± 0.38), with a variance of 12.45 (Fig. 8), depicting the hardiness of the GIS group, which was the mandate of the Department while importing the germplasm.

The benefit-cost ratio is a ratio used in a cost-benefit analysis to summarise the overall relationship between the relative costs and benefits of a proposed project. Benefit-cost ratio can be expressed in monetary or qualitative terms. If a project has a benefit-cost ratio greater than 1.0, the project is expected to deliver a positive net present value to the enterprise. Although trout farming in Kashmir has been a profitable venture, the losses to the farmer occur due to high conversion ratio and low survival rates. In order to curb these shortfalls, the GIS trout was introduced, which during this experiment reported higher values of 2.26 ± 0.02 in the GIS group compared to the NS group (2.15 ± 0.007), with a variance of 0.003 (Fig. 9). The calculations led to the conclusion that GIS rearing farmers had a 27% profit margin as a bonus compared to the NS farmers who are making a good profit from the enterprise.

4. Discussion

The physicochemical parameters of water play a dynamic role in the metabolic performance of rainbow trout, which also depends on different life stages. Temperature plays a pivotal role in determining the metabolic performance of the fish [4, 5]. In some studies, where rainbow trout has been reported to show negative allometric growth, has been explained has been attributed to the different life stages [6, 7]. In Indian conditions, K.D. Joshi, et al. (2005) [8] reported average growth of 200 g in 12 months at the thermal regime of 4.5-20°C. In another research work in the central Himalayas, average growth was recorded as 300 g (range 260-400 g) at a thermal regime of 5.0-22°C [9]. Several authors have reported higher growth rates at an optimum higher temperature [10], but beyond the optimum temperature, oxygen depletion occurs, which tells upon the fish metabolism [11-13].

Although there has been no work on the comparison between growth performance of genetically improved strain and the NS in the Kashmir valley. It is the first time that the cross-breeding to improve heterozygosity has been done in the Department of Fisheries. The final weight gain and average daily weight gain reported during the present research work cannot be correlatively compared with other works because of the uniqueness of the differentiation between the normally bred Kashmiri trout and the cross-bred genetically improved strains. However, more or less similar somatic growth parameters have been reported by K.D. Hopkins (1992) [14]; M. Kayim, et al. (2007) [15] and I. Ahmad, et al. (2019) [7].

S.L. Reza, et al. (2014) [16], while working on a comparison of the daily diet by weight in rainbow trout (*Oncorhynchus mykiss*) with concentrate feed to achieve a minimum conversion ratio reported that the food conversion rate and the efficacy lie with the strains, which was further documented [17, 18]. In intensive rainbow trout culture systems, the optimum feed conversion ratio is between 1 and 2, for commercial dry feed. The values of feed conversion ratio for both groups in the present study were within the ranges reported for commercial feed by other investigators [19, 20]. It was observed that the feed conversion ratio increased with increasing fish weight, and it was the best at the small size group. As it has been observed in the small size group, rainbow trout are able to utilise their feed very efficiently [21, 22].

For the well-being of the trout, the condition factor is expected to be higher than 1.0 [23]. According to C. Barnham, et al. (2003) [3], if the K value is 1.60; 1.40; 1.20; 1.0 and 0.80, these indicate excellent and trophy-class fish; a good and well-proportioned; a fair; poor long and thin; and finally extremely poor fish having disproportional large head and narrow skinny body, respectively. The condition factor is frequently used in evaluating the general health of the fish, on an individual and population level [24]. Since they include many stages of processes in the organism at numerous levels of organization, indices like Fulton's condition factor can indicate the nutritional status of individuals and the general health status of fish [25]. The condition factor presents a degree of the individual's response to the influences coming from

the environment such as the quantity and quality of nutrients, the presence of pathogens, pollutants, and toxic substances, which can result in a change in the mass of individuals and organs compared to individuals from the unchanged environment. The value of the condition factor is an expression of the physical condition of the fish and it is suitable for comparison of individuals of the same species and demonstrates differences in relation to sex, season, and place of sampling [26].

The cost of feed and seed emerges as critical factors in trout production, collectively absorbing almost 70% of the production costs, as pointed out by B. Tuburan, et al. (2001) [27], O. Oluwemimo, et al. (2013) [28], and I.E. Ele, et al. (2013) [29]. J. Lazard, et al. (2010) [30] also highlights the numerous challenges trout face, including environmental, social, and economic constraints. Given that trout are often cultured in intensive systems, it is imperative to implement better management practices to reduce production costs and mitigate environmental damage. Many authors have reported a benefit-cost ratio of more than 2.00 [31-35], which lends further support to our findings.

5. Conclusions

The findings of the present research revealed that:

- The introduction of GIS of rainbow trout in Kashmir has improved the economic status of the trout farmers.
- The GIS showed better growth, food conversion efficiency, and other morphometric characteristics, which are appealing to the consumers.
- The benefit-cost ratio in the case of GIS is better than the NS, although that culture is also profitable.

CRedit author statement

Shyambir: Conceptualisation, Validation; Salman Rauoof Chalkoo: Data curation, Writing - Original draft preparation, Data analysis.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] F. Wynne (2015), "Winter culture of caged rainbow trout in the south", *Aquaculture Publication Fact Sheet*, Collage of Agriculture, Food and Environment.
- [2] I. Okumus, M.S. Celikkale, I.Z. Kurtoglu, et al. (1999), "Growth performance, food intake and feed conversion ratios in rainbow (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) reared as a single and mixed species", *Turkish Journal of Veterinary and Animal Sciences*, **23**, pp.123-130.
- [3] C. Barnham, A. Baxter (2003), *Condition Factor, K, for Salmonid Fish 1998*, State of Victoria, Department of Primary Industries.
- [4] E.R. d'Orbecastel, J.P. Blancheton, J. Aubin (2009), "Towards environmentally sustainable aquaculture: Comparison between two trout farming systems using life cycle assessment", *Aquacult. Eng.*, **40**, pp.113-119, DOI: 10.1016/j.aquaeng.2008.12.002.

- [5] M. Khodadadi, N. Abbasi, T.J. Adorian, et al. (2017), "Growth performance, survival, body composition, hematological parameters, intestinal histomorphology, and digestive enzymes' activity in juvenile rainbow trout (*Oncorhynchus mykiss*) fed dietary immunogen", *Journal of Applied Aquaculture*, **30**, pp.174-186, DOI: 10.1080/10454438.2017.1420515.
- [6] R.K. Sharma, R.A. Bhat (2015), "Length-weight relationship, condition factor of rainbow trout (*Oncorhynchus mykiss*) from Kashmir waters", *Annals of Biological Research*, **6**(8), pp.25-29.
- [7] I. Ahmad, I. Ahmed (2019), "Length weight relationship and condition factor of cultured rainbow trout, *Oncorhynchus mykiss* (Walabum, 1792)", *Journal of Ecophysiology and Occupational Health*, **19**(1, 2), pp.24-27, DOI: 10.18311/jeoh/2019/22304.
- [8] K.D. Joshi, K.K. Vass, H.S. Raina (2005), "Experiments on the rearing of rainbow trout *Oncorhynchus mykiss* (Walbaum) in central Himalayas", *Indian Journal of Fisheries*, **52**(3), pp.331-337.
- [9] K.K. Vass, H.S. Raina, R.S. Haldar (2010), "Prospects of rainbow trout - *Oncorhynchus mykiss*, Walbaum culture in mid altitudes of central Himalayas, India", *Journal of Inland Fisheries Society*, **42**(1), pp.1-7.
- [10] N.N. Pandey, S. Ali (2015), *Rainbow Trout Farming in India: R&D Perspectives*, Bulletin No. 23, Indian Council of Agricultural Research, 33pp.
- [11] D.L. Breitburg, D.W. Hondorp, L.A. Davias, et al. (2009), "Hypoxia, nitrogen, and fisheries: Integrating effects across local and global landscapes", *Annual Review of Marine Science*, **1**, pp.329-349, DOI: 10.1146/annurev.marine.010908.163754.
- [12] D. Chabot, R. Koenker, A.P. Farrell (2016a), "The measurement of specific dynamic action in fishes", *Journal of Fish Biology*, **88**, pp.152-172, DOI: 10.1111/jfb.12836.
- [13] D. Chabot, J.F. Steffensen, A.P. Farrell (2016b), "The determination of standard metabolic rate in fishes", *Journal of Fish Biology*, **88**, pp.81-121, DOI: 10.1111/jfb.12845.
- [14] K.D. Hopkins (1992), "Reporting fish growth: A review of the basics", *Journal of The World Aquaculture Society*, **23**(3), pp.173-179, DOI: 10.1111/j.1749-7345.1992.tb00766.x.
- [15] M. Kayim, M. Suiçmez, Y. Güner, et al. (2007), "Growth of rainbow trout (*Oncorhynchus mykiss*, W. 1792) in net cages in Almus dam lake (Tokat), Pakistan", *Journal of Biological Sciences*, **10**(6), pp.964-967, DOI: 10.3923/pjbs.2007.964.967.
- [16] S.L. Reza, F. Mohsen, J. Houshang, et al. (2014), "Comparison of the daily diet by weight in rainbow trout (*Oncorhynchus mykiss*) with concentrate feed to achieve a minimum FCR and effective period of growth in Beyza regional climate", *Biomedical & Pharmacology Journal*, **7**(1), pp.357-362, DOI: 10.13005/bpj/498.
- [17] R.T. Kenneth, D.W. Carl, M.M. Ann, et al. (2000), "Effect of different feeding frequencies on growth, body composition, fillet composition of juvenile sunshine bass, *Morone chrysops* x *M. saxatilis*, growth indoors", *Journal of Applied Aquaculture*, **10**(2), pp.55-62, DOI: 10.1300/J028v10n02_05.
- [18] T.G. Galano, J.C. Pevez, G.Y.A. Gaxiola (2003), "Effect of feeding frequency on food intake, gastric evacuation and growth in juvenile snook, *Centropomus undecimalis* (Bloch)", *Rev. Invest.*, **24**(2), pp.145-154.
- [19] S.E. Papoutsoglou, E.P. Papoutsoglou, M.N. Alexis (1987), "Effect of density on growth rate and production of rainbow trout (*Salmo gairdneri*) over a full rearing period", *Aquaculture*, **66**, pp.9-17, DOI: 10.1016/0044-8486(87)90279-1.
- [20] O. Yıldırım, M.S. Celikkale, C. Erkebay, et al. (1998), "Possibility of feeding rainbow trout (*Oncorhynchus mykiss*) with fishmonger by-products", *The Proceedings of The First International Symposium on Fisheries and Ecology*, pp.2-4.
- [21] T. Storebakken, E. Austreng (1987), "Ration level for salmonids II. growth, feed intake, protein digestibility, body composition, and feed conversion in rainbow trout weighting 0.5-1.0 kg", *Aquaculture*, **60**, pp.207-221, DOI: 10.1016/0044-8486(87)90288-2.
- [22] N.A. Shah, H. Khan, H.U. Rehman, et al. (2018), "Evaluation of specific growth rate (SGR) in the rainbow trout", *International Journal of Biosciences*, **12**(6), pp.367-374, DOI: 10.12692/ijb/12.6.367-374.
- [23] B. Akbulut, T. Sahin, N. Aksungur, et al. (2002), "Effect of initial size on growth rate of rainbow trout, *Oncorhynchus mykiss*, reared in cages on the turkish black sea coast", *Turkish Journal of Fisheries and Aquatic Sciences*, **2**, pp.133-136.
- [24] M.T. Hoque, F.M. Yusoff, A.T. Law (1998), "Effect of hydrogen sulphide on liver-somatic index and Fulton's condition factor in *Mystus nemurus*", *Journal of Fish Biology*, **52**(1), pp.23-30, DOI: 10.1111/j.1095-8649.1998.tb01549.x.
- [25] S.M. Adams, W.D. Crumby, J.M.S. Greeley, et al. (1992), "Relationships between physiological and fish population responses in a contaminated stream", *Environmental Toxicology and Chemistry*, **11**, pp.1549-1557, DOI: 10.1002/ETC.5620111105.
- [26] R. Dekić (2010), *Circa-Annual Research on The Hematological Status of Barbus Peloponnesius as a Function of Habitat*, Doctoral Dissertation, Faculty of Science and Mathematics, University of Banja Luka (in Croatian).
- [27] B. Tuburan, E.B. Coniza, E.M. Rodriguez, et al. (2001), "Culture and economics of Wild grouper (*Epinephelus coioides*) using three feed types in ponds", *Aquaculture*, **201**(3), pp.229-240, DOI: 10.1016/S0044-8486(01)00744-X.
- [28] O. Oluwemimo, A. Damilola (2013), "Socio-economic and policy issues determining sustainable fish farming in Nigeria", *International Journal of Livestock Production*, **4**(1), pp.1-8, DOI: 10.5897/IJLP12.013.
- [29] I.E. Ele, O.W. Ibok, E.A.A. Obong, et al. (2013), "Economic analysis of fish farming in Calabar, Cross River State, Nigeria", *Greener Journal of Agricultural Sciences*, **3**(7), pp.542-549, DOI: 10.15580/GJAS.2013.3.061013653.
- [30] J. Lazard, B. Aurele, S. Mathe, et al. (2010), "Aquaculture system diversity and sustainable development: Fish farms and their representation", *Aquatic Living Resources*, **23**(2), pp.187-198, DOI: 10.1051/alr/2010018.
- [31] R.L.B. Andrade, R.L. Wagner, I. Mahl, et al. (2005), "Nile tilapia (*Oreochromis niloticus*) production costs in a farm model of the west region of the State of Paraná, Brazil", *Cienc. Rural*, **35**(1), pp.1982-2003, DOI: 10.1590/S0103-84782005000100032 (in Portuguese).
- [32] G.F. Amaral (2007), *Analysis of The Trout Segment: Production Chain Approaches and Rural Tourism*, Dissertation in Federal University of Rio de Janeiro (in Portuguese).
- [33] C.R. Engle (2010), *Aquaculture Economics and Financing: Management and Analysis*, John Wiley & Sons, DOI: 10.1002/9780813814346.
- [34] N.B. Bhandar, K. Prajuli (2016), *Production Cost and Marketing of Rainbow Trout in Nepal*, Ministry of Agriculture Development, Department of Agriculture, Government of Nepal, Lalitpur.
- [35] S.B. Andressa, G.P. Raquel, A.R. Laurindo, et al. (2020), "Economic analysis of family trout farming in Southern Brazil", *Aquaculture International*, **28**, pp.2111-2120, DOI: 10.1007/s10499-020-00580-7.

Hepatectomy using a bipolar vessel-sealing scalpel with Takasaki's Glissonean pedicle transection method in hepatocellular carcinoma: A single-centre short-term outcome

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Abstract:

Purpose: The study aimed to evaluate the results of a bipolar vessel-sealing (LigaSure™) scalpel application for parenchymal resection combined with Glissonean pedicle transection using the Takasaki method in the treatment of hepatocellular carcinoma (HCC). **Patients and methods:** A prospective descriptive study was performed on 67 patients from August 2021 to April 2022 (6 months). **Results:** The mean age was 56±12 years (14-76). Durations for right and left Glisson pedicle control were 3.44±1.65 and 4.58±4.05 minutes, respectively, while those for anterior and posterior pedicle control were 6.12±4.05 and 6.08±3.79 minutes, respectively. The mean blood loss during pedicle control was 21.88±14.02 ml; there were no complications during pedicle transection. The average time of parenchymal resection was 33.96±12.32 minutes, the average intraoperative blood loss was 211.79±125.41 ml, and there was one patient requiring intraoperative blood transfusion. All 3 cases of intraoperative complications were hepatic vein laceration. The overall rate of postoperative complications was 2.98%, of which postoperative liver failure accounted for 1.49%. The average number of days in hospital postoperatively was 8 days, the shortest was 6 days and the longest was 15 days. The rate of in-hospital mortality was 0%. **Conclusion:** The bipolar vessel-sealing (LigaSure™) scalpel application for parenchymal resection combined with Glissonean pedicle transection using the Takasaki method in the treatment of HCC is safe and effective.

Keywords: bipolar vessel-sealing scalpel, hepatocellular carcinoma, Takasaki method.

Classification number: 3.2

1. Introduction

Hepatocellular carcinoma is the most common primary liver cancer, ranking top in incidence and mortality rate worldwide [1]. Hepatectomy remains the most basic curative treatment for HCC. However, hepatectomy is also associated with many complications including bleeding, acute liver failure, and bile leakage, which may adversely affect survival and mortality. First described by K. Takasaki (1998) [2], Takasaki pedicle transection allowed a precise anatomical method of liver resection based on the ischemic line on the liver surface, as well as minimal intraoperative blood loss. This method is now widely applied around the world, especially in Vietnam and East Asia. Intraoperative

bleeding is common in liver parenchymal resection, raising questions about the optimal bleeding-control cutting device. Among current instruments like the LigaSure™, Harmonic Scalpel™, the Cavitron Ultrasonic Surgical Aspirator® (CUSA), and staplers, which allow the least blood loss and are the most cost-effective, etc., is still a matter of controversy.

Many studies confirm the role of the LigaSure™ scalpel in reducing operative time, morbidity rate of bile leakage, and bleeding compared with other means. On the other hand, many studies have shown LigaSure™'s comparable results over other devices (Harmonic Scalpel™, CUSA scalpel, Aquamantys®, or Stapler) in parenchymal resection

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and decrease in intraoperative blood loss [3-5]. However, there are currently no studies evaluating the efficacy of the combination of Takasaki's pedicle transection method with the LigaSure™ scalpel for parenchymal resection. Our study aimed to find out whether this combination would enhance the effectiveness of hepatectomy for HCC, by illustrating highlights pictures as well as short-term outcomes.

2. Materials and methods

These case series have been reported in line with the process guideline at the end of the methods section (and included in the references section) [6] and were registered in accordance with the Declaration of Helsinki. The study was conducted on 67 patients undergoing liver resection with the Takasaki technique using a LigaSure™ scalpel at Bach

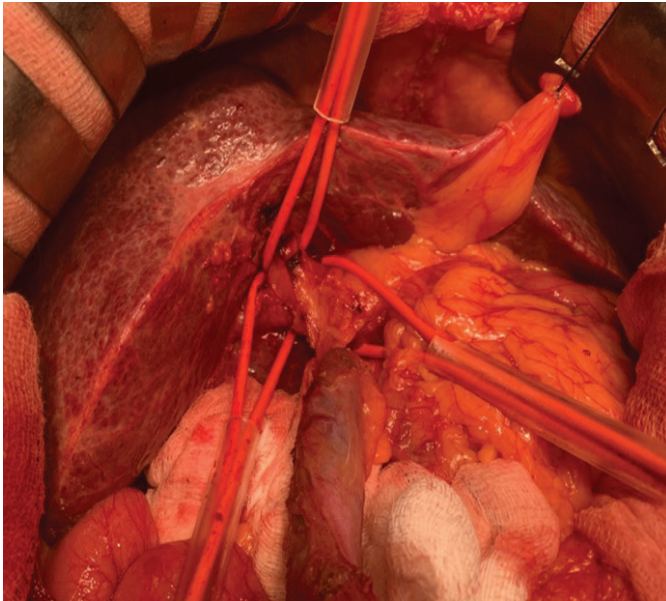


Fig. 1. Pedicle control with Takasaki's method.

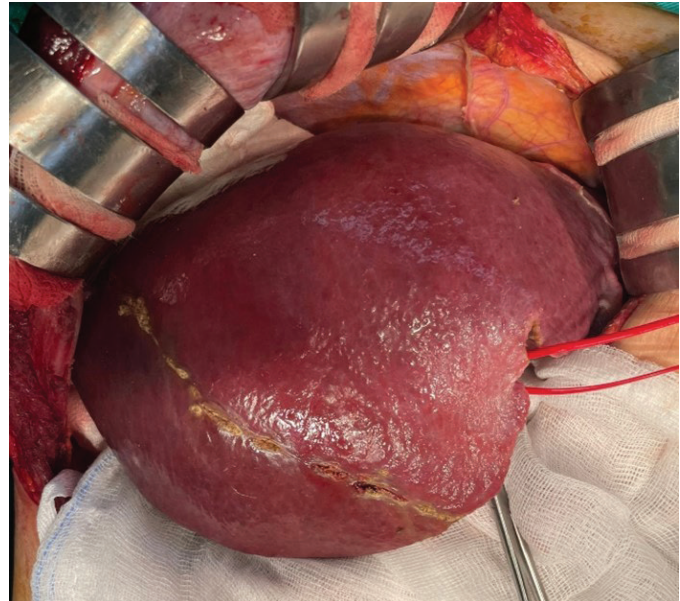


Fig. 2. Posterior hepatectomy transection plan marked based on ischaemic line on liver surface after posterior pedicle control.

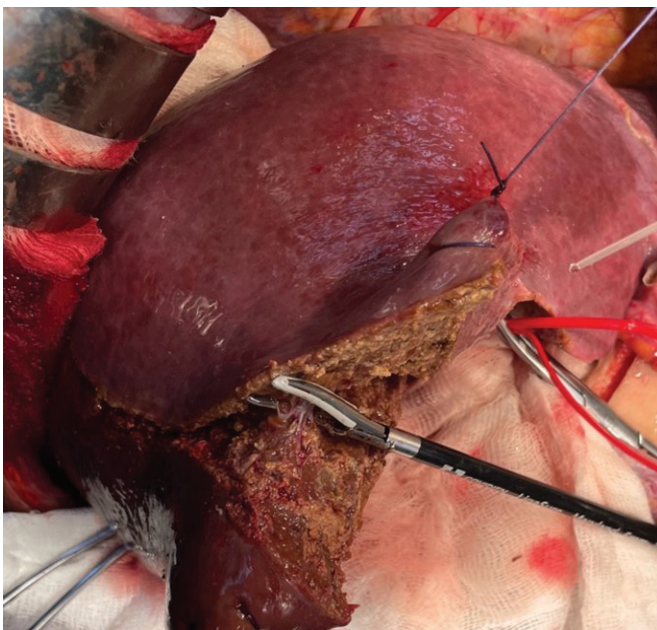


Fig. 3. Segment 6 hepatic vein cut and sealed with vessel sealing LigaSure™ scalpel.

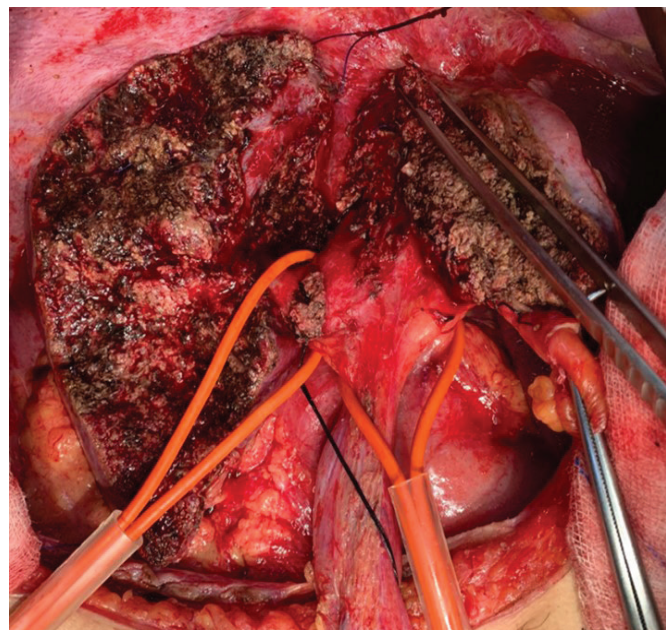


Fig. 4. Liver resection margin after performing liver parenchymal resection with LigaSure™ scalpel (Central hepatectomy).

Mai Hospital from August 2021 to April 2022 (6 months). The preoperative diagnosis was histologically confirmed as HCC, and staging was classified based on the Barcelona Clinic Liver Cancer Staging and Treatment Strategy in 2022 [7] and the Guideline for Diagnosis and Treatment of HCC of the Ministry of Health of Vietnam in 2020.

Inclusion criteria include: Patients are selected for surgery according to the Barcelona Clinic Liver Cancer 2022 criteria and Guideline for Diagnosis and Treatment of HCC of the Ministry of Health of Vietnam in 2020; Postoperative pathology confirmed HCC; and hepatectomy was performed with Takasaki pedicle transection method with a LigaSure™ scalpel.

Exclusion criteria include: secondary liver tumour resected, simultaneous extrahepatic biliary tract resection and biliary-enteric anastomosis, and hepatectomy with intraoperative radiofrequency ablation for other liver lesions.

Research method: Descriptive study.

3. Surgical protocol

Stage 1: Abdominal exploration:

- Open the abdomen with the right J line or the middle white line above and below the umbilicus.
- Evaluate the liver tumour and its invasion into the surrounding organs. Assess peritoneal metastasis.

Stage 2: Cholecystectomy, lower gallbladder bed and liver hilum.

Stage 3: Mobilise the liver depending on the type of surgery to be performed.

Stage 4: Pedicle control with Takasaki's method (Fig. 1). Selective clamping of the hepatic pedicle, defining the resection border of the liver parenchyma according to the ischemic line on the liver surface (Fig. 2).

Stage 5: Liver parenchymal resection with LigaSure™ scalpel (Fig. 3). Determine the transection plane based on ischemia line on liver surface. Cut the liver parenchyma with Kelly forceps and LigaSure™ scalpel. Dissect blood vessels less than 5 mm with LigaSure™ scalpel. Larger vessels are sutured with 3.0 nonabsorbable monofilament suture.

Stage 6: Cut the corresponding Glisson pedicle and hepatic vein with a stapler or suture with a nonabsorbable, monofilament suture.

Stage 7: Apply haemostats, check for bile leaks, place drainage at the transection plane, close abdomen (Fig. 4).

4. Data analysis

Collected data included: demographical characteristics such as age, gender, past medical history of liver disease, and other comorbidities, clinical and subclinical characteristics of tumour location, size, number, and liver function classified according to Child-Pugh classification; Technical characteristics and developments in surgery such as pedicle control time, parenchymal resection time, blood loss, and intraoperative complications; Postoperative complications, for example, liver failure, bile leak, bleeding, ascites, and mortality; and Length of hospital stay after surgery.

Using the criteria of the International Study Group on Liver Surgery, a biliary leak was defined as bile detected in abdominal drainage or incisional separation. Similarly, post-hepatectomy liver failure was defined as exogenous coagulation prothrombin less than 50% and elevation of total bilirubin above 50 µmol/l from the 5th postoperative day.

Data were analysed using SPSS 24.0. All qualitative data were presented in percentage. Quantitative data were presented as mean and standard deviation (SD).

5. Results

Sixty-seven patients (male/female ratio is 4:1) underwent liver resection with Takasaki's technique using the LigaSure™ scalpel. The mean age was 56±12 years. 73% had a history of hepatitis B. All patients were classified as Child-Pugh A, with platelet counts above 100,000 per mm³ and normal coagulation status. The median values of preoperative tumour markers - AFP, AFP-L3, PIVKA II - were 8.2 ng/ml, 6.5%, and 138.5 mAU/ml, respectively.

The morphologies of liver resection in our study group varied, from segmentectomy to multi-segmentectomy, encompassing segments 1 to 8. Minor hepatectomy accounted for the majority (68.7%) (Table 1). Right posterior segmentectomy contributed the highest proportion (26.9%), followed by left hepatectomy (19.4%).

Table 1. Morphology of liver resection.

Morphology		Number of patients (n)	Percentage (%)
Major hepatectomy (21)	Right hepatectomy	5	7.5
	Left hepatectomy	13	19.4
	Central hepatectomy	3	4.5
Minor hepatectomy (46)	Left sectionectomy	2	3.0
	Anterior sectionectomy	11	16.4
	Posterior sectionectomy	18	26.9
	Bisegmentectomy	8	11.9
	Segmentectomy	7	10.4

Table 2. Pedicle control and parenchymal resection time.

Pedicle control time			
Pedicle	Mean time (minutes)	Min (minutes)	Max (minutes)
Right	3.44±1.65	2	10
Left	4.58±4.05	1	15
Right anterior	6.12±4.05	1	20
Right posterior	6.08±3.79	1	20
Parenchymal resection time [(n) Mean time±SD] (minutes)			
Mean parenchymal resection time		(67) 33.96±12.32	
Major hepatectomy		(38) 36.05±13.26	
Minor hepatectomy		(29) 31.21±10.58	
Mean operative time		(67) 130.79±29.62	
Major hepatectomy		(38) 131.53±28.93	
Minor hepatectomy		(29) 129.83±30.98	

The mean time for right and left pedicle control was 3.44±1.65 minutes and 4.58±4.05 minutes, respectively, for anterior and posterior segment pedicles was 6.12±4.05 minutes and 6.08±3.79 minutes, respectively. The mean time of overall parenchymal resection was 33.96±12.32 minutes. The mean time for major hepatectomy was 36.05±13.26 minutes; minor hepatectomy was 31.21±10.58 minutes (Table 2). Three patients (4.48%) experienced complications during parenchymal resection, all of which were hepatic vein lacerations, well managed intraoperatively. The mean operative time was 130.79±29.62 minutes, with 131.53±28.93 minutes for major hepatectomy and 129.83±30.98 minutes for minor hepatectomy. After surgery, all our patients were able to sit up and start feeding on the first day post-surgery.

The results from Table 3 showed that the mean blood loss during pedicle control was 21.88±14.02 ml, the overall mean intraoperative blood loss was 211.79±125.41 ml, and only one patient required an intraoperative blood transfusion.

In Table 4, the postoperative overall complication rate was 2.98%: One case of postoperative liver failure and one case of wound infection. Both cases were graded Clavien-Dindo II without the need for surgical intervention.

Table 3. Intraoperative blood loss.

	Mean±SD (ml)
Blood loss during pedicle control	21.88±14.02
Intraoperative blood loss	211.79±125.41
Major hepatectomy	202.63±133.21
Minor hepatectomy	164.14±107.22

Table 4. Postoperative complications.

Postoperative morbidities	Cases	Percentage (%)	Clavien-Dindo
Bleeding	0	0	
Biliary leakage	0	0	
Ascites	0	0	
Wound infection	1	1.49	II
Liver failure	1	1.49	II
Total	2	2.98	

6. Discussion

In liver resection surgery, most authors acknowledge that three factors need to be controlled to limit blood loss and reduce intra- and postoperative complications: (1) inflow control, i.e., control of blood entering the liver; (2) outflow control, i.e., control of the hepatic veins and central venous pressure; (3) control of blood vessels during parenchymal resection.

6.1. Takasaki's technique of pedicle control

Takasaki's technique of pedicle control is one of the most common inflow control methods being applied. This technique not only determines a precise transection plan based on the ischaemic line on the liver surface but also facilitates anatomical liver resection. First introduced in 1986 in a case of right hepatectomy, Takasaki's technique is now well adopted worldwide in both laparoscopic and open surgery [2]. The technique was defined as successful when the surgeon could well expose the entire pedicle and place the control device precisely on the corresponding pedicle (left, right anterior, right posterior, and even segmental pedicles).

Our study reported a rate of successful pedicle control of 100% of the cases. The mean time to control the right and left pedicles was 3.44 ± 1.65 and 4.58 ± 4.05 minutes, respectively; for anterior or posterior segmental pedicles it was 6.12 ± 4.05 minutes. Blood loss during pedicle control is negligible. Assessing the amount of blood loss during this technique is also very difficult and relatively precise due to minimal blood loss. Dissection into the correct anatomical layer between the Glisson capsule and the Laennec capsule will not cause bleeding.

Our research results were consistent with domestic and international studies: K. Takasaki (2007) [8]'s data on more than 2,000 cases of HCC report a pedicle control success rate of 100% and no adverse events. In Vietnam, a study by V.Q. Vu, et al. (2022) [9] on 106 patients showed a success rate of 100% and a complication rate of 1.9% due to right portal vein laceration. Portal vein laceration is the most common complication during pedicle dissection, possibly due to portal hypertension in cirrhotic patients. According

to K. Takasaki (2007) [8], during pedicle control, it is advisable to completely control the pedicle first to reduce bleeding from small vessels in the hilar area, especially in cirrhotic patients with portal hypertension. This approach not only helps limit bleeding but also maintain a clean surgical field, and therefore, avoiding further complications in pedicle dissection.

6.2. The role of LigaSure™ scalpel

First introduced in 1998, LigaSure™ by Covidien (Medtronic, USA) utilises a combination of pressure and bipolar radiofrequency energy to induce vascular sealing up to 7 mm in diameter. The application of the LigaSure™ system in liver parenchymal resection offers significant benefits, including reduced blood loss, blood transfusion rates, operating time, and shortened postoperative hospital stays in established surgeries, which has been confirmed in many studies [3, 10-12].

During hepatectomy, after the liver parenchyma was crushed with a Kelly forceps, the blood vessels and small biliary tracts were left exposed. Instead of traditional suturing, we resected the connective tissue and small blood vessels (less than 5 mm) (mainly small liver veins) with a LigaSure™ scalpel. This technique not only reduced bleeding due to vessel laceration during suture binding but also shortened parenchymal resection time. LigaSure™ has also been proven to reduce the risk of biliary leakage due to its ability to seal small biliary tracts [3]. When performing the Takasaki pedicle control technique before parenchymal resection, the transection plan between anatomical units was clearly defined to minimise the risk of large blood vessel injury. The most important vascular component commonly encountered is the hepatic vein, which can be effectively sealed with LigaSure™. Therefore, the combination of pedicle control technique along with the application of LigaSure™ scalpel makes liver resection safe and effective.

According to our study, the mean time for parenchymal resection was 33.96 ± 12.32 minutes, and the mean intraoperative blood loss was 211.79 ± 125.41 ml, which was comparable with other studies [3, 13]. Several studies worldwide have shown no difference in the duration of liver parenchymal resection between LigaSure™ scalpel

and other devices, even in laparoscopic liver resection [11]. A recent systematic review with data from 22 randomised controlled trials involving 2360 patients has shown that bipolar cautery techniques (with LigaSure™ scalpel as a typical example) provide the best outcomes in reducing blood loss and shortening operating time [14]. In our study, three cases encountered complications during parenchymal resection; all the morbidities were hepatic vein lacerations during parenchymal crushing with Kelly forceps in cases with tumours near dilated blood vessels due to cirrhotic portal hypertension. These complications were not related to the use of LigaSure™. In our opinion, liver cirrhosis or a tumour adjacent to large blood vessels were prognostic factors for difficult parenchymal resection.

The LigaSure™ scalpel seals tissue and creates adhesion. Therefore, if applied to parenchymal resection, liver tissue would become adhesive, which prevents the scalpel tip from moving flexibly and increases the risk of vessel injury. Consequently, we recommend using Kelly forceps, instead of LigaSure™, to break the parenchyma and before using the LigaSure™ scalpel to cut connective tissues and blood vessels.

6.3. Short-term outcome

The mean postoperative hospital stay was 7.9 days (6-15 days). This result is consistent with other authors applying Takasaki's hepatectomy method and the SonaStar Ultrasonic Aspiration System for parenchymal resection, reported a mean hospital stay of 8 ± 5.2 days [9]. The mean postoperative blood loss was 211.79 ± 125.41 ml, compared to Y. Sakamoto, et al. (2004) [12]'s result of 200 ml when using the LigaSure™ scalpel, and V.L. Ho, et al. (2021) [13]'s result of 250 ml when using an ultrasonic scalpel.

Our postoperative complications included two cases (accounting for 2.98%), all of which were mild complications and did not require surgical intervention. No mortality was observed. This result is consistent with studies worldwide: M. Ikeda, et al. (2009) [10] studied 60 hepatectomy cases using the LigaSure™, and A. Saiura, et al. (2006) [15] reported the same number of patients, with neither witnessing intraoperative nor postoperative mortality.

Although the results in our study were very positive, this was only a retrospective observation of short-term outcomes on limited numbers of hepatectomy cases. Therefore, a longer follow-up period is required in future studies to elucidate the potential benefits of the LigaSure™ scalpel and the Takasaki technique for HCC.

7. Conclusions

Hepatectomy using the LigaSure™ scalpel combined with Takasaki's Glissonean pedicle control method in the treatment of HCC is feasible and effective. This approach allows liver resection to be precise with sufficiently safe margins, minimal intraoperative blood loss and blood transfusion, and a low complication rate. Further investigations and follow-up must proceed to evaluate the long-term outcomes of this technique.

Ethics approval and consent to participate

Ethics approval of this study was given by the Research Ethics Committees of Bach Mai Hospital. Written informed consent for publication of their clinical details and clinical images was obtained from the patients' family.

CRediT author statement

Ham Hoi Nguyen, Thanh Khiem Nguyen contributed equally as co-first authors, the main doctors to operate the patients, Conceptualisation; Van Duy Le, Kim Khue Dang, Van Minh Do: Performing the operations, Editing; Tuan Hiep Luong: Performing the operations, Writing; Hoan My Pham, Kim Khue Dang: Collecting data, Editing; Cong Long Nguyen, Ngoc Hung Nguyen: Conceptualisation, Data analysis, Editing.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] T. Akinyemiju, S. Abera, M. Ahmed, et al. (2017), “The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: Results from the global burden of disease study 2015”, *JAMA Oncology*, **3**(12), pp.1683-1691, DOI: 10.1001/jamaoncol.2017.3055.
- [2] K. Takasaki (1998), “Glissonean pedicle transection method for hepatic resection: A new concept of liver segmentation”, *Journal of Hepato-Biliary-Pancreatic Surgery*, **5**(3), pp.286-291, DOI: 10.1007/s005340050047.
- [3] F. Romano, C. Franciosi, R. Caprotti, et al. (2004), “Hepatic surgery using the ligasure vessel sealing system”, *World Journal of Surgery*, **29**(1), pp.110-112, DOI: 10.1007/s00268-004-7541-y.
- [4] R.F. Saidi, A. Ahad, R. Escobar, et al. (2007), “Comparison between staple and vessel sealing device for parenchymal transection in laparoscopic liver surgery in a swine model”, *HPB: The Official Journal of The International Hepato Pancreato Biliary Association*, **9**(6), pp.440-443, DOI: 10.1080/13651820701658219.
- [5] J.S. Hammond, W. Muirhead, A.M. Zaitoun, et al. (2012), “Comparison of liver parenchymal ablation and tissue necrosis in a cadaveric bovine model using the harmonic scalpel, the ligasure, the cavitron ultrasonic surgical aspirator and the aquamantys devices”, *HPB: The Official Journal of The International Hepato Pancreato Biliary Association*, **14**(12), pp.828-832, DOI: 10.1111/j.1477-2574.2012.00547.x.
- [6] R.A. Agha, C. Sohrabi, G. Mathew, et al. (2020), “The process 2020 guideline: Updating consensus preferred reporting of case series in surgery (process) guidelines”, *International Journal of Surgery (London, England)*, **84**, pp.231-235, DOI: 10.1016/j.ijssu.2020.11.005.
- [7] M. Reig, A. Forner, J. Rimola, et al. (2022), “BCLC strategy for prognosis prediction and treatment recommendation: The 2022 update”, *Journal of Hepatology*, **76**(3), pp.681-693, DOI: 10.1016/j.jhep.2021.11.018.
- [8] K. Takasaki (2007), *Glissonean Pedicle Transection Method for Hepatic Resection*, Springer Science & Business Media, DOI: 10.1007/978-4-431-48944-3.
- [9] V.Q. Vu, M.C. Truong, T.H.A. Vu (2022), “Evaluation of early results of major hepatectomy using Glisson pedicle control technique according to Takasaki to treat primary liver cancer”, *Journal of 108 - Clinical Medicine and Pharmacy*, **17**(7), pp.125-132, DOI: 10.52389/ydls.v17i7.1562 (in Vietnamese).
- [10] M. Ikeda, K. Hasegawa, K. Sano, et al. (2009), “The vessel sealing system (LigaSure) in hepatic resection: A randomized controlled trial”, *Annals of Surgery*, **250**(2), pp.199-203, DOI: 10.1097/SLA.0b013e3181a334f9.
- [11] F. Liu, Y. Wei, H. Li, et al. (2018), “Ligasure versus CUSA for parenchymal transection during laparoscopic hepatectomy in hepatocellular carcinoma patients with cirrhosis: A propensity score-matched analysis”, *Surgical Endoscopy*, **32**(5), pp.2454-2465, DOI: 10.1007/s00464-017-5947-5.
- [12] Y. Sakamoto, J. Yamamoto, N. Kokudo, et al. (2004), “Bloodless liver resection using the monopolar floating ball plus ligasure diathermy: Preliminary results of 16 liver resections”, *World Journal of Surgery*, **28**(2), pp.166-172, DOI: 10.1007/s00268-003-7167-5.
- [13] V.L. Ho, N.H. Pham, T.X. Nguyen, et al. (2021), “Hepatectomy with Takasaki’s technique using sonastar ultrasonic aspiration system: An experience from 58 cases”, *Clinical and Experimental Gastroenterology*, **14**, pp.297-302, DOI: 10.2147/CEG.S319434.
- [14] S.K. Kamarajah, C.H. Wilson, J.R. Bundred, et al. (2020), “A systematic review and network meta-analysis of parenchymal transection techniques during hepatectomy: An appraisal of current randomised controlled trials”, *HPB: The Official Journal of The International Hepato Pancreato Biliary Association*, **22**(2), pp.204-214, DOI: 10.1016/j.hpb.2019.09.014.
- [15] A. Saiura, J. Yamamoto, R. Koga, et al. (2006), “Usefulness of ligasure for liver resection: Analysis by randomized clinical trial”, *American Journal of Surgery*, **192**(1), pp.41-45, DOI: 10.1016/j.amjsurg.2006.01.025.

Association between HLA-DQB1, -DPB1 alleles and risk of carbamazepine-induced hypersensitivity reactions in Vietnamese patients with epilepsy

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Abstract:

Adverse drug reactions are a serious problem for health and one of the main reasons for treatment failure with antiepileptic drugs. In this study, we determined the HLA-DQB1 and -DPB1 alleles in the blood samples of 44 epileptic patients receiving carbamazepine treatment, including 21 patients with hypersensitivity and 23 patients with tolerance, using the next-generation sequencing method. We identified 19 HLA-DQB1 alleles, of which 13 were in the hypersensitive group and 15 were in the tolerant group. The frequency of the HLA-DQB1*03:01:01 allele was highest in both groups. Although the frequency of the HLA-DQB1*03:03:02 allele in the hypersensitive group was higher than that in the tolerant group ($p=0.0431$), this difference was not statistically significant after applying the Bonferroni correction ($p_c=0.5249$). However, the evidence of *in silico* interaction between CBZ and HLA-DQB1*03:03 at the peptide-binding cleft was suggested. For HLA-DPB1, 16 alleles were found in epileptic patients, with the HLA-DPB1*05:01:01 allele having the highest frequency; however, no allele was associated with the risk of hypersensitivity to carbamazepine in epileptic patients. Our study demonstrates that the HLA-DQB1*03:03:02 allele tended to behave as a susceptibility factor for carbamazepine-induced hypersensitivity reactions in Vietnamese patients with epilepsy.

Keywords: carbamazepine, epilepsy, HLA-DPB1, HLA-DQB1*03:03:02.

Classification numbers: 3.2, 3.5, 3.6

1. Introduction

Epilepsy is a common neurological disorder, affecting around 50 million people and accounting for 0.5% of the global disease burden [1]. The annual estimated proportion of epilepsy worldwide is 4-7 per 1,000 people; in Vietnam, this prevalence is 4.4 per 1,000 people [1, 2].

Carbamazepine (CBZ) is the first-line treatment for epilepsy, modulating voltage-gated sodium channels, inhibiting the action potential, and reducing synaptic transmission. Despite being an effective anticonvulsant, CBZ can cause hypersensitivity reactions of varying frequency and severity. The majority of hypersensitivity reactions are relatively mild skin rashes, which are usually

ordered by the doctor to be discontinued to resolve the symptoms of hypersensitivity [3]. However, CBZ also causes serious and potentially severe cutaneous adverse drug reactions such as toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome (SJS), hypersensitivity syndrome (HSS), drug rash with eosinophilia and systemic symptoms (DRESS) [4].

Human leukocyte antigen (HLA) molecules play a crucial role in adaptive immunity. Specifically, HLA class II molecules have been linked to various diseases, including autoimmune diseases, infections, allergies, and epilepsy [5-8]. In 2016, a previous study conducted a study on the genetic association of HLA class II alleles

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DRB1, DQA1, and DQB1 with mesial hippocampal sclerosis. Their results showed that the allele HLA DRB1*13:02 may act as a susceptibility factor in patients with pharmacoresistant temporal lobe epilepsy associated with mesial hippocampal sclerosis [7]. Another previous study analysed HLA typing results and found that class II HLA genes, particularly DRB4*03:01N, a null allele, may have a strong protective role in epilepsy patients in the Middle East [8]. However, there is limited data on the role of HLA class II in the Vietnamese population, especially in those with epilepsy treated with CBZ. Therefore, this study aimed to identify the association between HLA class II, specifically HLA-DQB1 and -DPB1, and the risk of CBZ-induced hypersensitivity reactions in Vietnamese patients with epilepsy.

2. Materials and methods

2.1. Biological materials

A total of 44 blood samples from epilepsy patients treated with CBZ were used in this study, comprising 2 groups: 21 hypersensitive patients and 23 tolerant patients provided by Tam Anh Hospital (Hanoi) with characteristics including age, gender, CBZ dose, drug allergy or tolerance status, and clinical features. The inclusion criteria were as follows: (i) The patients were examined and diagnosed with epilepsy; (ii) They had clear indications for treatment with CBZ; (iii) They had taken CBZ for less than 3 months and had allergic manifestations (hypersensitivity group) or more than 3 months and non-allergic manifestations (tolerance group); (iv) They had not been administered other drugs with a high allergy risk. The exclusion criteria were: (i) Patients with clinically significant allergic conditions before drug administration or allergic conditions present within 24 hours of drug administration; (ii) Pregnant women; (iii) Patients who had not undergone sufficient tests necessary for the study; (iv) Patients who refused to participate in the study.

The patients were informed of the study's purpose and agreed to provide the samples. All enrolled patients or their guardians signed an informed consent form. The study was approved by the Institutional Ethical Review Board of Hanoi Medical University, code 118/GCN-HDDNCYSH-DHYHN on July 8, 2020. Blood samples were collected in EDTA anticoagulant tubes and stored at -25°C until use.

2.2. Amplification of HLA-DQB1 and -DPB1 genes by long-range polymerase-chain-reaction

Total DNA was extracted from whole blood samples using the QIAamp DNA Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. Total DNA concentration and quality were measured by Qubit™ 4 Fluorometer (Invitrogen, USA), and then DNA samples were stored at -20°C until further experiments.

The HLA-DQB1 and -DPB1 genes were amplified using the QIAGEN Long-Range PCR Kit (Qiagen, USA) with locus-specific long-range PCR primers provided by Omixon Holotype HLA 96/7 RUO (Omixon, Hungary), according to the manufacturer's instructions. The HLA-DQB1 and -DPB1 loci were amplified in 25 µl of a reaction mixture consisting of 2 µl of primer mix, 2.5 µl of PCR long-range buffer (10X), 1.25 µl of mixed dNTPs (10 mM each), 0.4 µl Taq polymerase, and 20-30 ng/µl DNA. The thermal cycle used for amplification was 95°C for 3 mins; 35 cycles (95°C for 15 s; 60°C for 30 s; 68°C for 9 mins) and 68°C for 10 mins. The Qubit™ 4 Fluorometer (Invitrogen, USA) was used to measure the nucleic acid's ultraviolet light absorption density of PCR products at 260 nm (A_{260}). The PCR products were then diluted to a final concentration of 100 ng/µl for library construction.

2.3. Library preparation and next-generation sequencing

The library was constructed in 4 main steps: (1) DNA fragmentation to form short fragments approximately 300 bp in length by restriction enzyme; (2) DNA fragment repair and adenylation by adding an A nucleotide; (3) The DNA fragments were attached to two heads by adapter 1 and adapter 2 with specific sequencing; and (4) Library size selection using AMPure XP beads (Beckman Coulter, USA) performed with the Omixon Holotype HLA 96/7 RUO kit (Omixon, Hungary) according to the manufacturer's procedure.

Library DNA concentrations were quantified by measuring the A_{260} value using a Qubit™ 4 Fluorometer (Invitrogen, USA) for optimal use of the output of the Illumina MiSeq. All samples were barcoded,

aggregated, and then sequenced on the MiSeqFGx instrument (Illumina, San Diego, CA) using the Miseq Reagent Kit v3.

Sequencing data was generated from the Illumina Miseq platform as FASTQ files. The raw reading results of the sequencing system (FASTQ file format) were checked by FASTQC software to evaluate the quality criteria and analysed using Omixon HLA Twin v2.1.3 software (Omixon, Hungary), which assisted in aligning reads with specific HLA loci to generate the best-matched alleles at each read. Interpretation of the HLA genotyping format results was performed using Omixon Target software v1.7 (Omixon Biocomputing Kit, Budapest, Hungary) and Omixon Target software (Omixon) v1.8 with updated reference sequences on the IMGT/HLA database.

2.4. *In silico analysis*

HLA-DQB1*03:03 protein sequence was collected from the HLA database (<https://www.ebi.ac.uk/ipd/imgt/hla/alleles/>) and a protein structure model was built using SWISS-MODEL software (<https://swissmodel.expasy.org/interactive/>) [9], with the structure of the HLA-TCR complex (SMTL ID: 6px6.1) as the template. The appropriate model selection was based on the structural evaluation measures of SWISS-MODEL, especially with two important parameters, GMGE and QMEANDisCo Global [9-14]. The structure of carbamazepine was collected from the LIGANDs Database Open and eXtensible (LigandBox) with LigandBoxID of D00252. The chemical binding sites between the target (HLA-DQB1*03:03 molecule) and the ligand (CBZ) were predicted using SwissDock (<http://www.swissdock.ch/>) based on EADock DSS, and results were visualised by UCSF Chimera (<https://www.cgl.ucsf.edu/chimera/>) [13-15].

2.5. *Statistical analysis*

Differences in clinical and subclinical characteristics of the groups and frequency of HLA alleles were statistically analysed using GraphPad Prism version 8.4.2 for Windows (GraphPad Software, San Diego, California, USA). Continuous variables that followed a normal distribution were represented as mean and

standard deviation. In contrast, the Mann-Whitney U test was used if the data did not follow a normal distribution. For categorical variables, statistical comparisons were performed using Fisher's exact test.

Hardy-Weinberg equilibrium tests were conducted for each locus using the Basic Statistics of Gene[rate] Tools (one-locus summary; HLA-net, University of Geneva, Switzerland; <https://hla-net.eu/tools/basic-statistics/>) [16]. Alleles at each locus were considered in Hardy-Weinberg equilibrium if the observed and expected (estimated) frequencies did not differ significantly ($p > 0.05$). The comparison of allele frequencies in this study was performed using Fisher's exact test. The Bonferroni-Dunn method for multiple testing correction was applied when the p values were significant.

HLA-DQB1 allele frequencies in Vietnam were based on information from the Allele Frequency Net Database (www.allelefrequencynet.net) [17]. The HLA-DQB1*03:03 allele frequencies data of this study were compared to those in previous research on the Vietnamese population using Fisher's exact test.

The HLA allele-related risk estimation was expressed as an odds ratio (OR) and 95% confidence interval (CI) from logistic regression. A p-value and p-correction (p_c) less than 0.05 were considered to be statistically significant.

3. Results

3.1. *Clinical and subclinical characteristics of the patient groups with epilepsy*

The clinical and subclinical data of CBZ-treated patients with epilepsy are presented in Table 1. Statistical comparison results showed that, in the hypersensitivity group, the average dose used by patients with severe cutaneous adverse drug reactions (SJS/TEN) was higher than that of mild cutaneous adverse drug reactions (MCADR) ($p = 0.0394$), and the hypersensitivity group had a significantly lower dose of the drug than the tolerant group ($p < 0.0001$). Furthermore, the hypersensitivity group's haemoglobin content (g/l) was lower than that of the tolerant group ($p = 0.0233$). The remaining characteristics between the two groups were not significantly different ($p > 0.05$).

Table 1. Clinical and subclinical characteristics of Vietnamese patients with epilepsy.

Characteristics	MCADR (n=16)	SJS/TEN (n=5)	p-value	Hypersensitive cases (n=21)	Tolerant cases (n=23)	p-value
Average dose (mg/day)	315.6	500.00	0.0394[†]	359.53	595.65	<0.0001[†]
Age (year)						
Min-max	7-71	16-40		7-71	13-65	
Average	33	27	0.4834 [†]	32	28	0.5484 [†]
Gender (n, %)						
Male	9 (42.86%)	2 (40.00%)	0.6351 ^{††}	11 (52.38%)	14 (60.87%)	0.7613 ^{††}
Female	7 (43.75%)	3 (60.00%)		10 (47.62%)	9 (39.13%)	
Time of allergy appearance (day)	21	24	0.4332 [†]	19		
Skin lesions (n, %)						
Maculopapular eruptions	9 (56.25%)	0 (0.00%)		4 (19.05%)		
Skin rashes and/or itches	7 (43.75%)	0 (0.00%)		8 (38.09%)		
Blisters and erosions, in which skin detachment	0 (0.00%)	5 (100.00%)		9 (42.68%)		
Subclinical features (X±SD)						
ALT (IU/ml)	23.86±12.14	42.44±26.61	0.0728 [†]	28.28±17.83	24.97±13.09	0.5257 [†]
AST (IU/ml)	24.82±9.85	45.42±36.79	0.1842 [†]	29.72±20.59	22.20±7.50	0.1618 [†]
Urea (mmol/l)	4.24±1.04	3.97±1.53	0.9837 [†]	4.17±1.14	4.71±4.70	0.2594 [†]
Creatinine (μmol/l)	59.73±11.45	51.38±7.22	0.1471 [†]	57.74±11.05	63.90±13.77	0.2308 [†]
Glucose (mmol/l)	5.39±0.52	7.36±3.90	0.3031 [†]	5.86±2.00	5.46±0.63	0.6876 [†]
Potassium (mmol/l)	3.65±0.28	3.77±0.29	0.6423 [†]	3.67±0.28	3.72±0.24	0.5864 [†]
RBC (T/l)	4.77±0.76	4.53±0.48	0.5582 [†]	4.71±0.69	4.73±0.46	0.5333 [†]
Hb (g/l)	133.30±10.05	128.20±9.86	0.2471 [†]	132.10±10.01	139.17±13.58	0.0233[†]
PLT (G/l)	248.60±79.76	216.20±80.12	0.4950 [†]	240.90±79.09	252.96±49.43	0.6541 [†]
WBC (G/l)	5.95±1.99	7.06±1.75	0.1297 [†]	6.22±1.95	7.46±2.39	0.0710 [†]

*MCADR: mild cutaneous adverse drug reactions; ALT: alanine aminotransferase; AST: aspartate transaminase; RBC: red blood cell; Hb: haemoglobin; PLT: platelet; WBC: white blood cells; X: average; SD: standard deviation; [†] Mann-Whitney U test; ^{††} Fisher's exact test, the p-value in bold was statistically significant.

3.2. Frequency of the HLA-DQB1 and -DPB1 alleles in carbamazepine-treated patients with epilepsy

There is no significant deviation from Hardy-Weinberg equilibrium for loci of HLA-DQB1 and -DPB1 in both groups ($p>0.05$). In total, we identified 19 HLA-DQB1 alleles in 44 Vietnamese epilepsy patients treated with CBZ (Table 2). Among these 19 alleles, HLA-DQB1*03:01:01 was the most frequent allele in both hypersensitivity and tolerance groups, with frequencies of 0.4048 and 0.5090, respectively. Additionally,

the HLA-DQB1*03:03:02 allele appeared higher in the hypersensitivity group than in the tolerant group ($p=0.0431$); however, the difference was not significant after applying Bonferroni correction ($p_c=0.5249$).

For HLA-DPB1, 16 alleles were found in epileptic patients (Table 3), of which the HLA-DPB1*05:01:01 allele had the highest frequency. None of the 16 alleles were found to be associated with CBZ-induced hypersensitivity in epileptic patients.

Table 2. Frequency of the HLA-DQB1 allele in epilepsy patients treated with carbamazepine.

No.	HLA-DQB1 allele	Hypersensitive case (n=21)		Tolerant cases (n=23)		OR (95% CI)	p-value	p _c -value
		NA	AF	NA	AF			
1	02:01:01	1	0.0238	3	0.0652	0.35 (0.03-2.45)	0.6178	0.5771
2	02:02:01	1	0.0238	1	0.0217	1,10 (0.06-21.27)	>0.9999	0.9989
3	03:01:01	17	0.4048	15	0.3261	1,40 (0.58-3.30)	0.5090	0.9338
4	03:02:01	-	-	4	0,0870			
5	03:03:02	8	0.1905	2	0.0435	5.18 (1.11-25.05)	0.0431	0.5249
6	03:276N	1	0.0238	-	-			
7	03:358N	-	-	2	0.0435			
8	04:01:01	1	0.0238	2	0.0435	0.57 (0.04-4.78)	>0.9999	0.6868
9	04:02:01	1	0.0238	-	-			
10	05:01:01	4	0.0952	3	0.0652	1.51 (0.38-6.26)	0.7049	0.8525
11	05:01:24	3	0.0714	2	0.0435	1.69 (0.33-9.86)	0.6664	0.7979
12	05:02:01	2	0.0476	3	0.0652	0.72 (0.12-3.67)	>0.9999	0.8000
13	05:03:01	-	-	2	0.0435			
14	05:11:01	1	0.0238	-	-			
15	05:66:01	1	0.0238	-	-			
16	06:01:01	1	0.0238	4	0.0870	0.26 (0.02-1.68)	0.3631	0.5268
17	06:02:01	-	-	1	0.0217			
18	06:03:01	-	-	1	0.0217			0.5771
19	06:09:01	-	-	1	0.0217			0.9989

*: NA: number of alleles; AF: allele frequencies. The p-value obtained with Fisher's exact test, the bolded value was statistically significant. p_c: p-value after applying Bonferroni correction.

Table 3. Frequency of the HLA-DPB1 allele in epilepsy patients treated with carbamazepine.

No.	HLA-DPB1 allele	Hypersensitive case (n=21)		Tolerant cases (n=23)		OR (95% CI)	p-value	p _c -value
		NA	AF	NA	AF			
1	02:01:02	5	0.1190	3	0.0652	1.94 (0.45-7.651)	0.4710	0.7529
2	02:02:01	2	0.0476	1	0.0217	2.25 (0.25-33.24)	0.6039	0.6848
3	03:01:01	4	0.0952	2	0.0435	2.32 (0.51-12.58)	0.4192	0.6848
4	04:01:01	3	0.0714	5	0.1087	0.63 (0.16-2.66)	0.7158	0.7550
5	04:02:01	1	0.0238	-	-			
6	05:01:01	15	0.3571	15	0.3261	1.15 (0.45-2.71)	0.8238	0.9989
7	104:01:01	1	0.0238	2	0.0435	0.54 (0.04-4.78)	>0.9999	0.6868
8	105:01:01	-	-	1	0.0217			
9	107:01	-	-	1	0.0217			
10	13:01:01	5	0.1190	6	0.1304	0.90 (0.28-3.23)	>0.9999	0.9067
11	135:01	1	0.0238	-	-			
12	14:01:01	4	0.0952	3	0.0652	1.51 (0.38-6.26)	0.7049	0.8525
13	19:01:01	1	0.0238	1	0.0217	1.11 (0.06-21.27)	>0.9999	0.9989
14	21:01	-	-	3	0.0652			
15	235:01	-	-	1	0.0217			
16	31:01:01	-	-	2	0.0435			

*: NA: number of alleles; AF: allele frequencies; the p-value obtained with Fisher's exact test; p_c: p-value after applying Bonferroni correction.

Table 4. Comparison of HLA-DQB1*03:03 allele frequencies in some Vietnamese groups.

Group	Sample size	Genotyping method	AF	(4)	(5)		References
				<i>p-value</i>	<i>OR (95% CI)</i>	<i>p-value</i>	<i>OR (95% CI)</i>
Hypersensitive (1)	21	NGS	0.19	0.2311	1.69 (0.80-3.62)	0.3270	1.59 (0.69-3.83)
Tolerant (2)	23	NGS	0.04	0.1149	0.33 (0.08-1.18)	0.1236	0.31 (0.07-1.21)
Epilepsy (3)	44	NGS	0.11	>0.9999	0.92 (0.46-1.74)	0.8471	0.87 (0.42-1.88)
Vietnam Kinh DQB1 (4)	2076	SSOP	0.12				[18]
Vietnam Kinh (5)	101	NGS	0.13				[19]

*: (3)=(1) + (2); AF: allele frequencies.

Previous studies have reported data on the HLA-DQB1*03:03 allele using various research methods. To compare the frequency of this allele in Vietnamese groups, we searched information from the Allele Frequency Net Database and compared it to our own study. The results indicated that the frequency of the HLA-DQB1*03:03 allele in our study was consistent with previous publications (Table 4).

So far, we have not found any published data on the frequency of the HLA-DPB1 allele in the Vietnamese population, especially in Vietnamese patients with epilepsy.

3.3. Prediction of the carbamazepine interaction site on HLA-DQB1*03:03 molecular structure

The molecular structure of HLA-DQB1*03:03 was not available in the Protein Database bank (<http://www.rcsb.org/pdb/>), so SWISS-MODEL was used to model the structure of HLA-DQB1*03:03 based on the structure of the HLA-TCR complex (SMTL ID: 6px6.1) with sequence similarity up to 92.82% (Fig. 1A) [20]. The quality of the structure was confirmed using the SWISS-MODEL structural evaluation metrics [10]. The values of parameters GMQE=0.70 and QMEANDisCo Global=0.80±0.05 indicated that the model was sufficiently reliable and of high quality [11, 12]. The model quality was also evaluated based on the local similarity of the predicted protein to the corresponding target of each model residue (Fig. 1B) [21]. The results showed that the predictive model had good quality

(quality scores ranged from 0.8 to 1.0), however, there were two areas (positions 70-80 and 135-145) with low quality (scores lower than 0.6). Besides these, the QMEAN Z-Scores were also used to evaluate and validate the model [22]. The QMEAN Z value was equal to -0.98 and the set of Z values for various parameters including the interaction between the second carbon atoms (Cβ) was equal to -0.28; interaction between all atoms was 0.14; the solvation, -0.05, and the torsion, -0.97, were both small and close to 0, which indicated good quality of the model (Fig. 1C). Fig. 1D shows a histogram of the normalised QMEAN score based on protein size, relating the model quality score to the values obtained for experimental constructs of similar size. When compared to the non-redundant set of PDB structures, the HLA-DQB1*03:03 model's structure was found to be in the range |Z-score|<1, confirming reliability. In addition, Ramachandran analysis revealed that 95.86% of the amino acid residues were in regions corresponding to the quadratic structure of the Ramachandran plot, 0.00% of the radicals were in the outliers, and only 0.30% of the side chains of the sequence with low energy were A120 SER, further showing that the model had good quality (Fig. 1E) [23, 24]. Based on the above parameters, the HLA-DQB1*03:03 model was considered reliable.

Following the construction of the structural model of HLA-DQB1*03:03, molecular docking analysis was performed to evaluate the possibility of interaction between DQB1*03:03 protein and CBZ. The chemical

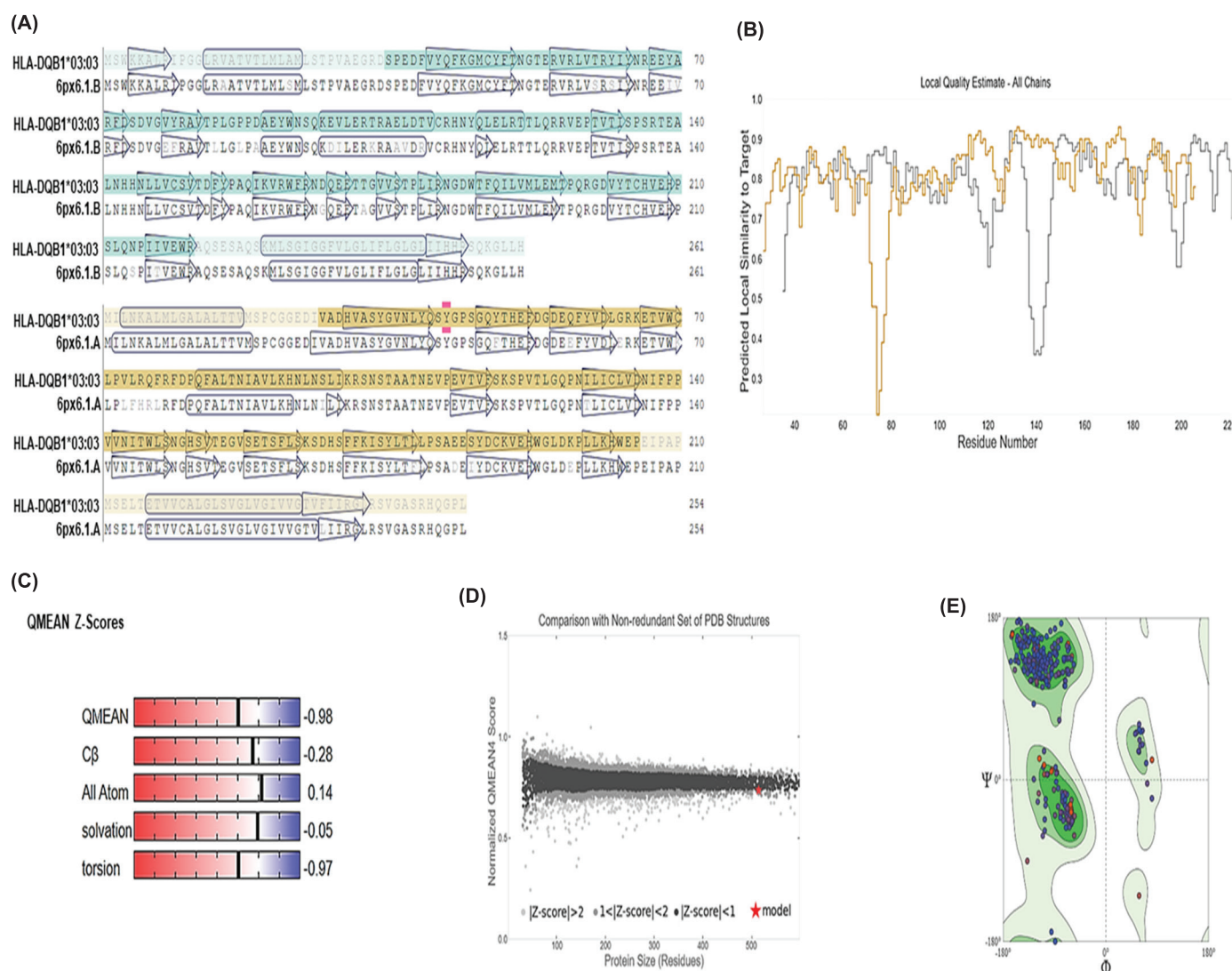


Fig. 1. HLA-DQB1*03:03 structure modelling using SWISS-MODEL Interactive Workspace. (A) Sequence alignment of target (HLA-DQB1*03:03) and template (HLA-TCR complex, SMTL ID: 6px6.1) by SWISS-MODEL; (B) Local quality estimate graph showing the values of the predicted local similarity to target (y-axis) plotted against the protein residue number (x-axis), this quality estimation was carried out on both chains of the target protein, with the yellow and grey lines representing chains (A) and (B), respectively; (C) QMEAN Z-Scores of the model; (D) Comparison with a non-redundant set of PDB structure (The x-axis shows protein length, represented by the number of residues). The y-axis was the normalized QMEAN score. Each dot represented an experimental protein structure, and the $|Z\text{-score}|$ indicated the standard deviation from the mean. DQB1*03:03 model is represented as a red star; (E) Ramachandran plot (the white area was the outlier, where there were almost no Φ/Ψ pairs; the light green area was the area created by the first contour line, where there were 99.7% of the Φ/Ψ pairs; the green area was the area created by the 2nd contour line, where 95.0% of the number of Φ/Ψ pairs were present, and the dark green area was the area of interest, created by the 3rd contour line, where there were 80.0% the number of Φ/Ψ pairs).

binding sites were predicted using the SwissDock software, and the results were visualised using the UCSF Chimera software [13-15]. In the results, 38 clusters were obtained with cluster rank, of which 6/38 clusters expressed CBZ interacting with HLA-DQB1*03:03 at the peptide binding cleft. The cluster with the lowest

molecular FullFitness and binding Estimated ΔG values were selected with indices of -1789.0076, kcal/mol and -6.703787 kcal/mol, respectively (Fig. 2A). CBZ binds to hydrophobic amino acids at the binding site and neutralises the potential on the β chain encoded by the HLA-DQB1*03:03:02 gene (Figs. 2B and 2C).

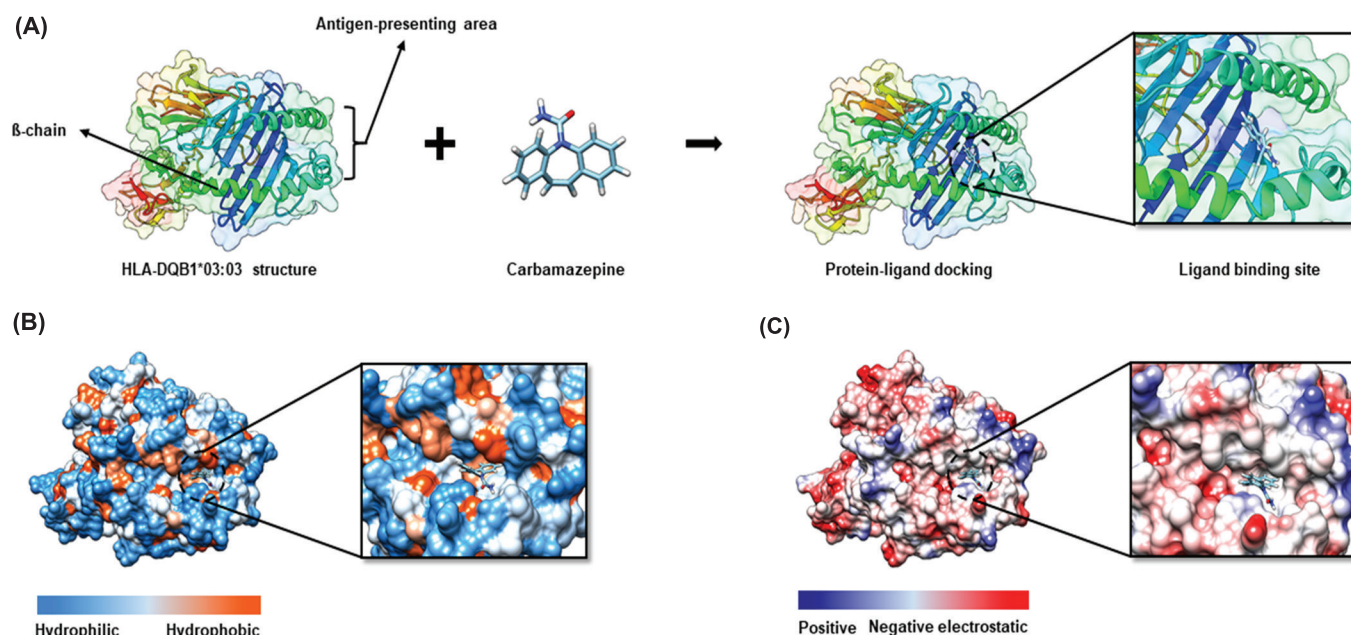


Fig. 2. Simulation of interaction between DQB1*03:03 protein and carbamazepine by molecular docking analysis. (A) The ribbon structure of the HLA-DQB1*03:03 molecule and its interaction site with carbamazepine; (B) The hydrophobic surface of the HLA-DQB1*03:03 molecule and interaction site with carbamazepine (the surface colour is mapped from red to blue to denote the most hydrophobic and hydrophilic regions, respectively); (C) The electrostatic surface of the HLA-DQB1*03:03 molecule and interaction site with carbamazepine (red the negative electrostatic potential energy values and in blue the positive values).

4. Discussion

CBZ is one of the most commonly prescribed antiepileptic drugs for the treatment of seizures. Patients who develop symptoms of hypersensitivity to the drug are often advised by their physician to discontinue its use to resolve the symptoms. For tolerant patients, the daily dose of CBZ may be increased, and the duration of dosing may be prolonged. Long-term use of antiepileptic drugs can lead to potentially toxic effects, especially haematological side effects. In this study, we found that patients in the SJS/TEN group who were taking carbamazepine had a significantly higher daily dose ($p=0.0394$) compared to the MCADR group. The observed severe drug reactions may be due to the high dose. This contradicts the results of a previous study by K.W. Chong, et al. (2014) [25], which showed that the SJS/TEN group had a lower median dose compared to the minor drug reactions group (0.2 mg/kg/day (range 4.6-7.4) and 6.7 mg/kg/day (range 3.6-20.0) for the two groups, respectively). However, our findings are consistent with those of V. Hariraj, et al. (2023) [26], who reported that carbamazepine-induced

severe cutaneous adverse drug reactions were 3.6 times more likely to occur in patients with a daily dose of 200 mg or less compared to those with a daily dose of 400 mg or more. Additionally, analytical results showed that haemoglobin (g/l) levels were increased in epileptic patients with high doses of CBZ. This contrasts with the results of O.S.E. Shimi, et al. (2021) [27], who suggested that haemoglobin content, platelet count, serum C4, and IgA levels were negatively correlated with CBZ use.

CBZ has been reported to cause serious adverse skin reactions in Vietnamese patients. In the HLA class I group, HLA-B*15:02 has been found to be associated with the incidence of severe cutaneous adverse reactions associated with CBZ treatment, while no association has been found between HLA-A and the reactions [28]. In our previous study (2023), we observed a higher prevalence of three alleles (HLA-B*46:01:01, HLA-DQA1*03:02:01, and HLA-DRB1*09:01:02) in the CBZ hypersensitivity group compared to the CBZ tolerant group when typing three loci of HLA class I (HLA-A, -B, and -C) and two loci of HLA class II (HLA-DQA1 and -DRB1). The

sensitivity and specificity of the HLA-B*46:01:01/HLA-DRB1*09:01:02 combination with respect to CBZ hypersensitivity diagnosis were 46.88 and 84.85%, respectively. These findings suggest that the presence of HLA-B*46:01:01/HLA-DRB1*09:01:02 may serve as a potential marker for CBZ-induced hypersensitivity reactions in Vietnamese patients [29]. In the present study, we found that the allele DQB1*03:03:02 was significantly associated with hypersensitivity symptoms in Vietnamese patients treated with CBZ ($p=0.0431$). However, this significance was lost after applying the Bonferroni correction. This is consistent with the findings of X.J. He, et al. (2013) [30] in the Han ethnic group of northeastern China ($p=0.022$, after applying Bonferroni correction $p_c>0.05$).

An association between the HLA allele and adverse drug reactions has been identified in several ethnicities. Several studies have evaluated the association between haplotype and drug-induced hypersensitivity reactions due to linkage disequilibrium in the HLA region. A previous study showed a relatively high risk (16.09) of haplotype HLA-A*24:02~B*59:01~C*01:02 carriers in 15 Japanese patients exhibiting CBZ-induced hypersensitivity reactions (10 patients with mild symptoms and 5 patients with SJS) [31]. In 21 Mexican Mestizo patients with epilepsy, haplotype A*02:01:01~B*35:01:01~C*04:01:01 was found to be associated with lamotrigine-induced macular rash ($p=0.0048$) [32]. In our study, the frequency of haplotype DQB1*03:03:02-DPB1*05:01:01 was 0.095 in the hypersensitivity group and 0.022 in the tolerance group; however, there was no statistically significant difference between the two groups ($p=0.1881$). Additionally, due to the lack of publicly available HLA-DPB1 data for the Vietnamese population, a comparison with the control group was not possible.

Currently, studies show that the hydrophobicity and electrical charge of amino acids greatly affect the strength of protein molecules, especially the hydrophobicity of the ligand-HLA complex. However, reports directly related to drug interactions of HLA class II molecules are still limited, necessitating additional research [33-35]. In general, the binding of CBZ to HLA is probably

weaker than natural and drug-target interactions because the HLA binding site does not spontaneously evolve to recognise the drug [28]. This poses challenges for drug combinations as fewer interactions can be formed between the drug and the HLA recognition site. However, finding specific alleles and modelling the interactions between them are essential because of their application in disease screening and treatment.

Several limitations need to be rectified by future research. Firstly, because the sample size is relatively small, larger studies should be conducted to clarify the findings in this study more thoroughly. Secondly, there is not enough data to analyse the haplotype, more data is needed for analysis. Thirdly, the exclusion of patients without genotyping results is another limitation, and future endeavours with more financial support should aim to include more patients to obtain a more comprehensive understanding and provide data that can reflect the whole population.

5. Conclusions

Genetic susceptibility to the risk of carbamazepine-induced hypersensitivity reactions in Vietnamese patients with epilepsy is not found in the HLA DPB1 region. The frequency of the HLA-DQB1*03:03:02 allele was higher in the hypersensitivity group than in the tolerant group ($p=0.0431$) although this difference was not statistically significant after applying the Bonferroni correction and the evidence of *in silico* interaction between CBZ and HLA-DQB1*03:03 at the peptide binding cleft suggested that HLA-DQB1*03:03:02 allele exhibited a tendency to behave as a susceptibility factor for developing carbamazepine-induced hypersensitivity reactions in Vietnamese patients with epilepsy.

CRedit author statement

Thi Thanh Nhan Le: Methodology, Formal analysis, Original draft preparation, Visualisation; Thi Tu Linh Nguyen, Lan Phuong Le, Doan Thuy Nguyen, Van Lieu Nguyen, Thi Van Anh Nguyen: Conceptualisation, Data curation, Investigation, Visualisation, Formal analysis; Hong Thai Trinh: Supervision, Validation, Writing - Reviewing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] World Health Organization (2022), *Epilepsy*, <https://www.who.int/en/news-room/fact-sheets/detail/epilepsy>, accessed 19 September 2022.
- [2] N.A. Tuan, L.Q. Cuong, P. Allebeck, et al. (2008), "The prevalence of epilepsy in a rural district of Vietnam: A population-based study from the EPIBAVI project", *Epilepsia*, **49(9)**, pp.1634-1637, DOI: 10.1111/j.1528-1167.2008.01663.x.
- [3] H. Arif, R. Buchsbaum, D. Weintraub, et al. (2007), "Comparison and predictors of rash associated with 15 antiepileptic drugs", *Neurology*, **68(20)**, pp.1701-1709, DOI: 10.1212/01.wnl.0000261917.83337.db.
- [4] F.J.F. Fernández, J.A. Garrido, M. Hauben, et al. (1995), "Severe adverse cutaneous reactions to drugs", *The New England Journal of Medicine*, **332(14)**, pp.959-960, DOI: 10.1056/NEJM199504063321414.
- [5] J. Trowsdale (2011), "The MHC, disease and selection", *Immunology Letters*, **137(1-2)**, pp.1-8, DOI: 10.1016/j.imlet.2011.01.002.
- [6] J.H. Karnes, L. Bastarache, C.M. Shaffer, et al. (2017), "Phenome-wide scanning identifies multiple diseases and disease severity phenotypes associated with HLA variants", *Science Translational Medicine*, **9(389)**, pp.139-148, DOI: 10.1126/scitranslmed.aai8708.
- [7] W.G. Horta, E. Paradela, A. Figueiredo, et al. (2015), "Genetic association study of the HLA class II alleles DRB1, DQA1, and DQB1 in patients with pharmacoresistant temporal lobe epilepsy associated with mesial hippocampal sclerosis", *Seizure*, **31**, pp.7-11, DOI: 10.1016/j.seizure.2015.06.005.
- [8] A. Fadda, M. Alsabbagh, D. Vasudeva, et al. (2022), "The complex etiology of Epilepsy: Genetic analysis and HLA association in patients in the Middle East", *Research Square*, pp.1-14, DOI: 10.21203/rs.3.rs-2358929/v1.
- [9] T. Schwede, J. Kopp, N. Guex, et al. (2003), "SWISS-MODEL: An automated protein homology-modeling server", *Nucleic Acids Research*, **31(13)**, pp.3381-3385, DOI: 10.1093/nar/gkg520.
- [10] A. Waterhouse, M. Bertoni, S. Bienert, et al. (2018), "SWISS-MODEL: Homology modelling of protein structures and complexes", *Nucleic Acids Research*, **46(Web Server Issue)**, pp.W296-W303, DOI: 10.1093/nar/gky427.
- [11] M. Biasini, M. Bertoni, A. Waterhouse, et al. (2014), "SWISS-MODEL: Modelling protein tertiary and quaternary structure using evolutionary information", *Nucleic Acids Research*, **42(Web Server Issue)**, pp.W252-W258, DOI: 10.1093/nar/gku340.
- [12] G. Studer, C. Rempfer, A.M. Waterhouse, et al. (2020), "QMEANDisCo-distance constraints applied on model quality estimation", *Bioinformatics*, **36(6)**, pp.1765-1771, DOI: 10.1093/bioinformatics/btz828.
- [13] A. Grosdidier, V. Zoete, O. Michielin (2011), "SwissDock, a protein-small molecule docking web service based on EADock DSS", *Nucleic Acids Research*, **39**, Suppl. 2, pp.W270-W277, DOI: 10.1093/nar/gkr366.
- [14] A. Grosdidier, V. Zoete, O. Michielin (2007), "EADock: Docking of small molecules into protein active sites with a multiobjective evolutionary optimization", *Proteins*, **67(4)**, pp.1010-1025, DOI: 10.1002/prot.21367.
- [15] E.F. Pettersen, T.D. Goddard, C.C. Huang, et al. (2004), "UCSF Chimera - A visualization system for exploratory research and analysis", *Journal of Computational Chemistry*, **25(13)**, pp.1605-1612, DOI: 10.1002/jcc.20084.
- [16] J.M. Nunes, S. Buhler, D. Roessli, et al. (2014), "The HLA-net GENE[RATE] pipeline for effective HLA data analysis and its application to 145 population samples from Europe and neighbouring areas", *Tissue Antigens*, **83(5)**, pp.307-323, DOI: 10.1111/tan.12356.
- [17] F.F.G. Galarza, S. Christmas, D. Middleton, et al. (2011), "Allele frequency net: A database and online repository for immune gene frequencies in worldwide populations", *Nucleic Acids Research*, **39**, Suppl. 1, pp.913-919.
- [18] T.N. Que, N.B. Khanh, P.D. Tung, et al. (2022), "Frequency and distribution of HLA-DQB1 alleles from 2076 cord blood samples of the Vietnamese cohort", *International Journal of Immunogenetics*, **49(5)**, pp.340-344, DOI: 10.1111/iji.12592.
- [19] D.M. Duc, L.H. G. Linh, N.T. Vinh, et al. (2020), "High-resolution HLA typing of HLA-A, -B, -C, -DRB1, and -DQB1 in Kinh Vietnamese by using Next-Generation sequencing", *Frontiers in Genetics*, **11**, pp.1-10, DOI: 10.3389/fgene.2020.00383.

- [20] H.M. Berman, J. Westbrook, Z. Feng, et al. (2000), "The protein data bank", *Nucleic Acids Research*, **28**(1), pp.235-242, DOI: 10.1093/nar/28.1.235.
- [21] P. Benkert, M. Biasini, T. Schwede (2011), "Toward the estimation of the absolute quality of individual protein structure models", *Bioinformatics*, **27**(3), pp.343-350, DOI: 10.1093/bioinformatics/btq662.
- [22] P. Benkert, S.C.E. Tosatto, D. Schomburg (2008), "QMEAN: A comprehensive scoring function for model quality assessment", *Proteins: Structure, Function, and Genetics*, **71**(1), pp.261-277, DOI: 10.1002/prot.21715.
- [23] V.B. Chen, W.B. Arendall, J.J. Headd, et al. (2010), "MolProbity: All-atom structure validation for macromolecular crystallography", *Acta Crystallographica. Section D, Biological Crystallography*, **66**(1), pp.12-21, DOI: 10.1107/S0907444909042073.
- [24] S. Liang, N.V. Grishin (2002), "Side-chain modelling with an optimized scoring function", *Protein Sci.*, **11**(2), pp.322-331, DOI: 10.1110/ps.24902.
- [25] K.W. Chong, D.W.S. Chan, Y.B. Cheung, et al. (2014), "Association of carbamazepine-induced severe cutaneous drug reactions and HLA-B*1502 allele status, and dose and treatment duration in paediatric neurology patients in Singapore", *Archives of Disease in Childhood*, **99**(6), pp.581-584, DOI: 10.1136/archdischild-2013-304767.
- [26] V. Hariraj, W.K. Wo, S.C. Lee, et al. (2023), "Carbamazepine-induced severe cutaneous adverse drug reactions: A 21-year comparison between children and adults in Malaysia", *The Journal of Clinical Pharmacology*, **63**(10), pp.1126-1132, DOI: 10.1002/jcph.2289
- [27] O.S.E. Shimi, A.A. Farag, A.M.E. Rebigi, et al. (2021), "Carbamazepine-induced hematological and immunological alterations in Egyptian children with idiopathic generalized seizures", *Journal of Child Science*, **11**(1), pp.e265-e272, DOI: 10.1055/s-0041-1736156.
- [28] N.V. Dinh, C. Vidal, H.C. Chu, et al. (2019), "Developing pharmacogenetic screening methods for an emergent country: Vietnam", *World Allergy Organization Journal*, **12**(5), DOI: 10.1016/j.waojou.2019.100037.
- [29] B.P. Thao, N.T.T. Linh, L.L. Phuong, et al. (2023), "Next-generation sequencing-based HLA typing reveals the association of HLA-B*46:01:01 and HLA-DRB1*09:01:02 alleles with carbamazepine-induced hypersensitivity reactions in Vietnamese patients with epilepsy", *Human Immunology*, **84**(3), pp.186-195, DOI: 10.1016/j.humimm.2023.01.005.
- [30] X.J. He, L.Y. Jian, X.L. He, et al. (2013), "Association between the HLA-B 15:02 allele and carbamazepine-induced Stevens-Johnson syndrome/toxic epidermal necrolysis in Han individuals of northeastern China", *Pharmacological Reports*, **65**(5), pp.1256-1262, DOI: 10.1016/s1734-1140(13)71483-x.
- [31] H. Ikeda, Y. Takahashi, E. Yamazaki, et al. (2010), "HLA class I markers in Japanese patients with carbamazepine-induced cutaneous adverse reactions", *Epilepsia*, **51**(2), pp.297-300, DOI: 10.1111/j.1528-1167.2009.02269.x.
- [32] I.F. Galindo, I.E.M. Juárez, N.M. Jaramillo, et al. (2014), "HLA-A*02:01:01/-B*35:01:01/-C*04:01:01 haplotype associated with lamotrigine-induced maculopapular exanthema in Mexican Mestizo patients", *Pharmacogenomics*, **15**(15), pp.1881-1891, DOI: 10.2217/pgs.14.135.
- [33] J. Kraml, A.S. Kamenik, F. Waibl, et al. (2019), "Solvation free energy as a measure of hydrophobicity: Application to serine protease binding interfaces", *Journal of Chemical Theory and Computation*, **15**(11), pp.5872-5882, DOI: 10.2217/pgs.14.135.
- [34] J. Kalayan, R.H. Henchman, J. Warwicker (2020), "Model for counterion binding and charge reversal on protein surfaces", *Molecular Pharmaceutics Journal*, **17**(2), pp.595-603, DOI: 10.1021/acs.molpharmaceut.9b01047.
- [35] D. Chowell, S. Krishna, P.D. Becker, et al. (2015), "TCR contact residue hydrophobicity is a hallmark of immunogenic CD8+ T cell epitopes", *Proceedings of The National Academy of Sciences of The United States of America*, **112**(14), pp.E1754-E1762, DOI: 10.1073/pnas.1500973112.

Chromosome 15q13.1q13.2 microdeletion in a foetus with severe congenital heart defects: A case report

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Abstract:

Congenital heart disease (CHD) is a complex condition affecting the heart's structure and function, leading to serious health problems. While the cause of CHD is not well understood, researchers have identified copy number variations (CNVs) as potential contributors to its pathogenesis. One such CNV is the rare chromosome 15q13 microdeletion, but its role in CHD remains unclear. In a 22-week gestation foetus, severe congenital heart defects were detected by colour Doppler ultrasound imaging. Array comparative genomic hybridisation (aCGH) was performed, revealing a 1.1 Mb deletion of the chromosome 15q13.1-q13.2 region containing five genes (*APBA2*, *NSMCE3*, *TJPI*, *FAM189A1*, *LOC100130111*). However, further chromosomal G-banding revealed no abnormal karyotype. Parental aCGH testing determined that this mutation was de novo. This report demonstrates the potential association between chromosome 15q13.1-q13.2 microdeletion and foetal congenital heart defects for the first time. The use of aCGH in foetuses with abnormal cardiac development diagnosed by routine cardiac colour Doppler ultrasound imaging is recommended for the early detection of congenital genetic abnormalities. This information can provide valuable insight for prenatal diagnostic consultation and decisions regarding pregnancy termination.

Keywords: array, chromosome, comparative, congenital, defects, genomic, heart, hybridisation, microdeletion, 15q13.1-q13.2.

Classification numbers: 3.2, 3.6

1. Introduction

Congenital heart disease is a significant health concern as it is the primary cause of birth defects and the leading cause of infant mortality after excluding infectious aetiologies [1]. With an incidence ranging from 19 to 75 per thousand live births and present in an even greater proportion of miscarriages, CHD is a major contributor to childhood morbidity and mortality worldwide. Despite its prevalence, the cause of the majority of CHD cases remains unknown, although genetic factors are increasingly recognised as playing a significant role [2]. Furthermore, approximately 30% of children with abnormal chromosomal numbers, or

aneuploidy, will develop CHD [3]. Understanding the genetic basis of CHD is crucial for developing effective prevention, diagnosis, and treatment strategies for this devastating condition.

The advancement of technology has led to the identification of submicroscopic CNVs associated with genetic disorders through the use of high-resolution microarrays in clinical analysis. Several studies have found that patients with CHD and other anomalies are highly likely to carry previously undetected CNVs, leading to the identification of new disease-related genes or loci for various cardiac defects [4, 5].

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Microdeletions, a type of CNV, can encompass multiple genes. Although they are uncommon in chromosome 15q13, their contribution to CHD remains incompletely elucidated.

In the presented case, an amniotic sample was obtained from a 22-week-old foetus exhibiting numerous congenital heart abnormalities. The sample was analysed using aCGH, which revealed a 1.1 Mb deletion on the long arm of chromosome 15 (46, XX [del (15). q13.1-q13.2]). Further investigation confirmed that

this deletion occurred spontaneously. It is therefore hypothesised that this mutation is solely responsible for the observed congenital heart defects.

2. Case presentation

A 29-year-old pregnant woman (G1P0) was admitted to the hospital for evaluation at 22 weeks of gestation. Foetal echocardiography was performed using 2-dimensional, M-mode, and Doppler imaging. A colour Doppler ultrasound revealed several heart abnormalities, including dextrocardia, a significant atrioventricular



Fig. 1. Colour Doppler ultrasound imaging showing (A) dextrocardia, significant atrioventricular septal defect and (B) aortic valve stenosis, (C) pulmonary valve stenosis.

septal defect (AVSD), and stenosis of the aortic and pulmonary valves (Fig. 1). The ventricular septum was examined carefully for cardiac wall defects, from the apex to the crux. Septal defects may be difficult to detect. The septum was detected when the ultrasound section was perpendicular to it, starting from a four-chamber view to obtain the normal cross-over of the aorta and main pulmonary artery at their origin. Details of the pulmonary artery bifurcation can also be seen. Other parts of the foetus showed no abnormalities. Ultrasound was performed on a GE Volution E10 machine from a healthcare company.

Approximately 20 ml of amniotic fluid was collected using ultrasound-guided transabdominal puncture. The fluid underwent conventional chromosomal G-banding karyotype analysis and aCGH analysis with CytoGenomic version 5.0, compared with GRCh37/hg19. The G-banding analysis revealed a 46, XY karyotype with no abnormalities detected. However, the aCGH analysis identified a 1.1 Mb deletion in the chromosome 15q13.1-q13.2 region (arr[GRCh37] 15q13.1q13.2(29253317_30366124)x1) containing five genes (*APBA2*, *NSMCE3*, *TJP1*, *FAM189A1*, *LOC100130111*). This deletion occurred between BP3-BP4 on chromosome 15q13.1-q13.2 (Fig. 2).

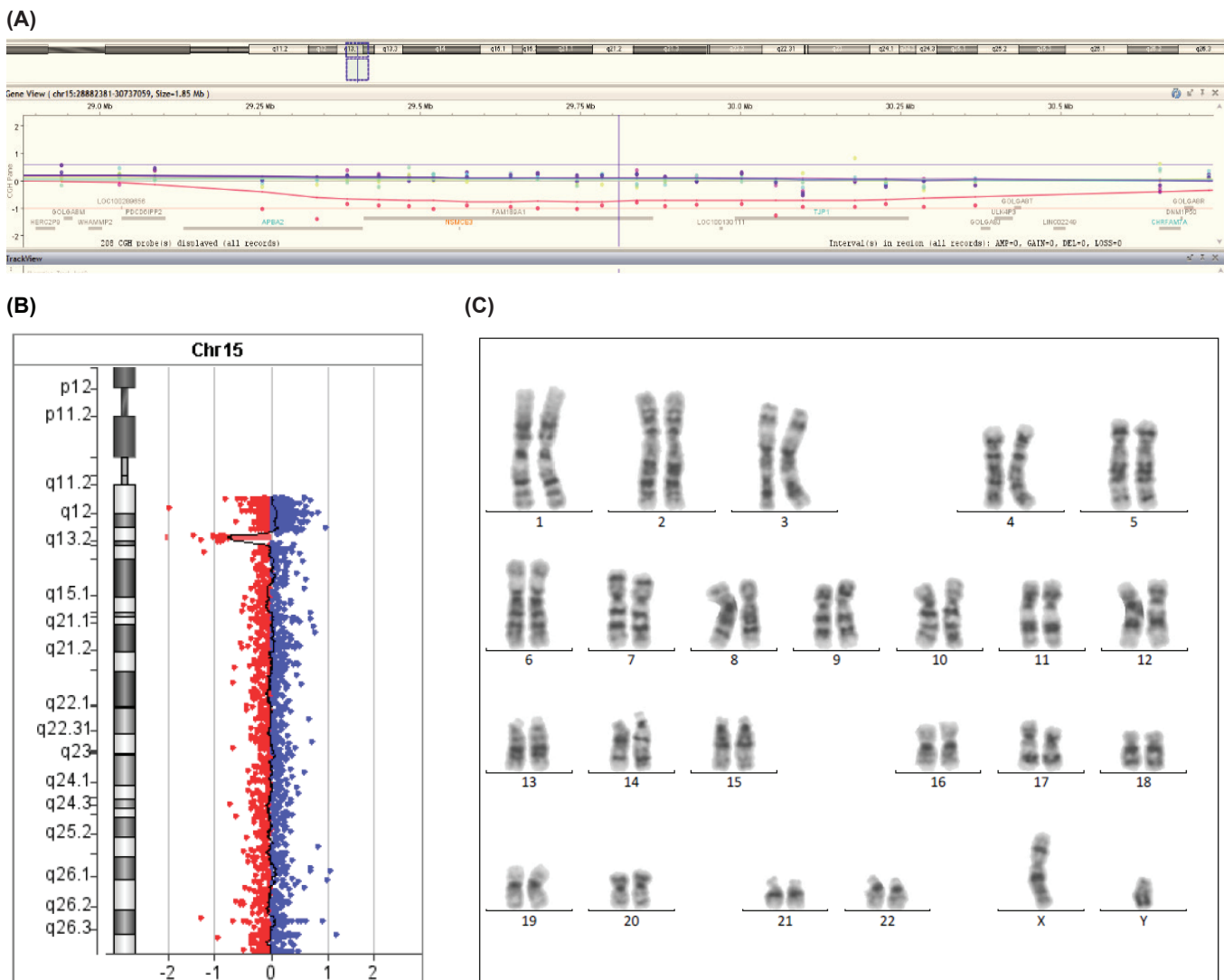


Fig. 2. Karyotype and array comparative genomic hybridisation analysis detects 1.1 Mb-deletion of the chromosome 15q13.1-q13.2 region containing 5 genes. (A) Comparison of 8 samples, (B) Deletion of chromosome 15, and (C) Normal foetus's karyotype.

Peripheral blood samples were taken from the foetus' parents and subjected to aCGH analysis, which did not identify any mutations. These results suggest that the foetus' chromosome with the microdeletion was not inherited from the parents. The pregnant woman reported having a regular menstrual cycle and a natural, uneventful pregnancy. She had no history of smoking, alcohol consumption, viral infections, or a family history of foetal anomalies. Additionally, she denied taking any oral medications during her pregnancy. Non-invasive prenatal testing was also conducted during the pregnancy, revealing a low risk. The pregnant woman and her family decided to terminate the pregnancy at 23 weeks of gestation. Unfortunately, we do not have the foetal pathology results after delivery because the family did not agree to an autopsy on the foetus.

3. Discussion

Genomic regions flanked by low-copy repeats (LCRs, or segmental duplications) with DNA sequence identity greater than 95-97% are prone to recurrent microdeletions, microduplications, and inversions mediated by non-allelic homologous recombination (NAHR) [6]. One of the most unstable regions in the human genome is the LCR-rich proximal region of chromosome 15 (15q11-q14), which is associated with various DNA copy-number variations (CNVs) such as deletions, duplications, inversions, translocations, and supernumerary inv dup(15) chromosomes [7]. The breakpoints of these rearrangements occur within complex sets of LCRs known as BP1 to BP6 [8].

Prader-Willi syndrome (PWS) and Angelman syndrome usually result from distinct parental-origin deletions that encompass the distal breakpoint BP3, along with proximal breakpoints BP1 or BP2 [8]. Another study on the UK Biobank revealed that the deletion of the 15q11.2 region spanning from BP1 to BP2, also known as the Burnside-Butler syndrome susceptibility locus, has been previously linked to various phenotypes, including developmental delay, autism, schizophrenia, and congenital cardiovascular malformation [9]. Supernumerary inv dup (15) chromosomes, also referred to as inverted duplications, are frequent rearrangements found in the 15q11-q13 region. These large inv dup (15) chromosomes are exclusively derived from the maternal side and have been observed in patients exhibiting facial dysmorphism, intellectual disability, severe epilepsy, and often autistic behaviour [10]. Duplications

and inv dup (15) chromosomes commonly involve the breakpoints BP4 and BP5, which are characterised by duplicons consisting exclusively of LCR15 elements. These duplicons at BP4 and BP5 play a significant role in triplications and inv dup (15) chromosome rearrangements [7].

The consequence of microdeletion between BP3 and BP4 is rarely reported. The presence of shared clinical features among individuals with this deletion and the existence of genes involved in development and nervous system function within the deleted region suggest that this deletion potentially contributes to abnormal phenotypes in certain individuals [11].

This patient with congenital heart defect (CHD) was found to have a 1.1 Mb deletion of the chromosome 15q13.1-q13.2 region between BP3-BP4. A previous study in 2019 reported a case with two microdeletions on 15q13.2 and 15q13.3, corresponding to two intervals between BP3 and BP4, BP4 and BP5. The patient exhibited symptoms of microdeletion 15q13.3 syndrome and CHD, specifically a redundant mildly prolapsed mitral valve and mildly thickened cardiac muscle [12]. In addition, a study in 2020 described four members of a family with a microdeletion from BP3 to BP5, two of whom had CHD [13]. Furthermore, C. Lowther, et al. (2015) [14] proved that no significant differences were identified when analysing the clinical features of patients in groups with deletions in BP4-BP5, BP3-BP4, and BP3-BP5. Therefore, it can be hypothesised that the heart defect in the present case is caused by the 15q13.2 microdeletion situated between BP3 and BP4.

4. Conclusions

This deletion variant on the long arm of chromosome 15 identified by aCGH technique in this paper is the first reported case in the literature. This variant may be associated with severe heart defects. It contributes to the common data sources, providing clinicians and geneticists with additional basis for genetic counselling in general, and especially in cases of prenatal diagnosis of heart defects.

CRedit author statement

Thi Ha Vu: Performing the study, Data collection and analysis, Original draft preparation; Thi Hai Hoang, Thi Sim Nguyen, Khanh Dung Ho: Clinical diagnosis, Data collection; Thi Kim Phuong Doan: Conceiving and designing the study, Writing - Reviewing and Editing.

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Availability of data and materials

The data reported in this study are available upon request by contact with the corresponding author.

Ethics approval and consent to participate

This study was approved by the Ethics Review Committee of Hanoi Medical University Hospital affiliated with Hanoi Medical University (Approval No. 106/GCN-HDDNCYSH_DHYHN, 24/06/2020). Written informed consent was obtained from the foetus's parents following a detailed description of the study's purpose. All experimental procedures described in this study were by international and national laws, regulations, and guidelines.

Consent for publication

The pregnant woman and her husband involved in this study agreed to publish related demographic and clinical features.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] A.C. Fahed, B.D. Gelb, J.G. Seidman, et al. (2013), "Genetics of congenital heart disease: The glass half empty", *Circ. Res.*, **112**(4), pp.707-720, DOI: 10.1161/CIRCRESAHA.112.300853.

[2] G.M. Blue, E.P. Kirk, G.F. Sholler, et al. (2012), "Congenital heart disease: Current knowledge about causes and inheritance", *Med. J. Aust.*, **197**(3), pp.155-159, DOI: 10.5694/mja12.10811.

[3] C. Liao, F. Fu, R. Li, et al. (2014), "Implementation of high-resolution SNP arrays in the investigation of fetuses with ultrasound malformations: 5-years of clinical experience", *Clin. Genet.*, **86**(3), pp.264-269, DOI: 10.1111/cge.12271.

[4] C.P. Schaaf, J. Wiszniewska, A.L. Beaudet (2011), "Copy number and SNP arrays in clinical diagnostics", *Annu. Rev. Genomics Hum. Genet.*, **12**, pp.25-51, DOI: 10.1146/annurev-genom-092010-110715.

[5] P. Quaresima, V. Fesslova, A. Farina, et al. (2023), "How to do a fetal cardiac scan", *Arch. Gynecol. Obstet.*, **307**(4), pp.1269-1276, DOI: 10.1007/s00404-023-06951-8.

[6] W. Gu, F. Zhang, J.R. Lupski (2008), "Mechanisms for human genomic rearrangements", *PathoGenetics*, **1**(1), DOI: 10.1186/1755-8417-1-4.

[7] M.A. Pujana, M. Nadal, M. Guitart, et al. (2002), "Human chromosome 15q11-q14 regions of rearrangements contain clusters of LCR15 duplicons", *Eur. J. Hum. Genet.*, **10**(1), pp.26-35, DOI: 10.1038/sj.ejhg.5200760.

[8] D.M. Cox, M.G. Butler (2015), "The 15q11.2 BP1-BP2 microdeletion syndrome: A review", *Int. J. Mol. Sci.*, **16**(2), pp.4068-4082, DOI: 10.3390/ijms16024068.

[9] S.G. Williams, A. Nakev, H. Guo, et al. (2020), "Association of congenital cardiovascular malformation and neuropsychiatric phenotypes with 15q11.2 (BP1-BP2) deletion in the UK Biobank", *Eur. J. Hum. Genet.*, **28**(9), pp.1265-1273, DOI: 10.1038/s41431-020-0626-8.

[10] C.M. Ravix, D. Depetris, J.J. Luciani, et al. (2007), "Recurrent rearrangements in the proximal 15q11-q14 region: A new breakpoint cluster specific to unbalanced translocations", *Eur. J. Hum. Genet.*, **15**(4), pp.432-440, DOI: 10.1038/sj.ejhg.5201775.

[11] J.A. Rosenfeld, L.E. Stephens, J. Coppinger, et al. (2011), "Deletions flanked by breakpoints 3 and 4 on 15q13 may contribute to abnormal phenotypes", *Eur. J. Hum. Genet. EJHG*, **19**(5), pp.547-554, DOI: 10.1038/ejhg.2010.237.

[12] M. Alsagob, M.A. Salih, M.H.A. Hamad, et al. (2019), "First report of two successive deletions on chromosome 15q13 cytogenetic bands in a boy and girl: Additional data to 15q13.3 syndrome with a report of high IQ patient", *Mol. Cytogenet.*, **12**(21), DOI: 10.1186/s13039-019-0432-6.

[13] P. Pavone, M. Ruggieri, S.D. Marino, et al. (2020), "Chromosome 15q BP3 to BP5 deletion is a likely locus for speech delay and language impairment: Report on a four-member family and an unrelated boy", *Mol. Genet. Genomic Med.*, **8**(4), DOI: 10.1002/mgg3.1109.

[14] C. Lowther, G. Costain, D.J. Stavropoulos, et al. (2015), "Delineating the 15q13.3 microdeletion phenotype: A case series and comprehensive review of the literature", *Genet. Med. Off. J. Am. Coll. Med. Genet.*, **17**(2), pp.149-157, DOI: 10.1038/gim.2014.83.

Antibacterial activity and preservative effect for pork tenderloin of chitosan - glucosamine Maillard reaction products prepared by gamma irradiation

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Abstract:

A mixed solution of chitosan (CTS) (1.6%) and glucosamine (GA) (0.4%) was irradiated by gamma rays from a Co-60 source to form Maillard reaction products (MRPs). Upon gamma-ray irradiation, the colour of the mixture solution became browner. The resultant MRP solution was characterised by spectrophotometric analyses at the wavelengths of 284 nm and 420 nm. The antibacterial activity of the CTS-GA MRP solution was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method, and the minimum inhibitory concentration (MIC) value against these bacteria was also determined. The results showed that the CTS-GA MRP solution exhibited high antibacterial activity against both bacteria. Furthermore, pork tenderloin soaked in CTS-GA MRP solution for 10 min could extend the storage time by 8 days at 5°C, with higher protein content and lower total aerobic microorganism count than the control. Therefore, this CTS-GA MRP solution has the potential to be applied as a natural preservative agent for meat and meat products.

Keywords: antibacterial effect, chitosan, glucosamine, Maillard reaction, pork tenderloin.

Classification numbers: 3.4, 3.5, 3.6

1. Introduction

Currently, foodborne illnesses caused by pathogens represent a global challenge with significant implications for human health. According to the World Health Organisation (WHO), foodborne illnesses are responsible for 420,000 deaths worldwide annually from 2007 to 2015 [1]. Bacteria, especially *Salmonella typhi*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7, are the causative agents for two-thirds of human foodborne illnesses. Additionally, meat, dairy products, and eggs are the main foods that expose people to these zoonotic bacteria [2]. On the other hand, food waste and food loss, estimated at 1.3 million tonnes annually, pose challenges to food security [3]. Hence, more effort is needed to develop new preservation methods, prevent foodborne

illnesses, and reduce food waste. Due to the widespread use of artificial preservatives and consumers' growing concerns about health issues, natural antibacterial agents have been explored as a potential solution for food preservation.

Chitosan, a cationic polysaccharide commercially prepared by alkaline deacetylation of chitin, has been receiving significant attention due to its excellent biological properties [4, 5]. Besides being biodegradable, biocompatible, and non-toxic, this polysaccharide also exhibits antibacterial, antioxidant [6, 7], fat uptake [8], emulsification [9], and edible film-forming properties [10]. Furthermore, CTS derived from shrimp has been recognised as Generally Recognized as Safe (GRAS) in the USA since 2001, thus broadening its applications in

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many fields, including food applications [7]. In fact, in recent decades, the applications of CTS as a preservative for many types of food have been widely reported in many studies, such as for fruits and vegetables [11, 12], seafood [13], and meat and meat products [14, 15]. Nevertheless, applications of CTS in many fields are still restricted due to its insolubility in water and its reduced biological activities at neutral or basic pH [4]. Therefore, many efforts have been made to improve the solubility and biological activities of CTS through chemical or enzymatic modifications, with chemical modifications generally being less preferred in food applications [5].

The Maillard reaction (MR), a non-enzymatic browning reaction, is a condensation reaction between the carbonyl groups of reducing sugars and the amino groups of amino acids, proteins, or any nitrogenous compounds through heating or irradiation [16]. This complex reaction occurs spontaneously during thermal food processing and forms a myriad of MRPs, which contribute to flavour formation and generate antibacterial and antioxidant compounds for food [17]. Additionally, it is generally agreed that a substantial amount of MRPs is taken up in the human daily diet [18]. Hence, MR is considered a desirable green method to improve the properties of CTS for food applications. Among the MRPs of CTS with various reducing polysaccharides, CTS-GA MRPs prepared by heat-induced MR were reported to exhibit impressive solubility and antibacterial activity in comparison with other MRPs and the native CTS [4]. More interestingly, it was also found that besides the heat-induced method, MR could take place rapidly by gamma-ray irradiation at room temperature without forming undesirable toxic by-products, such as 5-hydroxymethylfurfural [19-21]. In this study, the CTS-GA MRPs were prepared by the gamma Co-60 ray irradiation method, and the *in vitro* antibacterial activity and the preservative effect on fresh pork tenderloin during refrigerated storage were investigated.

2. Materials and methods

2.1. Materials

Shrimp-shell CTS with an average molecular weight of ~97 kDa and a degree of deacetylation of ~90% was supplied by Sun Eco Green Import Export Company Limited, Vietnam. GA was purchased from Merck, Germany. *Escherichia coli* ATCC 51813 and *Staphylococcus aureus* ATCC 25923 were provided by the Metabolic Biology Laboratory, University of Science, Vietnam National University - Ho Chi Minh

City. The Mueller Hinton (MH) medium and agar were purchased from Himedia Laboratories Ltd, India. 5-Hydroxymethylfurfural was supplied by Sigma-Aldrich, USA. Other chemicals such as lactic acid, NaOH in analytical grade, and distilled water were used for all experiments.

2.2. Preparation of chitosan-glucosamine maillard reaction product solution

The CTS-GA MRP solution was prepared according to our previous study [22]. Briefly, CTS was dissolved in a 1% lactic acid solution to obtain a 2% CTS solution. A 2% GA solution in distilled water was also prepared. Four volumes of 2% CTS solution were mixed with one volume of 2% GA solution to obtain a mixture of CTS (1.6%) and GA (0.4%). This mixture was then exposed to γ -rays from a Co-60 source of a Gamma-cell 5000 (BRIT, India) with a dose of 25 kGy at a dose rate of 1.3 kGy/h to perform the MR. The irradiated solution was characterised by spectrophotometric analyses, with the absorbance of the solution after appropriate dilution measured respectively at 284 nm (intermediate MRPs) and 420 nm (late MRPs) using a UV-Vis spectrophotometer (Jasco-V630, Japan) [19, 20].

2.3. Evaluation of antibacterial activity

E. coli ATCC 51813 and *S. aureus* ATCC 25923 were used to evaluate the antibacterial activity of the CTS-GA MRP solution in both qualitative and quantitative tests. In the qualitative test, the antibacterial activity was investigated by the agar well diffusion method [23]. Briefly, MH agar plates, after being spread with *E. coli* or *S. aureus* ($\sim 10^4$ CFU/ml), were punched aseptically to form wells with a diameter of ~5 mm. CTS-GA MRP solution (100 μ l) was introduced into a well. GA solution and CTS-GA mixture solution were also added to other wells. The plates were then incubated overnight at 37°C and monitored for inhibition-zone formation. In the quantitative test, the antibacterial activity was evaluated by the MIC determination method at an alkaline pH condition (pH 7) using the broth dilution technique [24]. MIC is defined as the lowest concentration of antimicrobial agent required to prevent the growth of microorganisms after subculture on antibiotic-free media [25]. The CTS-GA MRP solution was dispensed into sterile MH broth by a two-fold serial dilution technique to obtain broths with dilutions from 2^{-1} to 2^{-8} . A broth containing only the nutrient broth without the MRPs served as the control. The 0.1 ml bacterial suspension containing about 10^8 CFU/ml was aseptically inoculated

into 10 ml of dilution broths separately. The broths were then incubated at 37°C for 24 h and observed for turbidity development by unaided eyes or by measuring the optical density at a wavelength of 600 nm (OD_{600}). The MIC was determined as the lowest CTS-GA MRP concentration in the tube that prevents visible growth [24].

2.4. Preservative effect of chitosan-glucosamine Maillard reaction product solution on pork tenderloin at cold storage

The pork tenderloin was sliced with an average weight of 25 ± 5 g. The CTS-GA MRP solution was diluted in water to final concentrations of 500 and 1000 mg/l, respectively. Pork slices were soaked in the diluted solutions for 15 minutes before being packed in plastic containers. Water-soaked pork slices were performed in parallel to serve as the control. The pork slices were then stored in a refrigerator at $5 \pm 1^\circ\text{C}$. After 0, 7, and 14 days, the pork slices were tested for total crude protein and total plate count (TPC) using the Kjeldahl method [26] and the pour plate technique [27], respectively. The shelf-life or storage time of meat and meat products is the duration during which they retain their qualitative characteristics until the arrival of spoilage phenomena, including off-flavours, discolouration, gas production, and slime formation [28].

3. Results and discussion

3.1. Preparation of the chitosan-glucosamine Maillard reaction product solution and UV-Vis spectrophotometric analyses

The changes in colour and UV-Vis absorbance of the CTS-GA mixture solution after irradiation are presented in Fig. 1. Particularly, upon gamma-ray irradiation, the colour of the mixture solution became browner (Fig. 1A). This result was also confirmed by UV-Vis spectrophotometric analyses shown in Fig. 1B, where the absorbance intensity at 284 nm and 420 nm of the irradiated CTS-GA mixture solution increased in comparison to the non-irradiated one. Similar results have been obtained in other studies where protein-sugar mixture solutions were treated by heat [5, 7] or gamma-ray irradiation [19-21]. In the MR, intermediate-stage products can be detected by UV absorbance at 284 nm, while absorbance at 420 nm is preferred for detecting final-stage products [20]. Therefore, the increase in absorbance at 284 and 420 nm indicates that MRP was effectively formed by irradiation treatment at 25 kGy.

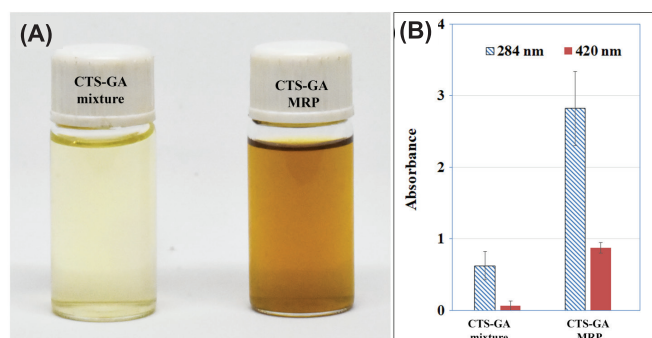


Fig. 1. Visual colour (A) and absorbance intensity (B) of the chitosan-glucosamine mixture and chitosan-glucosamine Maillard reaction product solution.

3.2. Antibacterial activity

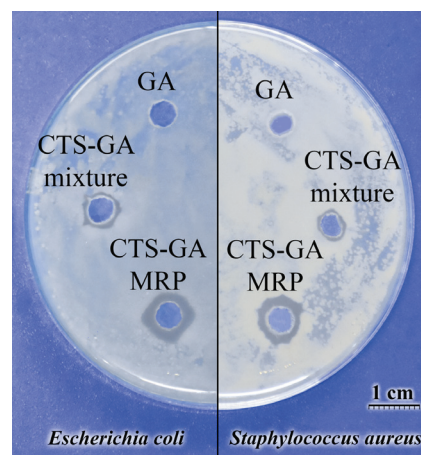


Fig. 2. Photograph of inhibition zone formation on *E. coli* and *S. aureus* plates by agar well diffusion test.

The antibacterial activity of the CTS-GA mixture and CTS-GA MRP solution against *E. coli* and *S. aureus* is displayed in Fig. 2. On both plates of *E. coli* and *S. aureus*, the CTS-GA mixture or CTS-GA MRP solution were able to form growth inhibition zones, while the control (the GA solution) did not. Interestingly, although the GA solution did not show antibacterial activity, the CTS-GA mixture exhibited this activity against both tested bacteria. This result proves that the antibacterial ability of the CTS-GA mixture is due to the role of CTS, as reported in previous studies [6, 29, 30]. Moreover, the antibacterial ability of different antibiotics can be relatively evaluated by the diameter of their growth inhibition zones formed on a bacterial plate, with larger diameters representing higher antibacterial activity [23]. Therefore, results in Fig. 2 indicate that the antibacterial activity of CTS was enhanced by the irradiation-induced MR.

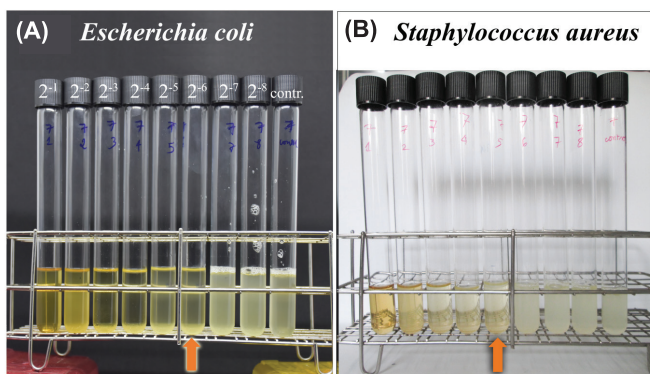


Fig. 3. Serial dilution tubes (A) and minimum inhibitory concentration values (B) of chitosan-glucosamine Maillard reaction product solution against *E. coli* and *S. aureus*.

Table 1. Minimum inhibitory concentration values of chitosan-glucosamine Maillard reaction product solution against *E. coli* and *S. aureus*.

	<i>E. coli</i>	<i>S. aureus</i>
Dilution ratio for Minimum inhibitory concentration	2 ⁻⁶	2 ⁻⁵
Minimum inhibitory concentration (mg/l)	312.5	625

The antibacterial activity of CTS and its derivatives has been widely studied. Generally, there are many factors affecting their antibacterial activity, including the type of microorganism [30]. This study investigated the antibacterial activity of the CTS-GA MRP solution on both *E. coli* and *S. aureus*. The MIC values of the CTS-GA MRP solution against these bacteria at pH 7 are shown in Fig. 3 and Table 1. After incubation, *E. coli* did not cause turbidity from the dilution $\geq 2^{-6}$, while that of *S. aureus* was observed from dilution $\geq 2^{-5}$ (Fig. 3). Hence,

the MIC values of the CTS-GA MRP solution against *E. coli* and *S. aureus* were evaluated as 312.5 and 625 mg/l, respectively (Table 1). The higher MIC value represents the lower antibacterial activity. Thus, the results in Table 1 reveal that the CTS-GA MRP solution exhibited higher antibacterial activity against *E. coli* than against *S. aureus*. A similar phenomenon has also been reported in other studies [4, 19], where *E. coli* was found to be more sensitive to MRP of CTS and glucose/GA than *S. aureus*.

3.3. Preservative effect of chitosan-glucosamine Maillard reaction product solution on pork tenderloin at cold storage

During storage at 5°C, the pork tenderloin slices treated by the diluted CTS-GA MRP solutions at 500 and 1000 mg/l became discoloured and formed slimy liquid as well as foul odour on the 12th and 14th day, respectively, while the control showed spoilage on the 6th day (Fig. 4). This result indicated that the diluted

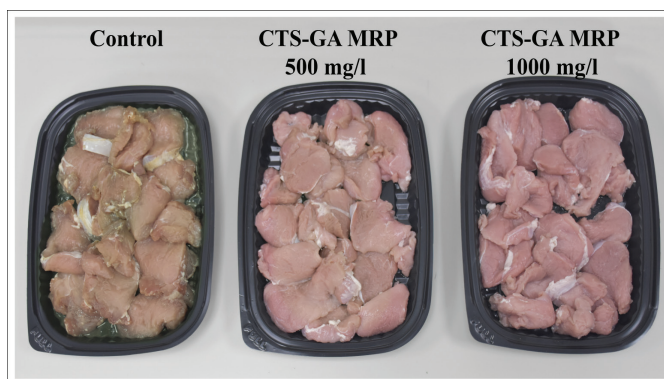


Fig. 4. Pork tenderloin slices treated by the diluted chitosan-glucosamine Maillard reaction product solutions after 6 days of storage at 5°C.

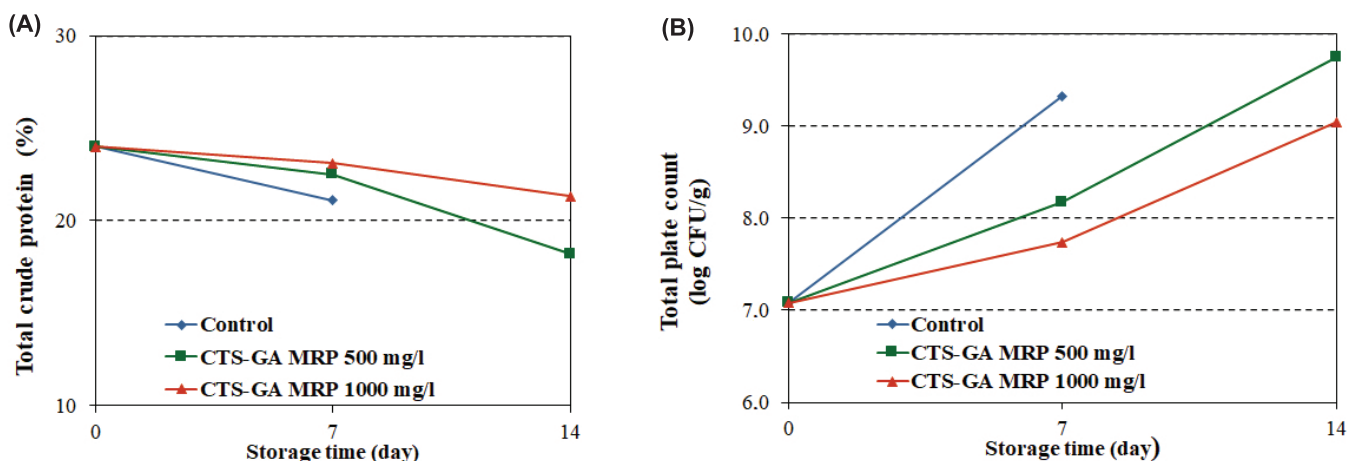


Fig. 5. Total crude protein (A) and total plate count of treated and untreated pork (B) tenderloin as a function of storage time at 5°C.

CTS-GA MRP solutions at 500 and 1000 mg/l could extend the shelf-life of the pork tenderloin at 5°C by 6 and 8 days, respectively, compared to the control. Furthermore, the total crude protein and TPC of the tenderloin, as presented in Fig. 5, also revealed that the diluted CTS-GA MRP solutions reduced protein loss and effectively inhibited microbial growth. These findings are consistent with the results of other studies [5, 31]. S.R. Kanatt, et al. (2008) [5] reported that adding a heat-induced CTS-glucose complex increased the shelf-life of lamb meat by more than two weeks during chilled storage and extended the shelf-life of pork cocktail salami to 28 days.

4. Conclusions

MR is considered a green strategy to improve the biological activities of CTS. In this study, the CTS-GA MRP solution prepared by the gamma Co-60 ray irradiation exhibited high antibacterial activity against *E. coli* and *S. aureus* at pH 7. Furthermore, the diluted CTS-GA MRP solutions at 500 and 1000 mg/l effectively extended the shelf-life of pork tenderloin for 6 and 8 days at cold storage, respectively. Therefore, the CTS-GA MRP solution could be used as a novel natural preservative for the food industry, particularly for meat and meat products. In addition, the gamma-ray irradiation method is considered a green method, which is favourable for large-scale production, including the production of MRPs.

CRediT author statement

Anh Quoc Le: Conceptualisation, Performed the experiment, Writing - Original draft preparation; Van Phu Dang: Performed the experiment, Original draft preparation; Ngoc Duy Nguyen: Performed the experiment; Chi Thuan Nguyen: Formal analysis, Writing, Reviewing and Editing; Quoc Hien Nguyen: Conceptualisation, Formal analysis, Writing - Reviewing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] World Health Organization (WHO) (2015), *WHO Estimates of The Global Burden of Foodborne Diseases: Foodborne Disease Burden Epidemiology Reference Group 2007-2015*, 265pp.
- [2] E. Abebe, G. Gugsu, M. Ahmed (2020), "Review on major food-borne zoonotic bacterial pathogens", *Journal of Tropical Medicine*, **2020**, DOI: 10.1155/2020/4674235.
- [3] J. Gustavsson, C. Cederberg, U. Sonesson, et al. (2011), *Global Food Losses and Food Waste - Extent, Causes and Prevention*, Food and Agriculture Organization of The United Nations, 37pp.
- [4] Y.C. Chung, C.L. Kuo, C.C. Chen (2005), "Preparation and important functional properties of water-soluble chitosan produced through Maillard reaction", *Bioresource Technology*, **96**, pp.1473-1482, DOI: 10.1016/j.biortech.2004.12.001.
- [5] S.R. Kanatt, R. Chander, A. Sharma (2008), "Chitosan glucose complex - A novel food preservative", *Food Chemistry*, **106**, pp.521-528, DOI: 10.1016/j.foodchem.2007.06.036.
- [6] P.K. Dutta, J. Dutta, V.S. Tripathi (2004), "Chitin and chitosan: Chemistry, properties and applications", *Journal of Scientific & Industrial Research*, **63**, pp.20-31, DOI: 10.1016/j.progpolymsci.2006.06.001.
- [7] N. Mahae, C. Chalat, P. Muhamud (2011), "Antioxidant and antimicrobial properties of chitosan-sugar complex", *International Food Research Journal*, **18(4)**, pp.1543-1551.
- [8] K.L. May, K.J. Tangso, A. Hawley, et al. (2020), "Interaction of chitosan-based dietary supplements with fats during lipid digestion", *Food Hydrocolloids*, **108**, DOI: 10.1016/j.foodhyd.2020.105965.
- [9] U. Klinkesorn (2013), "The role of chitosan in emulsion formation and stabilization", *Food Reviews International*, **29(4)**, pp.371-393, DOI: 10.1080/87559129.2013.818013.
- [10] J. Hafsa, M.A. Smach, M.B. Khedher, et al. (2016), "Physical, antioxidant and antimicrobial properties of chitosan films containing *Eucalyptus globulus* essential oil", *LWT - Food Science and Technology*, **68**, pp.356-364, DOI: 10.1016/j.lwt.2015.12.050.
- [11] P.J. Chien, F. Sheu, H.R. Lin (2007), "Coating citrus (Murcott tangor) fruit with low molecular weight chitosan increases postharvest quality and shelf life", *Food Chemistry*, **100(3)**, pp.1160-1164, DOI: 10.1016/j.foodchem.2005.10.068.
- [12] M.E.I. Badawy, E.I. Rabea (2009), "Potential of the biopolymer chitosan with different molecular weights to control postharvest gray mold of tomato fruit", *Postharvest Biology and Technology*, **51(1)**, pp.110-117, DOI: 10.1016/j.foodchem.2005.10.068.

- [13] G.J. Tsai, W.H. Su, H.C. Chen, et al. (2002), "Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation", *Fisheries Science*, **68**(1), pp.170-177, DOI: 10.1046/j.1444-2906.2002.00404.x.
- [14] S. Sagoo, R. Board, S. Roller (2002), "Chitosan inhibits growth of spoilage microorganisms in chilled pork products", *Food Microbiology*, **19**, pp.175-182, DOI: 10.1006/fmic.2001.0474.
- [15] P. Darmadji, M. Izumimoto (1994), "Effect of chitosan in meat preservation", *Meat Science*, **38**(2), pp.243-254, DOI: 10.1016/0309-1740(94)90114-7.
- [16] J. Hafsa, M.A. Smach, R.B. Mrid, et al. (2021), "Functional properties of chitosan derivatives obtained through Maillard reaction: A novel promising food preservative", *Food Chemistry*, **349**, pp.1-12, DOI: 10.1016/j.foodchem.2021.129072.
- [17] B. Gullón, M.I. Montenegro, A.I.R. Matuteb, et al. (2016), "Synthesis, optimization and structural characterization of a chitosan-glucose derivative obtained by the Maillard reaction", *Carbohydrate Polymers*, **137**, pp.382-389, DOI: 10.1016/j.carbpol.2015.10.075.
- [18] M. Hellwig, D. Bunzel, M. Huch (2015), "Stability of individual Maillard reaction products in the presence of the human colonic microbiota", *Journal of Agricultural and Food Chemistry*, **63**(30), pp.6723-6730, DOI: 10.1021/acs.jafc.5b01391.
- [19] M.S. Rao, S.P. Chawla, R. Chander, et al. (2011), "Antioxidant potential of Maillard reaction products formed by irradiation of chitosan-glucose solution", *Carbohydrate Polymers*, **83**, pp.714-719, DOI: 10.1016/j.carbpol.2010.08.045.
- [20] S.P. Chawla, R. Chander, A. Sharma (2009), "Antioxidant properties of Maillard reaction products by gamma-irradiation of whey protein", *Food Chemistry*, **116**, pp.122-128, DOI: 10.1016/j.foodchem.2009.01.097.
- [21] S. Oh, Y.S. Lee, J.W. Lee, et al. (2005), "The effect of γ -irradiation on the non-enzymatic browning reaction in the aqueous model solutions", *Food Chemistry*, **92**(2), pp.357-363, DOI: 10.1016/j.foodchem.2004.07.029.
- [22] L.A. Quoc, D.V. Phu, N.N. Duy, et al. (2021), "Study on the minimum bactericidal concentration (MBC) of Maillard reaction products of chitosan and glucosamine prepared by gamma-irradiation method", *Nuclear Science and Technology*, **11**, pp.44-51, DOI: 10.53747/nst.v11i3.371.
- [23] M. Balouiri, M. Sadiki, S.K. Ibsouda (2016), "Methods for *in vitro* evaluating antimicrobial activity: A review", *Journal of Pharmaceutical Analysis*, **6**, pp.71-79, DOI: 10.1016/j.jpha.2015.11.005.
- [24] J.M. Andrews (2001), "Determination of minimum inhibitory concentrations", *Journal of Antimicrobial Chemotherapy*, **48**(6), pp.5-16, DOI: 10.1093/jac/48.suppl_1.5.
- [25] C.I. Owuama (2017), "Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method", *African Journal of Microbiology Research*, **11**(23), pp.977-980, DOI: 10.5897/AJMR2017.8545.
- [26] S. Jamal, D.M. Jamil, Z.K. Khidhir (2020), "Protein determination in some animal products from Sulaymaniyah markets using Kjeldahl procedure", *Journal of Food and Dairy Sciences*, **11**(12), pp.343-346, DOI: 10.21608/jfds.2020.160394.
- [27] M.C. Kuncara, F.N. Yuliati, K.I. Rrahesti (2021), "The total plate count, *Staphylococcus aureus*, and pH value of raw chicken meat sold at the traditional markets in Maros regency", *IOP Conference Series: Earth and Environmental Science*, **788**, DOI: 10.1088/1755-1315/788/1/012157.
- [28] M.F. Iulietto, P. Sechi, E. Borgogni, et al. (2015), "Meat spoilage: A critical review of a neglected alteration due to ropy slime producing bacteria", *Italian Journal of Animal Science*, **14**(3), pp.316-326, DOI: 10.4081/ijas.2015.4011.
- [29] H.K. No, N.Y. Park, S.H. Lee, et al. (2002), "Antibacterial activity of chitosan and chitosan oligomers with different molecular weights", *International Journal of Food Microbiology*, **74**, pp.65-72, DOI: 10.1016/s0168-1605(01)00717-6.
- [30] A. Verlee, S. Mincke, C.V. Stevens (2017), "Recent developments in antibacterial and antifungal chitosan and its derivatives", *Carbohydrate Polymers*, **164**, pp.268-283, DOI: 10.1016/j.carbpol.2017.02.001.
- [31] H.L. Chang, Y.C. Chen, F.J. Tan (2011), "Antioxidative properties of a chitosan-glucose Maillard reaction product and its effect on pork qualities during refrigerated storage", *Food Chemistry*, **124**, pp.589-595, DOI: 10.1016/j.foodchem.2010.06.080.

Investigation of the status and the prospect of fuel pellet production and utilisation toward net zero emissions by 2050 in Vietnam

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Abstract:

The Vietnamese government has committed strongly to reducing greenhouse gas emissions to zero by 2050 at the United Nations Climate Change Conference in Glasgow (COP26). Vietnam possesses significant potential to develop renewable energy from wood biomass and agricultural residues. This study comprehensively evaluates the status and potential of fuel pellet production and utilisation in Vietnam. Factors affecting fuel pellet production were evaluated. Vietnam's total wood pellet output is approximately 5 million tonnes per year. Raw materials are mainly derived from residues of plantation forests (90%) and the wood processing industry (10%). Vietnam's wood pellet production potential could increase two to three times due to the extensive plantation forest resource of nearly 5 million hectares. Currently, Vietnam does not utilise any wood pellets or wood chips as fuel to produce electricity domestically. The Vietnamese government is strongly committed to achieving net-zero emissions by 2050 and has implemented numerous policies and mechanisms to encourage the development of renewable energy. Owing to the vast potential of biomass from both forestry and agricultural residues, the export of wood fuel, such as wood pellets and wood chips, is expected to continue increasing in the future due to rising global demand.

Keywords: biomass utilisation, net-zero emission, renewable energy, wood pellet.

Classification numbers: 5.1, 5.3

1. Introduction

Vietnam is regarded as a region with high potential for renewable energy sources, including wind, solar, and biomass [1]. The electricity industry has developed with an average annual growth rate of 10-12% in both production and consumption [2]. Vietnam is an active participant in agreements aimed at reducing greenhouse gas emissions as well as commitments related to energy, land, etc., with the ultimate goal of mitigating global warming [3]. At the COP26 Conference, Vietnam and nearly 150 countries committed to achieving net-zero emissions by 2050. The strong commitment of the Vietnamese government at COP26 has necessitated a significant shift in socio-economic development thinking within the country. The need for a green transition is stronger than

ever, opening numerous opportunities for cooperation on low-emission growth, promoting circular economic development, and adapting to climate change. Various international organisations, development partners, and large corporations, particularly financial institutions and major players in renewable energy have committed to and signed cooperation agreements with Vietnam. T.K. Tran, et al. (2023) [4] explored how natural resource rents and depletion, along with economic growth, foreign direct investment (FDI), industrialisation, and population growth, impact methane emissions and commitments to COP26 in the Vietnamese context. T.H. Le (2022) [5] analysed the economic and environmental impacts of non-renewable and renewable energy consumption and CO₂ emissions in Vietnam using innovative multivariate wavelet analysis tools.

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Technologies have been researched and developed to convert biomass energy into forms that are practical for Vietnam's conditions [6-12]. Besides energy conversion technology, several studies have addressed the current situation and solutions for developing renewable energy sources in Vietnam. A.H. Truong, et al. (2022) [13] assessed the technology of blending 5% rice straw in two existing coal power plants in Vietnam, concluding that co-firing is cheaper than coal but not significant enough to motivate stakeholders. K. Handayani, et al. (2022) [14] indicated that in power sector pathways under the net-zero emissions scenario, ASEAN member states need to swiftly capitalise on their currently underutilised renewable energy potential to achieve net-zero emissions by 2050. D.L. Bui, et al. (2023) [15] calculated that for the 664,500 tonnes of pellet yields, the total amounts of carbon dioxide (CO₂) emitted during production and transportation are 7,680.6 and 28,627.0 tonnes, respectively. T.N. Do, et al. (2023) [16] recommended strategies to phase out unabated coal power by the 2040s, including (1) reforming regulations to facilitate investments in clean energy, electricity transmission, and energy storage; (2) continuing political prioritisation; and (3) building broad-based support from the community and enterprises. By applying the principles of a circular economy for sustainable development, the wood industry can contribute to Vietnam's commitment to carbon neutrality by reducing the total net carbon emission from +552,750 tonnes CO_{2eq} to -1,145,940 tonnes CO_{2eq} per year [17]. Early preparation of transmission systems for solar and wind electricity is needed to maximise the potential for expanding the use of these technologies [18]. However, most studies have not provided reliable data on the status, potential, and factors affecting the development of biomass energy sources in Vietnam.

Vietnam currently has more than 3 million hectares of commercial forests, annually providing 40 million m³ of wood for the manufacturing industry. In addition to domestic wood sources, Vietnam also imports 322 types of wood to meet domestic needs and for export to 104 countries. Residues from industrial wood are utilised and become a rich source of raw materials for stable biomass energy development. Moreover, rice straw, rice husk,

sugarcane bagasse, and coffee husks are also inputs for available biomass energy in Vietnam. The combination of wood biomass resources from forests and plantations will undoubtedly play a crucial role in the development of energy alternatives [19].

The total amount of municipal solid waste (MSW) was 27 million metric tonnes in 2015 and is projected to reach 54 million metric tonnes by 2030. However, waste management and treatment in Vietnam are labour-intensive and inefficient. Vietnam consumed 65.5 million tonnes of coal in 2020, while a large amount of MSW was not utilised for energy production. Therefore, searching for alternative fuels that could replace fossil fuels and reduce gas emissions is an urgent need [6].

Vietnam has great potential to develop renewable energy from biomass resources. However, agricultural residues and other biomass are often unused or only used on a small scale. It is estimated that more than 40 million tonnes of rice straw are generated annually. However, this volume of straw has not been used effectively; on the contrary, it is one of the sources of serious air pollution due to the habit of burning in the rice fields. T.H. Le (2022) [5] produced rice straw pellets mixed with additives at a scale of 500 kg/day, opening the possibility of using pellets from agricultural residues as fuel. Other common sources of agricultural residues in Vietnam include approximately 8.6 million tonnes/year of rice husks, 3.5 million tonnes/year of sugarcane bagasse, and about 5 million tonnes/year from other sources. The total potential electricity generation capacity from this by-product source is over 1500 MW.

This study collected data from government statistical sources, timber and forest associations, and other reliable sources. The data were compiled and analysed to provide information on the status of raw materials for wood pellet production and biomass utilisation in Vietnam. The prospects for wood pellet production and biomass use were analysed comprehensively in the context of Vietnam's strong commitment to net-zero emissions by 2050. Factors affecting wood pellet production, such as raw material sources, forest land planning, sustainable certified forest development, and international and

domestic markets, were evaluated. Vietnam's biomass energy development policies were also discussed to assess the prospects for developing the wood pellet production industry and future use. For the first time, the study analyses the overall picture of all factors affecting the development of biomass energy pellets in Vietnam, from raw material types and sources to fuel pellet market development orientation, to Vietnam's policies with the goal of reducing net emissions to zero by 2050.

2. Subjects and methodology

Raw materials for producing fuel pellets primarily come from wood, agricultural residues, and municipal solid waste. Fig. 1 presents the scope of the study's assessment, which includes raw material sources, fuel pellet production, and market. Factors affecting raw material sources encompass geographical location, forest development policies, sources of agricultural residues, plantation forests, and the wood processing industry. Vietnam has forest resources that are unevenly distributed among regions. Moreover, in wood pellet production, the logistics costs of raw materials account for a significant proportion. Vietnam's wood processing industry utilises wood from domestically grown forests

but mainly relies on imported wood sources. Residues from the wood processing industry provide raw materials for wood pellet production.

Fuel pellet production is considered in terms of technological aspects, policies, manufacturing enterprises, capacity, and geographical location. The fuel pellets currently produced and traded are mainly pellets from wood raw materials. There are some projects producing fuel pellets from agricultural residues such as rice husks, straw, or municipal solid waste, but the quantities are small. Therefore, the pellet production section mainly considers wood pellet production.

Factors affecting the fuel pellet consumption market include the international and domestic markets. Future market development trends are also discussed. Additionally, common factors affecting fuel pellets include policies on Vietnam's energy and electricity mix in the future with a vision to 2030 and 2050, and policies on developing Vietnam's wood processing industry. Vietnam's commitments at COP26 and the development of Vietnam's carbon credit market also influence the production and consumption of fuel pellets in the domestic market.

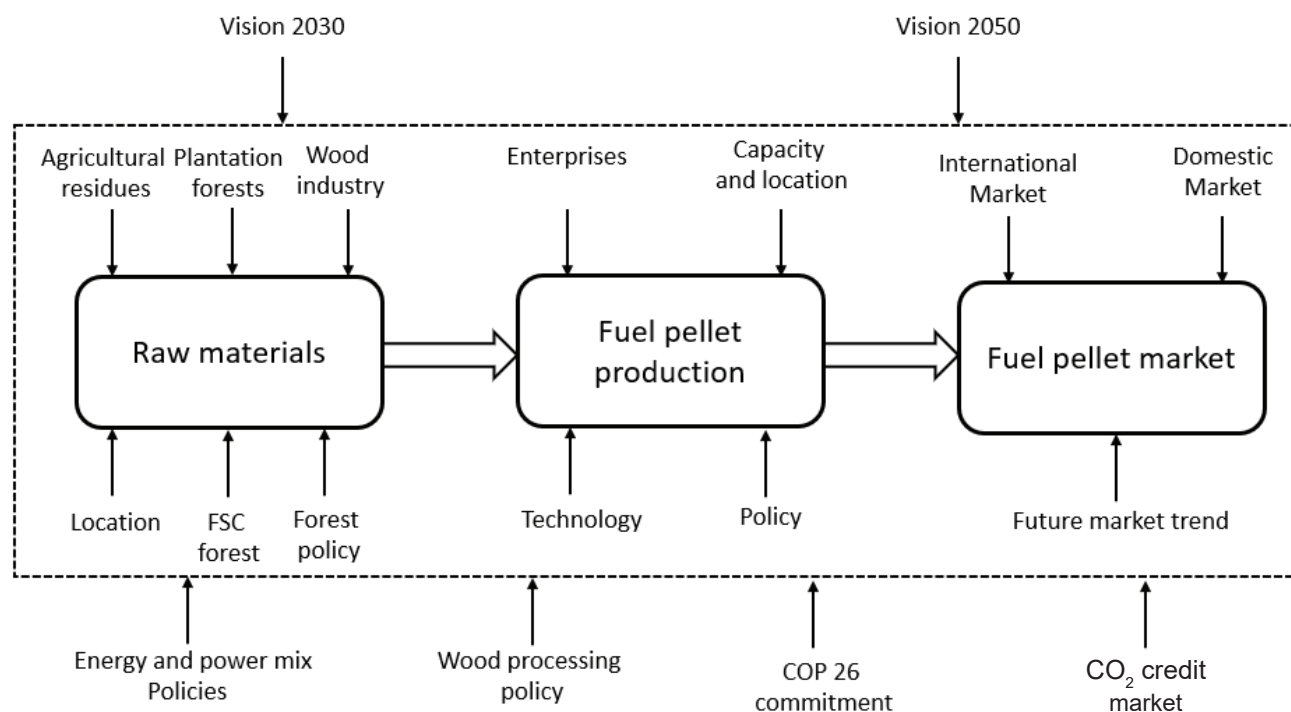


Fig. 1. Boundary of investigation of the status and the prospect of fuel pellet production and utilisation in Vietnam.

3. Results and discussion

3.1. Status of fuel pellet production in Vietnam

The status of fuel pellet production in Vietnam encompasses factors such as raw material sources, production volume and value, manufacturers, and markets.

3.1.1. Raw material

In Vietnam, wood pellets are typically made from waste wood such as sawdust, shavings, and tree branches. These materials are compressed under high pressure into pellet form. Some informants noted that small-scale producers often use any timber available locally, with a network of traders purchasing materials from villagers. However, some large-scale producers have their own plantations, typically of acacia or eucalyptus wood, some of which have Forest Stewardship Council (FSC) certification. Raw materials for wood pellets from domestically grown forest materials account for about 90% of the total supply of raw materials for wood pellet production. Raw materials from sawmills and wood processing factories account for less than 10% of the total source for the entire wood pellet industry (Fig. 2).

Official statistics from the Ministry of Agriculture and Rural Development indicated that Vietnam currently has nearly 14.7 million hectares of forest. According to function, this area is divided into protection forests (4.7 million hectares, 31.8% of the total area), special-use forests (2.2 million hectares, 14.8%), and production forests (7.8 million hectares, 53.4%). Production forests, which are the source of raw wood for the processing industry, are mainly located in the Northeast (2.42 million hectares), the North Central region (1.66 million hectares), and the Central Highlands (1.53 million hectares). According to origin, forests are classified into natural forests (10.3 million hectares) and plantation forests (4.4 million hectares). Fig. 3 shows the area of natural and plantation forests in different regions of Vietnam. Of the 4.4 million hectares of plantation forests, they are mainly located in the Northeast with 1.58 million hectares, the North Central region with 0.92 million hectares, and the Central Coast with 0.87 million hectares [21].

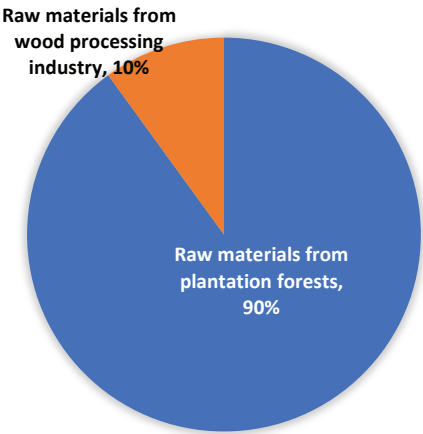


Fig. 2. Biomass feedstock for wood pellets production [20].

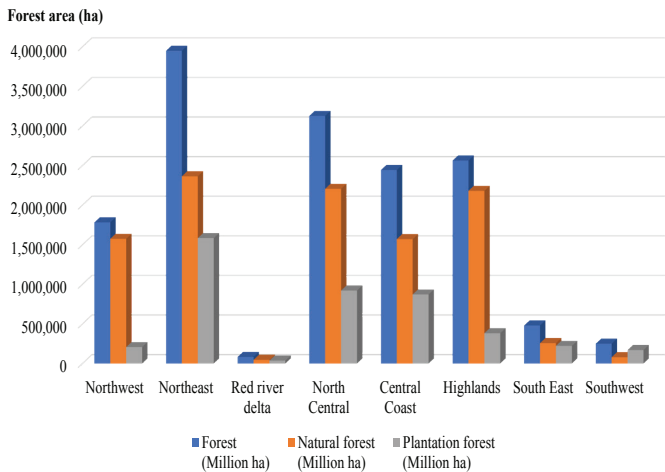


Fig. 3. Distribution of natural forests and plantation forests by region in Vietnam [21].

Table 1. Statistics of certified forest areas in Vietnam [21].

Location	Number of certificates	Certified forest area by 2021			Tree type
		Total	Natural forest	Plantation forest	
Northwest	1	6,314	0	6,314	acacia
Northeast	23	85,549	5,178	80,371	acacia
North Central	21	79,046	30,889	48,157	acacia, natural forest, bamboo
South Central region	9	33,503	499	33,004	acacia
Highlands	7	24,718	486	24,232	acacia, rubber
Southeast	12	77,227	3,043	74,184	acacia, rubber
Southwest	1	752	0	752	acacia
Vietnam	74	307,109	40,095	267,014	

The government has been encouraging the development of plantation forests with sustainable forest management certificates (FSC, PEFC) because the demand for certified wood used as input materials for export products is high and increasing. Statistics from the General Department of Forestry indicated that by the end of August 2021, the total area of certified forests in Vietnam reached over 307,000 hectares, including 40,000 hectares of natural forests; the rest is plantation forests as shown in Table 1. The area of certified forests accounts for only 8.4% of the total plantation forest area of the country.

3.1.2. Wood pellet production volume and value

Most wood pellets produced in Vietnam are exported. There is no statistical data on the amount of wood pellets consumed domestically. Based on a survey of wood pellet factories, most of the pellets produced are sold to trading companies for export. Hence, the amount of wood pellets consumed domestically is very small. Additionally, thermal power plants in Vietnam all use coal, natural gas, or biomass such as sugarcane bagasse and rice husk. There are no power plants that use wood pellets or co-firewood pellets with coal to produce electricity. Therefore, in this study, data on exported wood pellets were used to represent data on domestically produced wood pellets.

Figure 4 shows the volume and value of wood pellets produced from 2013 to the end of 6 months, 2023. In 2013, the production of wood pellets was only 175 thousand tonnes, and the amount increased rapidly due to demand from Japan and Korea, especially after 2018, peaking in 2022 at nearly 5 million tonnes. In the first six months of 2023, the production of wood pellets was nearly 2.1 million tonnes. Although it is lower than in 2022, it is still at a high level. In 2022, the export value of wood pellets was nearly 800 million USD, nearly double that of 2021, although the output of wood pellets increased by 40%.

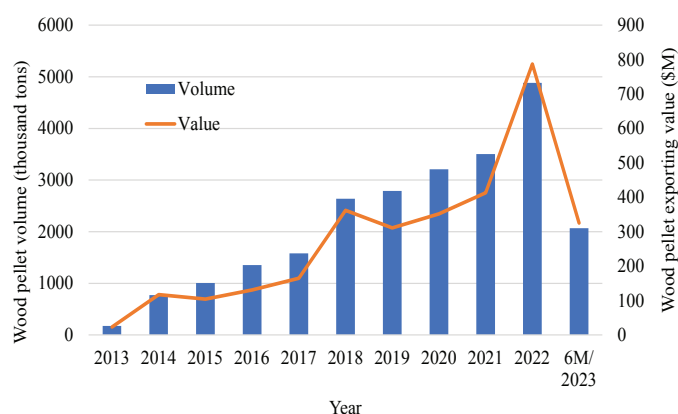


Fig. 4. Volume of pellets exported from Vietnam from 2019 to the end of 6 months in 2023 [22].

3.1.3. Wood pellet manufacturers

Currently, Vietnam has more than 100 enterprises directly involved in exporting wood pellets and over 300 large and small wood pellet manufacturers. However, wood pellet export is almost concentrated in a few large enterprises. Among the enterprises directly involved in exporting wood pellets today, there are only eight companies with an export volume of over 100,000 tonnes annually. The export volume of these eight enterprises accounts for nearly 67% of the total export volume of wood pellets in Vietnam. Among them, there are three enterprises with an export volume of over 400,000 tonnes, and particularly one enterprise with a very large export volume of wood pellets, nearly 714,000 tonnes, accounting for about 20% of the total export volume of wood pellets of Vietnam.

3.1.4. Wood pellet exporting markets

Korea and Japan are the two largest wood pellet import markets of Vietnam. The volume of wood pellets exported from Vietnam to these two countries accounts for over 95% of the total volume of wood pellets exported from Vietnam to all markets. The source of raw materials to produce pellets exported to Korea and Japan differs. Wood pellets exported to Korea are mainly made from the by-products of the wood processing industry such as sawdust, shavings, and wood chips. Enterprises exporting pellets to Korea are mainly located in the Southeast region, where furniture factories are concentrated. Pellets exported to Japan must be made from domestic

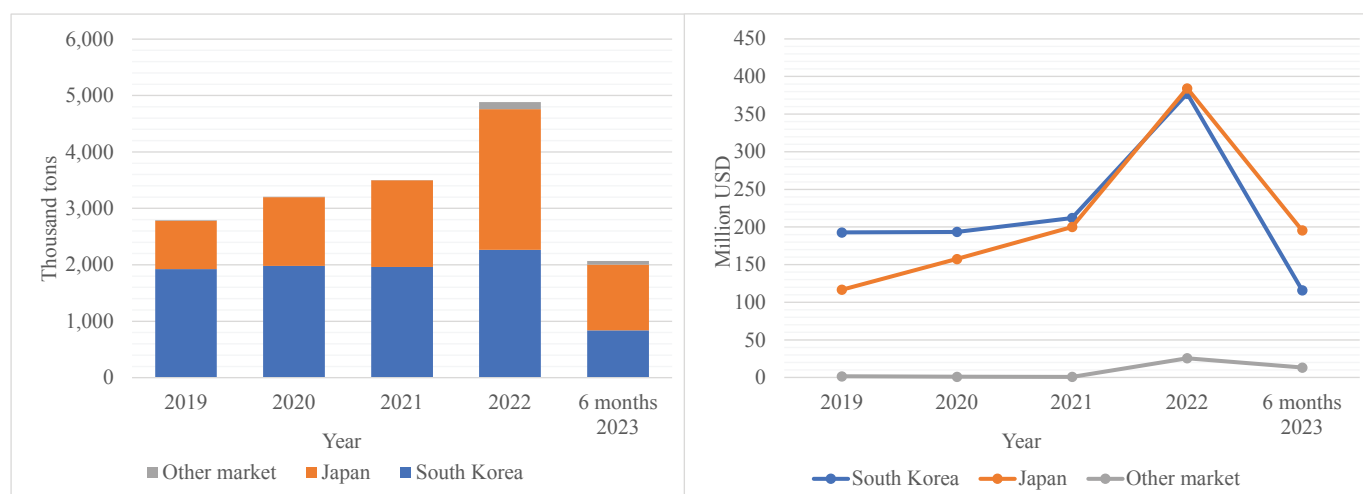


Fig. 5. Pellet export volume and volume by markets from 2019 to 6 months in 2023.

plantation timber with an FSC certificate. This material is only available where there are large regions of plantation forests, particularly from the Central region to the North of Vietnam.

Figure 5 shows the volume and value of pellets exported from Vietnam from 2019 to the end of 6 months in 2023, respectively. In 2022, exports increased sharply when the export volume reached nearly 4.9 million tonnes and the export value reached 0.79 billion USD.

3.2. Prospects of fuel pellet production

Prospects for developing fuel pellet production are considered, focusing on three aspects: raw material sources, markets, and related policies.

3.2.1. Raw materials sources

Potential of wood, agricultural, and municipal solid waste biomass: According to the Institute of Energy, Ministry of Industry and Trade, the total potential of wood biomass for energy is nearly 25 million tonnes/year, equivalent to 8.8 million tonnes of crude oil. The agricultural biomass energy potential, including straw, rice husk, sugarcane bagasse, and other agricultural products, is up to nearly 53.5 million tonnes/year, equivalent to 12.8 million tonnes of crude oil. The biomass energy in Vietnam includes wood biomass (25 million tonnes) from forest residues and the wood processing industry, MSW (27 million tonnes), and agricultural residues (53.5 million tonnes). Agricultural residues consist of

40 million tonnes of rice straw, 8.6 million tonnes of rice husks, 3.5 million tonnes of sugarcane bagasse, and others of 1.4 million tonnes.

Plantation forest: Vietnam has a total plantation forest area of 4.4 million hectares. Of which, households, forestry companies, and commune People's Committees manage 1.45 million hectares, 0.62 million hectares, and 1 million hectares, respectively. The remaining areas are plantation forests for protection and special use. Generally, forest lands of households and commune People's Committees are used inefficiently due to several difficulties such as lack of access to investment capital, science and technology, and management qualifications. Connecting with businesses can help minimise any restrictions, with the potential for large timber plantations in the future. Part protection and special-use forests are bare land and degraded forests. The government needs to put bare land areas and irreversibly degraded forests into developing plantation forests.

Vietnam's forestry development strategy for the period 2021-2030: Vietnam sets strategic goals for forest development and wood processing in the 2021-2030 period as follows: The growth rate of forestry production value is from 5.0 to 5.5%/year in the period 2021-2025 and remains stable until 2030. Export value of wooden furniture and non-wood forest products: reaching 18-20 billion USD by 2025; reaching 23-25 billion USD by 2030. Domestic wood exploitation output reaches

45 million m³/year by 2025, over 62 m³/year by 2030, basically meeting the demand for raw materials for wood and forest product processing. Improve the quality and efficiency of plantation forests and agroforestry systems; issue certificates of sustainable forest management for 0.5 million hectares in the period 2021-2025, reaching 1 million hectares in the period 2026-2030. Thus, compared to the status of Vietnam's forest product exploitation in 2020, by 2025 the output will double, and by 2030 the output will triple. Thus, the potential to provide raw materials for wood pellet production in Vietnam can increase 2-3 times in the period 2025-2030, equivalent to an increase in wood pellet output of 8-12 million tonnes.

Other popular biomass sources: The cinnamon area in Vietnam covers about 150,000 hectares, accounting for 17% of the global cinnamon area, located mainly in the provinces of Lao Cai and Yen Bai. Currently, Vietnam is the world's third-largest producer and exporter of cinnamon in terms of output, after Indonesia and China. The cinnamon branches and wood are estimated at 700,000 tonnes/year, of which cinnamon branches after separating essential oil are estimated at 400,000 tonnes/year and cinnamon wood at 300,000 tonnes/year. Currently, there are no large-scale factories processing this residue. This is a great potential source of biomass for wood pellet production.

Statistics from the Vietnam Rubber Association showed that Vietnam's rubber area in 2021 reached nearly 939,000 hectares, of which the area of smallholders' accounts for about half. In particular, the rubber industry is expected to contribute to Vietnam's wood industry by about 7-8 million m³ annually from the replantation of rubber tree area of over 25,000-30,000 hectares to provide large timber for about 20% of raw material needs.

The bamboo plantation area is 1,480,000 hectares, accounting for 10.5% of the total forest area of the country, of which pure forests have 240,925 hectares, mixed forests (wood and bamboo) of 1,152,864 hectares. Exploitation output is 40 million trees/year, equivalent to 0.92 million tonnes/year. In 2030, 57,000 hectares, exploitation output of 76 million trees/year equivalent to 1.75 million tonnes of bamboo/year. Bamboo wood

pellets have the highest quality among wood pellets due to their very low ash content. It is estimated that the number of by-products from the bamboo products processing industry will be about 200,000 tonnes in 2020 and increase to 350,000 tonnes in 2030.

3.2.2. Overseas and domestic markets

Exports are currently on the rise. The world's demand for pellets is projected to increase by about 250% over the next decade, reaching 36 million tonnes from 14 million tonnes in 2017. In recent years, demand has increased mainly in European countries, Japan, and Korea. However, Vietnam's pellet industry could expand the market when consumption demand in Vietnam begins to show signs of rapid growth as the government is making efforts to fulfill its commitments to reduce greenhouse gas emissions and use clean energy, including pellets to replace coal in energy production. The Power Masterplan VIII, which was approved by the government on 15 May 2023, prioritises the development of renewable energy sources, including biomass power to replace high-emission coal power. Therefore, the demand for wood pellets in the domestic market may expand very quickly in the future.

3.3. Status and prospects of biomass utilisation in Vietnam

3.3.1. Energy and power mix in Vietnam

Figure 6 describes Vietnam's energy mix in 2020 and 2021. Vietnam's main energy sources include coal, natural gas, hydroelectric, and renewable energy. The proportion of renewable energy increased significantly from 5 to 11%, mainly from wind and solar energy. Coal still accounts for the largest proportion of Vietnam's energy mix, about nearly 50%. Hydroelectric accounts for 31% of Vietnam's energy structure, the rest is natural gas. In the period from 2011 to 2021, the energy consumption rate increased by an average of 7.2% per year, among the highest in the world.

The lower heating value of some fuels such as coal is 17-25 MJ/kg, natural gas 42-55 MJ/kg, biomass 12-16 MJ/kg, and MSW 7-9 MJ/kg. The amount of coal and

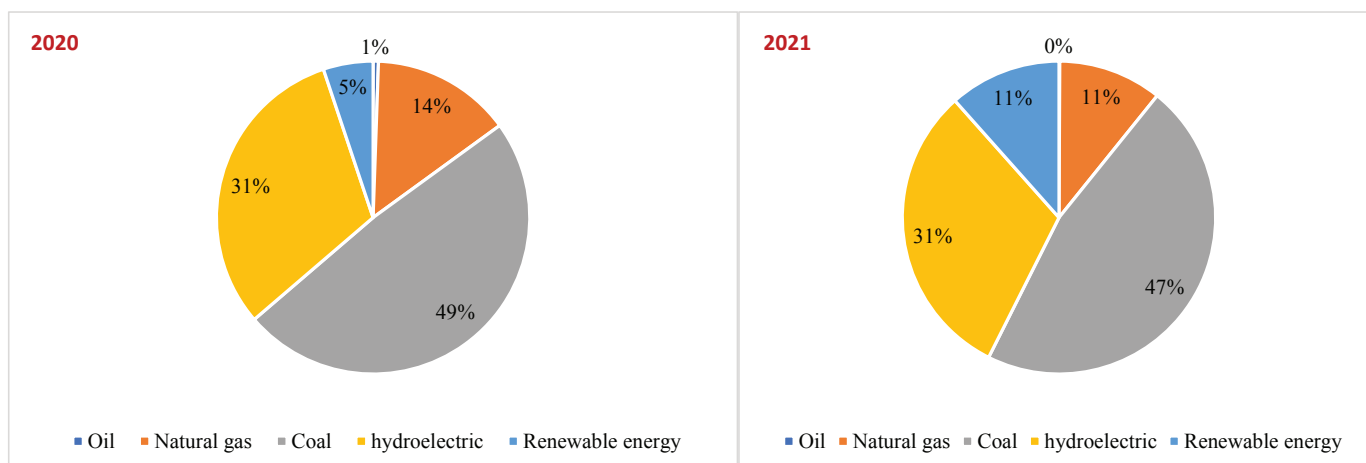


Fig. 6. Vietnam's energy mix in 2020 and 2021 [23].

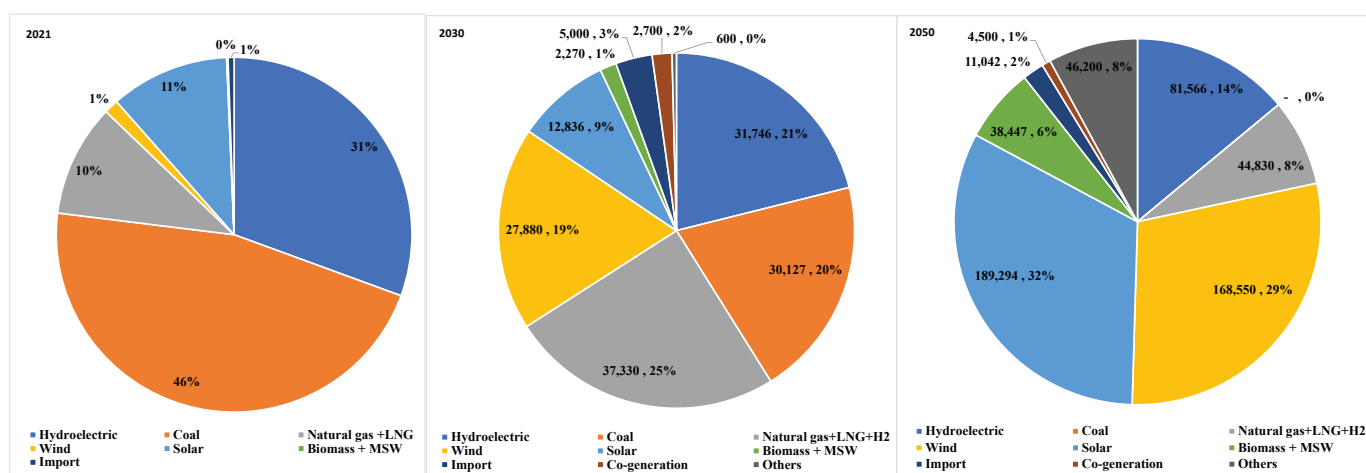


Fig. 7. Vietnam's power mix in 2021 and vision to 2030 and 2050 [24].

natural gas that could be replaced by biomass can be roughly estimated based on calorific value. At the current energy mix in Vietnam, agricultural residues, woody biomass, and MSW can replace more than 25 million tonnes of coal (12 million tonnes of natural gas); 12.5 million tonnes of coal (6 million tonnes of natural gas), 9 million tonnes of coal (4 million tonnes of natural gas), respectively.

In the period 2021-2050, the growth rate of energy demand is about 6.5-7.5% per year. The total final energy demand is 107 million tonnes of oil equivalent in 2030 and will reach 165-184 million tonnes of oil equivalent by 2050. The total primary energy supply will be 155 million tonnes of oil equivalent in 2030 and 294-311 million tonnes of oil equivalent by 2050.

By the end of December 2021, the electricity output from renewable energy was expected to reach 110 billion kWh, accounting for nearly 43% of the electricity generation output of Vietnam's power system, of which 31% is hydropower and 12% is wind, solar, and biomass power (Fig. 7) [24].

The ratio of coal power will decrease from 46% in 2021 to 20% in 2030 and to zero by 2050. The proportion of other renewable energy will increase from 8.5 to 32.4% (wind power) and from 8.5 to 32.4% (solar power). Hydroelectric will increase output from 31,746 to 81,566 MW, but the proportion in the electricity mix will decrease from 21.1 to 14%. By 2050, the total biomass potential is over 120 million tonnes, and MSW is about 60 million tonnes, equivalent to 40 million tonnes and 10 million tonnes of oil equivalent, respectively. Wood biomass and

agricultural residues can replace 60 million tonnes of coal, and MSW can replace 20 million tonnes of coal. In the power production mix, the total power generation capacity from biomass and waste will be about 38,500 MW. By 2050, Vietnam will no longer produce electricity from coal, but will still produce electricity from natural gas. However, to evaluate the CO₂ emissions of biomass pellet products, lifecycle analyses are necessary under Vietnamese conditions. The carbon footprint of wood pellets ranges from 30 to 106 kg CO₂/MWh depending on the type of wood used to produce the pellets, its source, and the drying method. The carbon footprint of coal is about 850 kg CO₂/MWh. The CO₂ emission reduction from using wood pellets can be from 88-96% compared to coal.

3.3.2. Biomass power plants

Vietnam has issued a number of decisions related to biomass power development such as Decision No. 24/2014/QĐ-TTg supporting the development of biomass power projects in Vietnam, Decision No. 51/VBHN-BCT on the mechanism to support the development of

biomass power projects, and Decision No. 500/QĐ-TTg of the Prime Minister approving the National Electricity Development Plan for the period 2021-2030, with a vision to 2050 (Power Plan VIII). According to Decision No. 500/QĐ-TTg, Vietnam will develop between 1,230 and 2,270 MW of biomass power sources and electricity from waste by 2030. It is expected that the proportion of electricity produced from these power sources will reach about 1.5%. It will develop about 38,447 MW, accounting for about 6.6% of total electricity production by 2050 as shown in Fig. 7.

Figure 8 lists Vietnam's biomass and municipal solid waste power plants and their respective capacities. Some factories are in operation, while others are under construction. Biomass and municipal solid waste power plants have small capacities ranging from just a few MW to the largest of nearly 100 MW. The main reason for small capacity is that factories are built only to take advantage of available raw materials around them without mobilising large biomass sources such as wood pellets and wood chips.

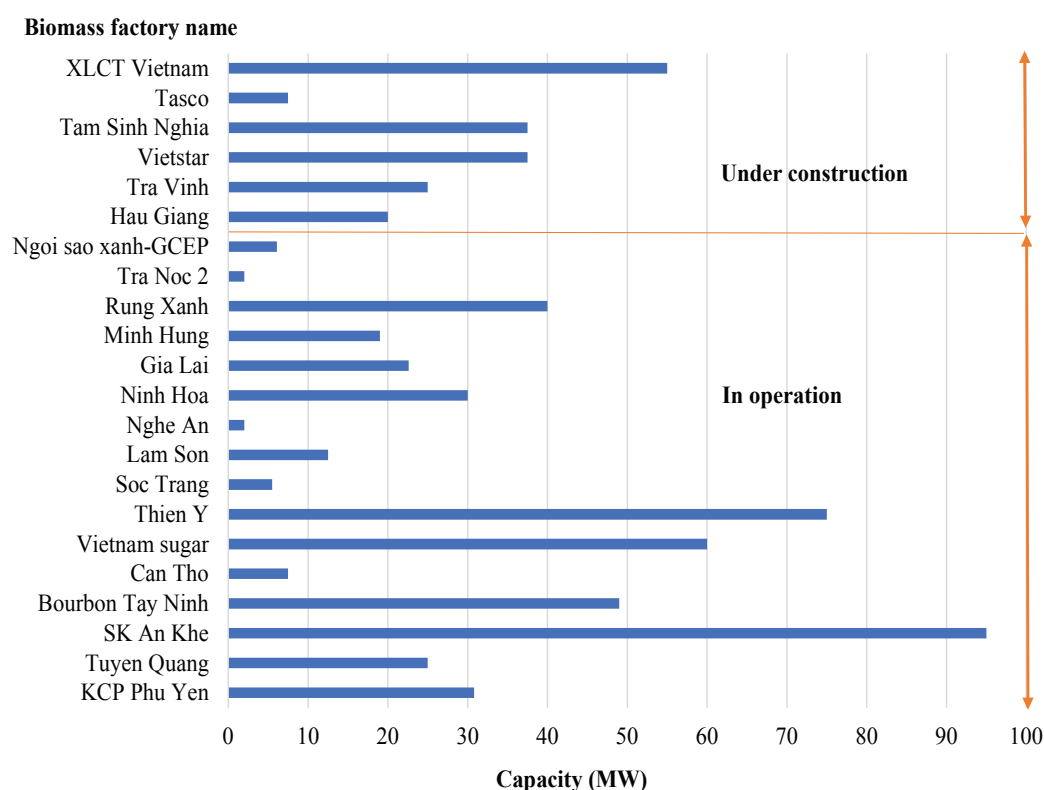


Fig. 8. List of biomass factories in operation and construction in Vietnam.

3.3.3. Action towards a net zero emissions commitment by 2050 of Vietnam

Vietnam has taken concrete policy actions to gradually realise a net zero emissions commitment by 2050, including: (1) National Steering Committee to implement Vietnam's commitments at COP26; (2) Project on tasks and solutions to implement the results of the COP26 Conference (Decision No. 888/QD-TTg dated 25 July 2022); (3) National Strategy on Climate Change for the period up to 2050 (Decision No. 896/QD-TTg dated 26 July 2022); (4) Action plan to reduce methane emissions until 2030 (Decision No. 942/QD-TTg dated 5 August 2022).

For the implementation of emission reduction activities nationwide to meet commitments at the COP26 Conference, on 7 January 2022, the government issued Decree No. 06/2022/ND-CP regulating the mitigation of greenhouse gas emissions and protection of the ozone layer. Accordingly, the greenhouse gas emission mitigation target approved by the Prime Minister in the Nationally Determined Contribution (NDC) includes the greenhouse gas emission mitigation target for the energy sectors, agriculture, land use and forestry, waste management, and industrial processes in accordance with the country's socio-economic development conditions and international commitments to which Vietnam is a member.

Vietnam's updated NDC has set targets for reducing greenhouse gas emissions by 2030 for sectors contributing to achieving net zero emissions by 2050. On 18 January 2022, the Prime Minister issued a list of fields and establishments emitting greenhouse gases that must conduct greenhouse gas inventories (Decision No. 01/2022/QD-TTg) with nearly 2,000 facilities with annual greenhouse gas emissions of 3,000 tonnes of CO₂ equivalent or more. Sectors and establishments that emit greenhouse gases are responsible for reporting emission levels, formulating, and implementing emission reduction plans to achieve the national goal of reducing greenhouse gas emissions.

Based on the decisions issued by the Prime Minister, ministries and sectors have developed and issued sectoral action plans to implement commitments at COP26 with very specific targets for each field. The Ministry of Industry and Trade has researched and developed a programme to reduce the use of fossil energy in the energy industry, integrating it into the process of developing Power Plan VIII and the National Energy Development Strategy. The Ministry of Agriculture and Rural Development has developed a plan to implement the Glasgow Declaration on forests and land use and submit it to the Prime Minister for approval; reviewing forest carbon credit exchange projects and implementing initiatives in the agricultural sector that participated in the COP26 Conference. The Ministry of Transport has developed and submitted for promulgation an Action Programme on green energy conversion and reduction of carbon and methane emissions in the transport sector; develop mechanisms, policies, and roadmaps for developing electric vehicles (E-mobility) for the national and city levels. The Ministry of Natural Resources and Environment has developed and published a report on offshore wind and wave energy potential in Vietnamese waters to serve the development of renewable energy, especially offshore wind power projects.

In the updated NDC for 2022, Vietnam increased its unconditional emission reduction contribution to 2030 from 9% (NDC 2020) to 15.8%; and conditional contribution from 27 to 43.5% (compared to the business-as-usual scenario (BAU)). NDC has specific emission reduction targets for each sector, specifically 37 remedies in the energy sector, 14 remedies in the agriculture sector, 7 remedies in the forestry and land use sector, 5 remedies in the industrial processes sector, and 11 remedies in the waste management sector.

4. Conclusions

Currently, Vietnam's total wood pellet output is about 5 million tonnes/year. Raw materials are mainly from forest residues and residues of the wood processing industry. Vietnam's wood pellet production potential can increase 2 to 3 times due to the huge plantation forest resource of nearly 5 million hectares. Vietnam's plantation forest area

can be increased by taking advantage of land areas that are being used ineffectively or fallow. The area of forests with FSC certification is still small at about 200,000 hectares, however, the area of forests with FSC certification will increase in the future, thus providing a source of quality raw materials for wood pellet production. Wood pellet production also faces strong competition with wood chip production due to higher production costs but lower value. Vietnam currently does not use many wood pellets or wood chips as fuel to produce electricity domestically. Currently, the demand for domestic wood pellets for co-firing to generate electricity is not high, but in the future, it will compete with wood pellet exports due to the strong commitment of the Vietnamese government to net zero emissions by 2050.

Vietnam is a country with huge biomass potential from forestry and agricultural residues. By 2050, biomass energy and MSW could account for up to 16% of Vietnam's total energy mix. However, the application of bioenergy has many limitations in terms of technology, conversion efficiency, and economic competitiveness compared to fossil energy sources. Therefore, many policy mechanisms are needed to encourage the development of energy sources from biomass and household waste. Furthermore, the biomass pellet production process also emits greenhouse gases. Therefore, life cycle analysis is needed to predict the percentage of biomass energy that can replace fossil fuels.

Currently, Vietnam's biomass electricity mainly utilises sugarcane bagasse, rice husks, and municipal solid waste. There are no biomass power plants from wood pellets or wood chips in Vietnam. The proportion of biomass electricity in Vietnam in the future will increase but not significantly compared to other renewable energy sources. The Vietnamese government is strongly committed to reducing net-zero emissions by 2050 and has many policies and mechanisms to encourage the development of renewable energy. However, due to the huge potential of biomass from both forestry and agricultural residues, the export of wood fuel such as wood pellets and wood chips will continue to increase in the future as world demand is still growing.

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COMPETING INTERESTS

The author declares that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] A.T. Hoang, A. Pandey, E. Lichtfouse, et al. (2023), "Green hydrogen economy: Prospects and policies in Vietnam", *Int. J. Hydrogen Energy*, **48**(80), pp.31049-31062, DOI: 10.1016/j.ijhydene.2023.05.306.
- [2] T.H. Le, P. Han, H.C. Le, et al. (2023), "Electricity market development in Vietnam: Historical trends and future outlooks", *Utilities Policy*, **81**, DOI: 10.1016/j.jup.2023.101510.
- [3] H.T. Nguyen, S.V. Nguyen, V.H. Dau, et al. (2022), "The nexus between greenhouse gases, economic growth, energy and trade openness in Vietnam", *Environmental Technology & Innovation*, **28**, DOI: 10.1016/j.eti.2022.102912.
- [4] T.K. Tran, C.Y. Lin, Y.T. Tu, et al. (2023), "Nexus between natural resource depletion and rent and COP26 commitments: Empirical evidence from Vietnam", *Resources Policy*, **85**, DOI: 10.1016/j.resourpol.2023.104024.
- [5] T.H. Le (2022), "Connectedness between nonrenewable and renewable energy consumption, economic growth and CO₂ emission in Vietnam: New evidence from a wavelet analysis", *Renew. Energ.*, **195**, pp.442-454, DOI: 10.1016/j.renene.2022.05.083.
- [6] T.X. Do, T.T.N. Phan, T.V.D. Son (2021), "Process modeling and economic assessment of converting municipal solid waste into solid fuel via hydrothermal processing: A case study in Vietnam", *J. Mater. Cycles Waste Manage*, **23**, pp.2318-2335, DOI: 10.1007/s10163-021-01286-5.
- [7] T.X. Do, Y.I. Lim, H. Do, et al. (2018), "Techno-economic analysis of fry-drying and torrefaction plant for bio-solid fuel production", *Renew. Energ.*, **119**, pp.45-53, DOI: 10.1016/j.renene.2017.11.085.
- [8] T.X. Do, Y.I. Lim, H. Cho, et al. (2017), "Process modeling and energy consumption of fry-drying and torrefaction of organic solid waste", *Drying Technol.*, **35**, pp.754-765, DOI: 10.1080/07373937.2016.1211674.

- [9] T.X. Do, Y.I. Lim (2016), “Techno-economic comparison of three energy conversion pathways from empty fruit bunches”, *Renew. Energ.*, **90**, pp.307-318, DOI: 10.1016/j.renene.2016.01.030.
- [10] T.X. Do, Y.I. Lim, S. Jang, et al. (2015), “Hierarchical economic potential approach for techno-economic evaluation of bioethanol production from palm empty fruit bunches”, *Bioresour. Technol.*, **189**, pp.224-235, DOI: 10.1016/j.biortech.2015.04.020.
- [11] T.X. Do, Y.I. Lim, H. Yeo, et al. (2014a), “Techno-economic analysis of power plant via circulating fluidized-bed gasification from woodchips”, *Energy*, **70**, pp.547-560, DOI: 10.1016/j.energy.2014.04.048.
- [12] T.X. Do, Y.I. Lim, H. Yeo (2014b), “Techno-economic analysis of biooil production process from palm empty fruit bunches”, *Energ. Convers. Manage.*, **80**, pp.525-534, DOI: 10.1016/j.enconman.2014.01.024.
- [13] A.H. Truong, M.H. Duong, H.A. Tran (2022), “Economics of co-firing rice straw in coal power plants in Vietnam”, *Renew. Sust. Energ. Rev.*, **154**, DOI: 10.1016/j.rser.2021.111742.
- [14] K. Handayani, P. Anugrah, F. Goembira, et al. (2022), “Moving beyond the NDCs: ASEAN pathways to a net-zero emissions power sector in 2050”, *Appl. Energy*, **311**, DOI: 10.1016/j.apenergy.2022.118580.
- [15] D.L. Bui, L.T. Le, T.M.N. Vu, et al. (2023), “Economic and environmental analysis of biomass pellet supply chain using simulation-based approach”, *Asia Pacific Management Review*, **28(4)**, pp.470-486, DOI: 10.1016/j.apmr.2023.02.002.
- [16] T.N. Do, P.J. Burke (2023), “Phasing out coal power in a developing country context: Insights from Vietnam”, *Energy Policy*, **176**, DOI: 10.1016/j.enpol.2023.113512.
- [17] T.T.H. Do, T.B.T. Ly, N.T. Hoang, et al. (2023), “A new integrated circular economy index and a combined method for optimization of wood production chain considering carbon neutrality”, *Chemosphere*, **311**, DOI: 10.1016/j.chemosphere.2022.137029.
- [18] T.N. Do, P.J. Burke, H.N. Nguyen, et al. (2021), “Vietnam’s solar and wind power success: Policy implications for the other ASEAN countries”, *Energy for Sustainable Development*, **65**, pp.1-11, DOI: 10.1016/j.esd.2021.09.002.
- [19] B.M. Yudego, J. Arevalo, O.D. Yáñez, et al. (2017), “Wood biomass potentials for energy in northern Europe: Forest or plantations?”, *Biomass and Bioenerg.*, **106**, pp.95-103, DOI: 10.1016/j.biombioe.2017.08.021.
- [20] T.C. Cao, X.P. To, K.A. Luong (2022), “Vietnam’s pellet production and export: Current status and some policy aspects”, https://goviet.org.vn/upload/aceweb/content/Vien%20nen%20Xuat%20khau%20cua%20Viet%20Nam_Thuc%20trang%20va%20mot%20so%20khia%20canh%20chinh%20sach.pdf, accessed 20 August 2023 (in Vietnamese).
- [21] Vietnam Ministry of Agriculture and Rural Development (2020), *Vietnam Forestry Development Strategy Period 2021-2030, Vision Toward 2050* (in Vietnamese).
- [22] T.C. Cao, X.P. To, L.H. Tran (2023), *Wood Pellet Production and Export of Vietnam: Current Dynamics and Market Trends*, https://goviet.org.vn/upload/aceweb/content/Wood%20pellet%20production%20and%20export%20of%20Vietnam%20T7-2023_EN.pdf, accessed 20 August 2023.
- [23] Vietnam Energy Association (2023), “Vietnam renewable energy in 2022: Events, achievements and identifying challenges”, <https://nangluongvietnam.vn/nang-luong-tai-tao-viet-nam-nam-2022-cac-su-kien-thanh-tuu-va-nhan-dien-thach-thuc-30046.html>, accessed 20 August 2023 (in Vietnamese).
- [24] Vietnam Government (2023), *Vietnam’s Electricity Planning VIII*, <https://xaydungchinhachsach.chinhphu.vn/toan-van-quy-hoach-phat-trien-dien-luc-quoc-gia-11923051616315244.htm>, accessed 20 August 2023 (in Vietnamese).

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