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Formulation, physiochemical and anti-bacterial properties of Mangosteen (*Garcinia mangostana*) juice

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Dorndi, S., Greedumrongsak, S., Monchaising, J., Srivilai, D., Suwanposri, A., Boonna, S. and Charoensuk, K. (2024). Formulation, physiochemical and anti-bacterial properties of Mangosteen (*Garcinia mangostana*) juice.

Abstract Mangosteen (*Garcinia mangostana*) is a popular tropical fruit widely grown in South-East Asia. June to August is the harvest season of mangosteen in Thailand. The edible portion (aril) of the mangosteen fruit is 1 of 3 of the whole fruit. Due to it is highly nutritional value, thus, mangosteen juice was formulated from frozen mangosteen with different concentrations of 45% and 100 % w/w of mangosteen juice. The effects of different concentrations of mangosteen puree on various parameters (e.g., colour, pH, total soluble solids (TSS) and microbiological evaluation) were investigated. The juice was to be red brown in colour with depended upon the concentration of the puree. The results indicated that TSS of the juice increase varied by the puree concentration while pH was decreased. The suitable 1 serving size (100 mL) of sterilized mangosteen juice contain total calories of it was 50-76.3 Kcal, total carbohydrate expressed 12.2-18.6 grams, and some amount of iron (Fe). DPPH scavenging activity ranged from 21.0% to 28.2%. Mangosteen juice at the concentrations of 100% of puree inhibited *in vitro* of *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli*.

Keywords: Mangosteen, Puree, Juice, Biological properties

Introduction

Mangosteen (*Garcinia mangostana*) is a popular tropical fruit widely grown in South-East Asia. June to August is the harvest season of mangosteen in Thailand. Mangosteen often referred to as the "queen of fruits," is a tropical fruit cherished for its sweet, tangy flavour and its wide range of health benefits. It is native to Southeast Asia and has gained global popularity due to its rich nutrient profile and antioxidant properties. Mangosteen puree, made by blending the ripe fruit's flesh which is a powerful source of antioxidants, particularly xanthones. Xanthones are compounds known for their ability to fight oxidative stress, reducing cell damage and lowering the risk of chronic diseases (Bumrungpert *et*

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al., 2010; Marzaimi and Aizat, 2019). The xanthones in mangosteen have anti-inflammatory effects that may help relieve symptoms of inflammatory conditions such as arthritis and other chronic diseases (Xie *et al.,* 2015). Mangosteen puree contains essential vitamins like Vitamin C, which supports the immune system by enhancing the body's ability to fight infections (Matan *et al.,* 2024). With its fiber content and natural enzymes, mangosteen puree can improve digestion and alleviate common gastrointestinal concerns (Gutierrez-Orozco *et al.,* 2015). The antioxidants in mangosteen can improve heart health by combating free radicals and preventing the oxidation of LDL cholesterol, a known contributor to cardiovascular diseases (Alam *et al.,* 2023). Research suggests that the xanthones in mangosteen may possess anticancer properties by inhibiting the growth of cancer cells and reducing inflammation related to cancer progression (Midin *et al.,* 2018; Klein-Júnior *et al.,* 2020). Mangosteen fruit has been used as an antibacterial agent since age old times. The mangosteen pericarp has proven to have antibacterial effect (Janardhanan *et al.,* 2017). However, the effect of the same on mangosteen juice has not been explored. The present study was an attempt to gain a better understanding of the antibacterial and the physicochemical properties effect of mangosteen juice.

Materials and methods

Preparation of puree

The mangosteen used in this study was acquired from a local market in Chanthaburi Province, Thailand. The fruits were washed thrice, then manually dehusked (the rind was cut open). Afterward, a screw press conveyer (Zhengzhou Shuliy Machinery Co., Ltd, Model No. 0086 15093262873, China) was used to extract the juice to obtain a puree with a smooth texture (Charoensuk *et al.,* 2018).

Preparation of juice

Two variations of mangosteen juice were created using 2 different concentrations: 45% and 100% puree. The specific formulations for each variation can be found in Table 1.

Table 1. Different formulations of mangosteen juice

Sample	Mangosteen puree (%w/w)	Sugar (%w/w)	Drinking water (%w/w)
T1	45	2.5	52.5
T2	100	0.0	0.0

Subsequently, every mixture combined and subjected to batch pasteurization in a stainless-steel apparatus, wherein the juice's temperature was elevated to 70- 80 °C for a duration of 5 minutes. Following this, the heated juice was promptly filled into pasteurized bottles and securely sealed. To ensure the

desired shelf life of the juice, the filled bottles underwent sterilization at a temperature of 100°C for a period of 15 minutes. Representative samples were then taken for nutrition tests and microbial growth analysis, including coliform bacteria, *E. coli*, *S. aureus*, and *Salmonella* spp. Using standard procedures.

Physiochemical properties analysis

The colour was examined using the colour meter (Nippon Denshoku, ZE2000) and represented as L*, a*, and b*. The pH level and total soluble solids (TSS) were assessed using pH meter (Extech PH100ExStik pH meter) and portable Otago Hand Refractometer (N1) with a range of 0-32%, respectively. The TSS was indicated as degrees Brix (°Brix).

Chemical compositions and nutritional value determination

Chemical compositions including moisture content, protein, fat, ash, and calculated total carbohydrates were analysed according to the AOAC method (2000). The calories were measured using the in-house method TE-CH-169, which according to AOAC standard methods (Association of Official Agricultural Chemists, 2010). The nutritional facts of mangosteen juice were determined using the standard procedure outlined in Table 4 at the Central Laboratory Co. Ltd. Thailand.

Biological activity

The phenolic compound, antioxidant activity, and antimicrobial assay of the juice derived from the industrial sterilization process were investigated in this study.

Total phenolic contents

The quantification of total phenolic content was conducted using the Folin–Ciocalteu method. In this procedure, 0.2 mL of the juice sample, along with 0.2 mL of Folin–Ciocalteu reagent and 4 mL of 0.7 M Na_2CO_3, were combined and mixed for a duration of 5 minutes. After 30 minutes, the absorbance was measured at 750 nm using a Thermo Scientific Orion AquaMate 7000 spectrophotometer, with gallic acid as the standard. The results were expressed as gallic acid equivalents (µg/mL) (Mongkontanawat *et al.,* 2022).

Antioxidant activity

The measurement of antioxidant capacity was conducted by employing 1,1-diphenyl2-picryl hydrazyl (DPPH). To determine this, 15 µL of the juice was combined with 4 mL of DPPH dissolved in ethanol (100 mM). The absorbance was then determined at 515 nm using the Trolox reagent as a reference standard

to express the Trolox equivalents per litter (TE)/L of the sample. The percentage of radical scavenging activity was calculated using the provided equation (Mongkontanawat *et al.,* 2022).

DPPH radical scavenging activity (%) = $[(A_0 - A_1)/A_0]\times100$ (1)

when A_0 = the absorbance of control reaction (containing all reagents except the sample)

A_1 = the absorbance of test compound

Antimicrobial assay

The disk diffusion method and its various modifications were employed to determine the antimicrobial activity (Lim *et al.,* 2013). The test utilized *B. cereus, S. aureus,* and *E. coli* as the microorganisms. To prepare the microbial samples, they were diluted with sterile distilled water until the absorbance reached 0.2 at a wavelength of 600 nm. The diluted microorganisms were then applied to the nutrient ager (NA) medium using the spread plate technique. Sterilized discs were positioned on the surface of the culture medium, and 15 µL of each sample juice was carefully dropped onto the discs. The plates were then incubated at a temperature of 30 °C for a duration of 48 hours. Following incubation, the diameter of the inhibition zone (IZD) was measured in millimetres (mm) and sterile distilled water used as a negative control.

The antimicrobial assay was reported as follows:

Inhibition zone (mm) = IZD sample (mm) – IZD negative control (mm)

Statistical analysis

The setup for the experiment followed a completely randomized design and was conducted separately for each variable. To test for differences in means, a one-way ANOVA test was used. All calculations were done using a 95% confidence interval ($p\leq0.05$).

Results

Mangosteen juice formulation

The physiochemical characteristics of the prepared mangosteen juices, which were made using concentrations of 45% and 100% puree and stored in air-tight containers (Table 2). The juice with 45% puree had a total soluble solid content of 10 °Brix and a pH value of 4.1, whereas the juice with 100% puree had a total soluble solid content of 17 °Brix and a pH value of 3.5. The colour analysis of the juices revealed that as the concentration of mangosteen puree increased, the brightness (L*) decreased, while the redness (a*) and yellowness (b*) values increased. Furthermore, Table 3 displays the results of microbial growth analysis (CFU) for the sterilized mangosteen juices with 45% and 100% puree. It was

observed that subjecting the juices to a heat treatment of 80°C for 5 minutes, followed by sterilization at 100°C for 15 minutes, effectively controlled the number of coliform bacteria, *E. coli*, *S. aureus*, and *Salmonella* spp. This treatment ensured that the juices met the safety requirements specified for ready-to-drink fruit juices stored in air-tight container-packages.

Table 2. Physiochemical properties of the squeezed mangosteen juice

Sample	Color			TSS	pH
	L*	a*	b*		
T1	75.7±0.9	4.0±0.5	9.6±0.2	14.0	4.1
T2	30.2±0.5	6.5±0.6	18.1±0.5	17.0	3.5

Table 3. The microbial amount in 100 mL of sterilized mangosteen juice

Microorganisms	Mangosteen puree (%w/w)		Standard	Standard procedure
	(45)	(100)		
Coliform bacteria	<1.1	<1.1	<2.2	MPN
E. coli	none	none	none	APHA(Water): 2012 (9221 E)
S. aureus	none	none	none	ISO 6579: 2002/ Cor. 1: 2004
Salmonella spp.	<1	<1	<1	BAM: 2001

Mangosteen juice nutrition

The nutritional values of the samples of mangosteen juice were assessed using the standard procedure outlined in Table 4. The appropriate serving size for sterilized mangosteen juice, containing 45% and 100% puree, is 100 mL. This serving size provided a total of 50 and 76.3 Kcal of energy, respectively. In terms of carbohydrates, a serving of mangosteen juice contains 12.2-18.6 grams, which corresponds to 4.1-6.2% of the recommended daily intake (%RDI). Mangosteen is considered a super fruit due to its numerous nutritional benefits. The analysis also revealed that mangosteen juices were sodium-free (Table 4). As a result, these juices are recommended for health- conscious consumers as a non-alcoholic beverage option.

Biological activity

The study examined the total phenolic content and antioxidant activity of mangosteen juices. The results revealed that the mangosteen juice with 45% puree contained approximately 23.37±3.21% antioxidants, while the juice with 100% puree had a higher concentration of 56.26±2.50% (Table 5). This observation was consistent with the levels of total phenolic compounds present in the juices. Interestingly, the antimicrobial assay using the disk diffusion method showed that 45% puree juice inhibited only *E. coli*, while 100% puree juice inhibited all three

pathogenic bacteria (*B. subtilis*, *S. aureus,* and *E. coli)* as shown in Table 5. This finding was likely due to the puree content in the juices. Overall, the biological activity of mangosteen juices could be attributed to their antioxidant, antimicrobial activity, and total phenolic compound content.

Table 4. Nutritional facts of a 100 mL serving size of mangosteen juice

% Daily value	Mangosteen puree (45% w/w)		Mangosteen puree (100% w/w)		Standard Procedure
	100 mL	%RDI	100 mL	%RDI	
Total juice	100	-	100	-	
Calories (Kcal)	50	2.5	76.3	3.8	In-house method TE-CH169
Total fat (g)	0	0	0	0	AOAC (2016)922.06
Protein (g)	0	0	0.5	0.9	In-house method TE-CH042
Total Carbohydrates (g)	12.2	4.1	18.6	6.2	In-house method TE-CH169
Fiber	0.8	4.0	1.2	6.0	In-house method TE-CH169
Vitamin B1 (mg)	0	0	0	0	In-house method TE-CH057
Calcium (mg)	0	0	0	0	AOAC (2012)984.27
Iron (mg)	0.2	1.8	0.6	4	AOAC (2012)999.10
Sodium (mg)	0	0	0	0	AOAC (2012) 984.27
Moisture (g)	85.1	-	87.0	-	AOAC (2012) 925.45 (A)

Table 5. Biological activities of mangosteen juices

Sample	Total phenolic content (μg/mL)	DPPH radical scavenging (%)	Inhibition zone (mm)		
			B. subtilis	*S. aureus*	*E. coli*
T1	2374.4±75.6	23.37±3.2	0.0±0.0	0.0±0.0	0.7±0.3
T2	5498.6±71.4*	56.26±2.5*	2.2±0.0*	0.9±0.1*	1.6±0.3*

Notes: Significant difference ($p \leq 0.05$) between T1 and T2 for each test is indicated by an asterisk (*)

Discussion

Different concentrations of 45% and 100 % puree were used to formulate mangosteen juice. The colour of the juice, which was intended to be red-brown, depended on the concentration of the puree. The TSS (Total Soluble Solids) and pH of the juice varied according to the concentration of the puree as similar to Lee *et al.* (2013). A serving size of 100 mL of sterilized mangosteen juice made with 100% puree contained higher total calories, total carbohydrates, fiber and iron compared to the juice made with 45% puree. Additionally, the juice made with 100% puree exhibited higher levels of total phenolic content, antioxidant activity (measured by DPPH) as similar to Sukma *et al.* (2011), and antimicrobial activity against both Gram-positive and Gram-negative pathogenic bacteria compared to

the juice made 45% puree. To ensure the safety of the ready-to-drink fruit juice in an air-tight container, the mangosteen juice underwent heat treatment at 80°C for 5 minutes, followed by industrial sterilization at 100 °C for 15 minutes. This process effectively controlled the number of pathogenic microorganisms present in the juice. Based on these findings, it can be concluded that the sterilized mangosteen juice made with 45 and 100% puree is suitable for commercialization and can be served as a single-strength fruit drink. Furthermore, it would be interesting to conduct an in vitro anticancer assay comparing the effects on normal cells.

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The nutritional values of chicken feet and effect of cryogenic freezing technique on freezing rate and its frozen product quality

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Greedumrongsak, S., Dorndi, S. and Charoensuk, K. (2024). The nutritional values of chicken feet and effect of cryogenic freezing technique on freezing rate and its frozen product quality.

Abstract Chicken feet are cooked and eaten in many countries. In Thai cuisine, the chicken feet are served in a variety of dishes, such as in a version of chicken green curry. After an outer layer of hard skin is removed, most of the edible tissue consists of skin and tendons with no muscle. This gives the feet a distinct gelatinous texture different from the rest of the chicken meat is the edible. In this study, the nutritional value of chicken feet and effect of cryogenic freezing techniques on freezing rate and its frozen product quality were investigated. The effect of freezing methods on the freezing rate (°C /h) and physical properties of the chicken feet were evaluated. Chicken feet samples were frozen using cryogenic freezing (CF) at -100 °C and natural convection freezing (NF) at -20 °C. The freezing rate of the CF and NF was calculated when the core temperature of chicken feet reached -20°C. It was found that, the freezing rate of CF was 4 times higher than that of the NF. Then, the quality parameters, such as drip loss, cooking loss, colour and nutritional value of the CF and NF were elucidated after natural convection thawing at 4°C. The chicken feet quality of the CF and NF did not cause any remarkable change in the quality. These results demonstrate that the CF for chicken feet processing may have practical applications in the frozen chicken feet industry.

Keywords: Nutritional value, Chicken feet, Cryogenic freezing, Freezing rate

Introduction

Poultry meat production and consumption are central components of the global food system, driven by increasing demand for affordable, high-protein food (Kumar *et al*., 2022). During poultry slaughter, 10 to 13% of the live weight of poultry comprises skin, stomach, heart, feet and other by-products (Akimova *et al.,* 2023). Chicken feet are indeed a by-product of poultry meat production, and they have significant value in various global markets. Instead of being waste, chicken feet are often utilized and processed for human consumption, especially in regions where they are considered a delicacy or key ingredient in traditional dishes (Santana *et al*., 2020).

Chicken feet are rich in several nutrients, provide a good amount of protein, which is essential for muscle growth, tissue repair, and overall body function (Akimova *et al*., 2023). They still offer many health benefits and are a good source of calcium, important for bone health, especially since the small bones in chicken feet are often soft and consumed after cooking (Almeida *et al*.,

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2013). However, chicken feet are a unique product that is widely consumed in certain parts of the world, and marketing them requires an understanding of regional preferences, cultural importance, and market demand. Chicken feet are often sold fresh or frozen in supermarkets and local markets. it's essential to handle them with care and process them properly to reduce the risk of food poisoning (Elsesy *et al*., 2015).

The chilling and freezing process of chicken feet is essential for ensuring product safety, maintaining quality, and extending shelf life. These processes are critical in preparing chicken feet for both domestic consumption and export, as they help preserve the texture, freshness, and safety of the product by slowing down bacterial growth and enzymatic activities. Therefore, the aim of this study is to study the effect of the cryogenic freezing (CF) technique on the physicochemical properties of chicken feet product compared to the nature conventional freezing (NF).

Materials and methods

Chilling treatment processing and sample collection

Fresh chicken feet, averaging 34 g/piece, was sourced from Marel feet processing (Cargill meat Thailand co., ltd.) which consist of scalding process by bring chicken feet to soke in hot water that control temperature around 56-62 °C for 5 minutes. Then, deskinned process or peeling process by rubber finger blended until yellow membrane out of the chicken feet. After being deskinned, all of these feet were equally and randomly divided into three chilling treatments: conventional chilling (air inlet without cooling water) treatment as a control, Treatment I; conventional chilling (air inlet with spraying cooling water) treatment, and Treatment II; conventional chilling (air inlet pipe pass through chill water bath) treatment. Treatment III; conventional chilling (cooling tank with additional cube ice) treatment. All 4 chilling treatments are controlling the temperature of the air blowing under the cooling tank under same condition and parameter: overflow of 0.14 Liter/chicken, capability feet 14 kg/min, chicken feet temperature at the beginning of 40.6$\pm$ 1.5°C, air temperature of 21.0 $\pm$ 1.0°C, time in spin chilling 7 mins, chill water of 3 $\pm$ 1.0 °C and room temperature of 20 $\pm$ 1.0°C. The core temperature of the chicken feet at the average of each chilling treatment was measured.

Freezing treatment processing

Samples of chicken feet contained in 10 packaging of 2.0 Kg plastic bag (Sunvally brand, Cargill meat Thailand Saraburi limited.) were frozen by using cryogenics frozen (CF) at -100 °C in a cryogenic freezer (SPEEDCRYO, Guangzhou speed refrigeration equipment Co., Ltd., Guangzhou, China) and natural convection Frozen (NF) at -20 °C in a chest freezer (BC/BD-318HD, Qingdao Hair Special Electric Freezer Co., Ltd., Qingdao, China). The freezing

process was continued until the core temperature of the chicken feet reached -20°C, recording hours of both types of freezing.

Thawing rate

Chicken feet frozen sample which contained in 2 Kg plastic bag was thawed in warm salt-water with a concentration of 3.0% and the probe of the thermometer was inserted into the sample to measure the core temperature. When the temperature reached 5 °C, it was considered that the thawing was complete.

Drip loss

The volume of drip loss was determined (Marchel *et al*., 2013) by placing the chicken feet sample in a funnel fitted with a 5 mm thick grid spacer to prevent the leakage from getting in contact with the sample. The amount of leakage was calculated by the difference in weight before freezing and after thawing process using the formula:

$$\%\text{Drip loss} = \frac{\text{(Weight of fresh sample – Weight of thawed sample)}}{\text{Weight of fresh sample}} * 100 \quad (1)$$

Cooking loss

Samples were sealed in plastic bags and heated in a water bath (BF-30SB, BioFree, Seoul, Korea) at 70°C for 30 min. Then, the heated samples were cooled at an ambient temperature for 30 min to remove excess moisture. The weight of the samples was weighed before and after the heat treatment. Cooking loss is expressed as the ratio of weight loss over the initial weight, using the follow formula:

$$\%\text{Cooking loss} = \frac{\text{(Weight of fresh sample – Weight of thawed sample)}}{\text{Weight of fresh sample}} * 100 \quad (2)$$

Water-holding capacities (WHC) of pre- and post-rigor frozen chicken leg and breast muscles were determined by released water (RW) % following the method of Joo (2018) with minor modifications. The samples (3.0 g) of chicken muscles were placed in a previous desiccated and weighed filter-paper (Whatman No. 1 of 11 cm of diameter) with two thin plastic films. Then the meat samples placed in the filter-paper, and plastic film were transferred between plexiglass plates followed by a 2.5 kg load was applied for 5 min. The compressed meat sample was removed care-fully from the damp filter-paper and plastic films and weighed the damp filter-paper and plastic films immedi-ately. The WHCs of different cut muscles were calculate.

Quality evaluation

Colour

Physical analysis of chicken feet by measuring colour with a Minolta CR400 colorimeter and quantifying the amount and display in term of the value L*(lightness), a*(green-red) and b*(blue-yellow).

Moisture content

Moisture content determination was performed using the official method of AOAC (1990). Chicken feet sample (3 g) was used in the assessment. This was repeatedly done until the weight of the samples remained constant. The moisture content was measured in triplicate and estimated based on the following equation:

$$\% \text{ Dry Content} = \frac{\text{Weight of dry sample}}{\text{Original weight of sample}} * 100 \quad (3)$$

$$100\ \% \text{Moisture content} = 100 - \% \text{ dry content} \quad (4)$$

Ash content

The total ash was determined according to AOAC Official Method 923.03. Approximately 5 g of chicken feet were weighed into a shallow ash dish. The dish containing the chicken feet was ignited, cooled in desiccator and weighed after cooling to the room temperature. Then, the sample was ignited in a furnace at 550°C (dull red) until the weight become constant. In this condition, light grey ash was observed in the dish. Then the grey ash was weighed after the sample was cooled to the room temperature in the desiccator. The total ash content was calculated according to the following equation:

$$\% \text{ Total ash} = \frac{\text{Ash weight}}{\text{Original sample weight}} * 100 \quad (5)$$

Crude protein content

Weight 1.0 g of chicken feet sample into a nitrogen-free weighing boat (code CM0486000) and place it into a 250 ml test tube. For each sample, add in the test tube: 2 catalyst tablet VST (code A00000277; 3.5 g K_2SO_4 5.0 g) 20 ml concentrated sulphuric acid (96-98%) and 5 ml of hydrogen peroxide (30%). Prepare some blanks with all chemicals and without sample. Digest the samples for 30 minutes at 250 °C, plus 30 minutes at 350 °C and 60 minutes at 420 °C. Distillation and Titration by let the test tubes to cool down to 50-60 °C. Distil the samples and 50 ml HCl (0.1 N) as titrant solution, with NaOH (32 %): 70 ml H_3BO_3 (4 % with indicators): 30 ml. Distillation and titration analysis time: from 4 minutes for one test. be tested a minimum of three times in the Sprint Protein Analyzer to establish a calibration line.

$$\%\text{Protein content} = \frac{0.1 * (V1-V2) * 14.0067 * N}{1{,}000 * W} * 100 \quad (6)$$

Where V_1 -volume of acid used to titrate the sample (ml)
V_2 -Volume of acid used to titrate to blank (ml)
N -Factor (6.25)

W -Initial weight (g)

Total fat content

Weight 2-3 grams of sample into HYDROTHERM weigh paper (C. Gerhardt #1004939). Place sample into HYDROTHERM beaker and add 0.5 teaspoon of celite. Place HYDROTHERM filters (C. Gerhardt #1004092) into corresponding collection funnel. Run HYDROTHERM program utilizing 15% hydrochloric acid as the hydrolysis reagent. After completion of the HYDROTHERM program, remove filters and dry for 30 minutes at 100°C. Place dried filters into cellulose thimble for extraction. Weigh initial beaker weights for the corresponding thimbles. Place thimbles into the corresponding beaker. Add 90-100 ml of Petroleum ether to the beaker and extract on the SOXTHERM®. Following extraction, remove thimbles, and dry beakers for 30 minutes at 100°C. Cool and record final beaker weight. Total fat is calculated using the following calculation:

$$\text{Total Fat} = \frac{\text{Final beaker weight - Initial beaker weight}}{\text{Sample Weight}} * 100 \quad (7)$$

Total plate count

For this purpose, frozen chicken feet sample thawed at 4°C and then cut in pieces. Then, blended 25 g in a sterile polyethylene bag with 150 ml of peptone-water for 30 s, where 1 ml of the peptone-water was transferred to a Petri dish with a sterile medium and then poured after cooling to a temperature of 45 °C in determinations of aerobic plate count at 37°C for 24 h. The number of bacteria was developed in the dishes (Hussein *et al.*, 2020).

Statistical analysis

Two individual experimental trials were carried out. Data were analyzed using the general linear model procedures of the SPSS 19.0 software package (SPSS, Chicago, IL). The data were statistical analysed by Analyze of variance (ANOVA) and compared mean difference using Duncan's New Multiple Range Test (DMRT) at a 95% confidence level (p<0.05).

Results

Chilling treatment processing

The effect of different chilling systems on the core temperature of chicken feet as shown in Table 1. The treatment I which reduced the air inlet temperature by using spraying chill water had affected to the core temperature of chicken feet reached 3.5 $\pm$ 0.52 °C. The treatment II which reduced the air inlet temperature by introduced the air inlet pipe pass through the chill water bath had affected to the

core temperature of chicken feet reached 6.7 ± 0.61 °C. The treatment III which control chill water bath at 3.0 ± 0.5 °C was done by added cube ice directly to the chilling tank that had affected to the core temperature of chicken feet reached 3.3 ± 0.48°C. While conventional chilling treatment (Control) had affected to the core temperature of chicken feet reached 7.1 ± 0.80 °C.

Table 1. Mean of core temperature chicken feet chilled by different chilling methods

Treatment	Air temperature (°C)		Cube ice	Chilling time (min.)	Chilling water (°C)	Core temperture (°C)
	Inlet	Outlet				
Control	21.0	21.0	No	7	3	7.1 ± 0.80
Treatmrnt I	21.0	3.2	No	7	3	3.5 ± 0.52
Treatmrnt II	21.0	17.1	No	7	3	6.7 ± 0.61
Treatmrnt III	21.0	21.0	Yes	7	3	3.3 ± 0.48

Freezing experiments

The freezing time of the CF and NF methods which had affected to the core temperature of the chicken feet shows that freezing chicken feet using cryogenic freezing (CF) takes 15 minutes, causing the core temperature of the product to reach -20 °C which the calculated freezing rate of this was 1.6 °C/min, while freezing chicken feet using the natural convection freezing method takes 60 minutes which the calculated freezing rate of this was 0.4 °C/min as shown in Table 2 and Figure 1.

Table 2. Cryogenic and natural convection freezing process of chicken feet

Freezing Time (Min.)	Cryogenic freezing (CF) (°C)	Natural convection freezing (NF) (°C)
0	4.0	4.0
5	0.0	3.0
10	-7.0	0.0
15	-20.0	-1.0
20	-	-1.2
30	-	-3.0
40	-	-10.0
50	-	-17.0
60	-	-20.0

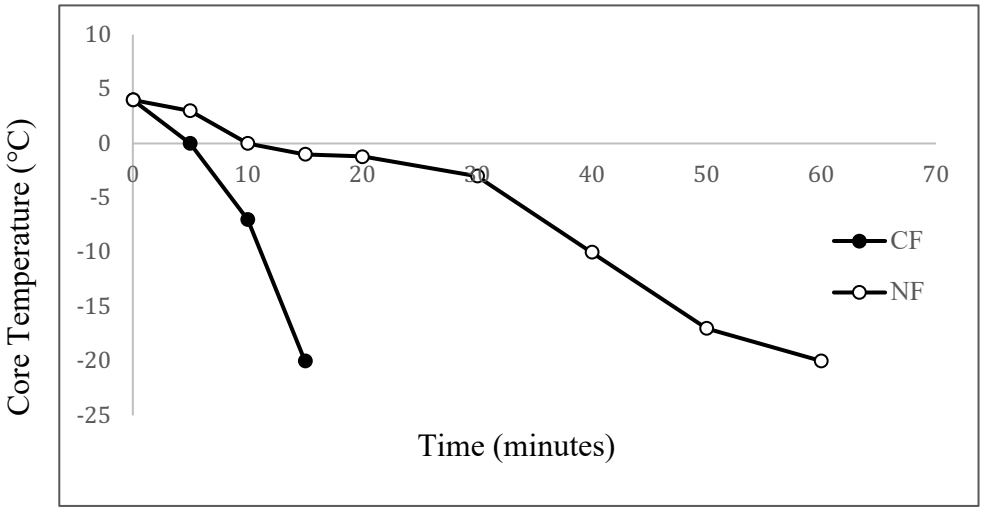

Figure 1. Freezing curve of cryogenic and natural convection feezing of chicken feet

Thawing experiments

The thawing experiment of the CF and NF methods of chicken feet in warm salt-water and recording thawing hours of both freezing types until the temperature reached 5 °C. The CF and NF methods which had affected to thawing time of the frozen chicken feet shows that cryogenic freezing (CF) takes 15 minutes, causing the core temperature of the product to reach 5.0 °C which the calculated of this thawing rate was 1.67 °C/min, while frozen chicken feet by using the natural convection freezing method takes 25 minutes which the calculated of this thawing rate was 1.00 °C/min as shown in Table 3 and Figure 2.

Table 3. Thawing experiment of cryogenic and natural convection freezing process of chicken feet

Thawing Time (min.)	Thawing Temperature (°C)	
	Cryogenic Freezing	National convection freezing
0	-20.0	-20.0
5	-2.2	-5.0
10	-0.1	-3.2
15	5.0	-1.0
20	-	2.0
25	-	6.0

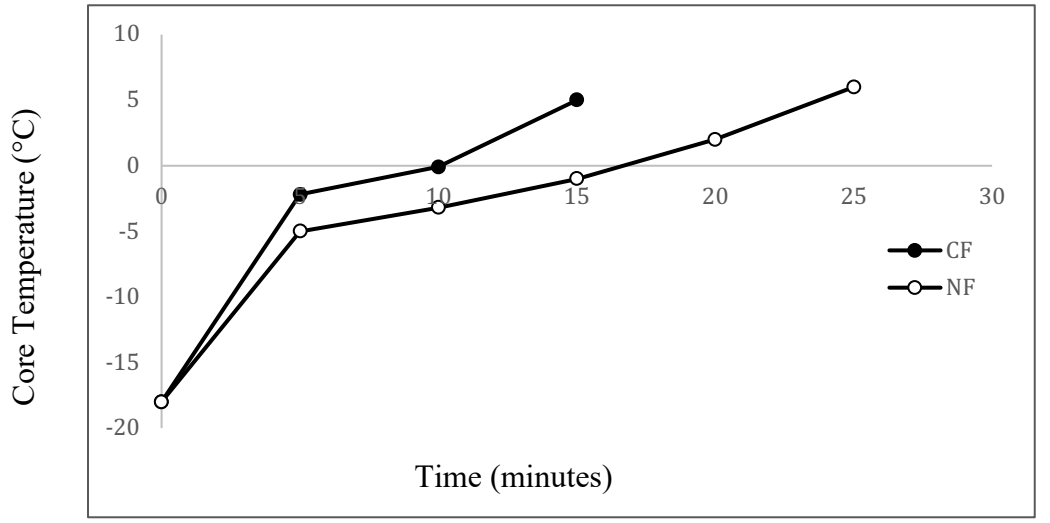

Figure 2. Thawing curve of CF and NF frozen chicken feet

Drip loss and cooking loss

It is generally accepted that frozen food will lose weight during thawing and cooking process. The amount of leakage was calculated by the percentage (%) of the difference in weight before and after of thawing and cooking process of the CF and NF frozen chicken feet. As the result, no different of drip loss and cooking loss of CF and NF which was 1.50 % and 1.00%, respectively, as shown in Table 3.

Table 4. Drip loss and cooking loos of cryogenic and natural convection freezing chicken feet

Samples	Sample weight (kg)		Drip loss^{ns}	Sample weight (kg)		Cooking loss^{ns}
	Before	After	(%)	Before	After	(%)
CF	2.0±0.00	1.97±0.01	1.50	2.0±0.00	1.98±0.01	1.00
NF	2.0±0.00	1.97±0.01	1.50	2.0±0.00	1.98±0.01	1.00

Quality evaluation

Colour and moisture content

This study was conducted to investigate the effects of freezing methods cryogenic freezing (CF) at -100 °C and natural convection freezing (NF) at -20 °C for 15 min and 60 min, respectively, and combined the natural convection thawing at 4°C on surface colour and moisture content of chicken feet which was compared to fresh chicken feet. After thawing, it is worth noting that the L*, a*, and b* and the moisture content values from the CF and NF, in the present study were much similar to that of fresh chicken feet (control) as shown in table 3 and all samples shown in Figure 3.

Table 5. Colour and moisture content of thawed CF and NF compared to fresh chicken feet

Sample	Colour			Moisture^{ns}
	L*	a*	b*	(%)
Fresh (control)	85.82	8.22	3.35	32.54
CF	83.46	8.92	4.42	31.09
NF	83.98	9.36	3.51	32.09

(a) (b) (c)

Figure 3. The apparence of chicken feet (a) fresh (b) thawed CF and (c) thawed NF

Nutritional value analysis

The effect of freezing methods on the nutrition value of chicken feet was evaluated. There were no significant differences in the nutrition value for the thawed CF and NF when compared to fresh chicken feet as shown in Figure 4.

Table 6. The proximate analysis of thawed CF and NF compared to fresh chicken feet

Samples	Proximate analysis (%)				
	Carb^{ns}	Fiber^{ns}	Protein^{ns}	Fat^{ns}	Ash^{ns}
Fresh	24.65 ± 1.64	3.82 ± 0.33	21.77 ± 2.59	17.80 ± 0.00	0.66 ± 0.18
CF	23.82 ± 1.64	3.91 ± 0.33	20.45 ± 2.12	16.92 ± 0.00	0.78 ± 0.11
NF	23.45 ± 1.64	4.01 ± 0.33	21.77 ± 3.00	16.81 ± 0.00	0.80 ± 0.18

Total plate count

A number of total aerobic bacteria was observed in comparing CF and NF with fresh chicken feet sample. There were no significant differences in the total plate count bacteria for the CF and NF when compared to fresh chicken feet which less than 5.0 x10^5 CFU/g as shown in figure 4.

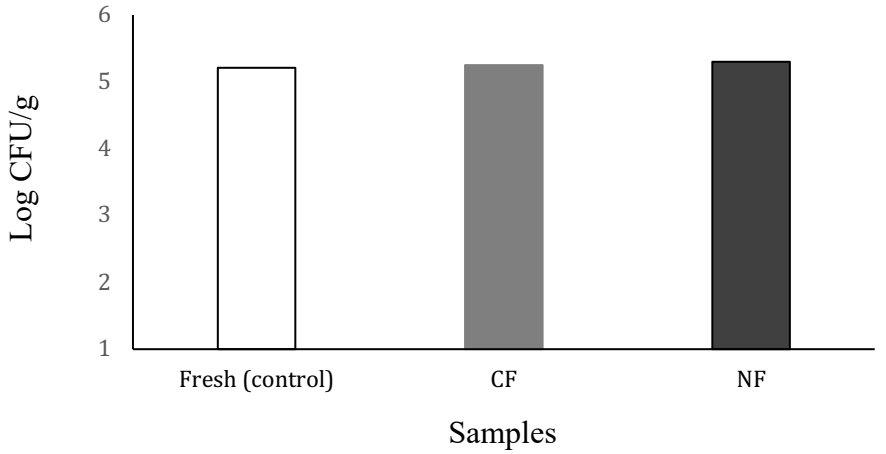

Figure 4. Total aerobic bacteria of CF and NF frozen chicken feet compared with fresh chicken feet

Discussion

The effect of different chilling processes on the quality and bacterial load of chicken is of continuing concern (Zhang *et al*., 2015). Chilling temperature refers to 0-4 °C as the temperature at which poultry products, such as chicken feet, are cooled and stored to maintain freshness and prevent the bacterial growth. In the present study, both methods were mentioned by reducing the air inlet temperature by directly spraying chilled water (Treatment I) and maintaining the chilling tank temperature by adding ice cubes (Treatment III) were effective in controlling the core temperature of chicken feet to below 4°C.

Offer interesting insights into the potential of CF for practical use in the frozen chicken industry (Streeter and Spencer, 1973). The results demonstrated that CF was superior in terms of freezing efficiency, with a freezing rate of 1.6°C/min compared to 0.4°C/min for NF. It made CF a more practical choice for the frozen chicken feet industry when speed and quality preservation are key factors. A faster freezing rate can be advantageous as it reduces the formation of large ice crystals, which can negatively affect texture (Choi *et al*., 2017). In the comparison between high-speed thawing rates of CF which was 1.67 °C/min and slow thawing rates was 1.0 °C/min of NF which may be significantly impact the quality, safety, and texture of chicken feet. However, no significant differences were observed in key quality factors like drip loss, cooking loss, colour, moisture, nutritional value, and total amount of bacteria among them (Choi *et al*., 2017). Studied the effect of freezing rate on the quality characteristics of pork loin was found that the qualities of pork within high freezing rate were not different from slow freezing rate.

In conclusion, our results revealed that both cooling techniques by application of chilled water through direct spraying helped rapidly reduce the air inlet temperature, which in turn controlled the core temperature of the chicken feet and the ice cube addition to maintain the chilling tank's temperature with the addition of ice cubes provided a stable and consistent cooling environment, keeping the core temperature below 4°C which can be integrated into chicken feet processing systems to optimize temperature control and ensure food safety to meet regulatory standards. Interestingly, the results suggest that CF could offer a practical advantage in the frozen chicken feet industry by improving freezing efficiency without compromising the quality and safety of the product. The application of CF could therefore be an effective strategy for optimizing frozen chicken feet production while maintaining product standards. In contrast, NF may be better suited for smaller-scale operations where speed is less critical and cost considerations are a priority.

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Growth and yield performance of DOA Songkhla 2 Bambara groundnut grown in the central plain of Thailand

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Kaokrathok, R. and Phakamas, N. (2024). Growth and yield performance of DOA Songkhla 2 Bambara groundnut grown in the central plain of Thailand.

Abstract In the Central Plain of Thailand, DOA Songkhla 2 (newly released variety) exhibited lower growth and yield performance than DOA Songkhla 1. Leaf dry weight accounted for 44.4% of yield variation at harvest and 42.3% at 85 DAP, highlighting its critical role in pod production. However, the dry pod yield of DOA Songkhla 2 was significantly lower than its genetic potential, reaching only 264.02 kg ha^{-1}. The low harvest index (0.18) indicated inefficient nutrient partitioning to pod formation. Key limiting factors include poor drainage and low soil aeration, which may have hindered root development. These findings highlight the need for improved agronomic practices to enhance yield potential in the region.

Keywords: Clay soil, Growth analysis, Dry matter accumulation, Multiple regression

Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an important crop in Africa due to its high nutrient content, including carbohydrates (55–70%), protein (17–25%), and dietary fiber (5.2–6.4%) (Maphosa *et al*., 2022). These traits make Bambara groundnut an affordable and essential protein source. The crop thrives in arid environments and contributes to soil fertility by fixing nitrogen, reducing the need for chemical fertilizers (Nwekw and Emch, 2013). In Thailand, the Bambara groundnut is mainly cultivated in southern provinces such as Songkhla, Narathiwat, and Pattani (Wongwichaiwat, 2023). However, its cultivation faces challenges, including restricted land availability, competition with rubber and oil palm, insufficient pest control, and climate-related shifts in rainfall patterns and pest pressure. Despite these challenges, Bambara groundnut remains a promising climate-resilient crop (Mayes *et al*., 2019). DOA Songkhla 2 was developed from the IITA-derived TVsu 89 variety and was introduced in 2023 by the Songkhla Field Crop Research Center. It matures within 85–90 days and provides higher yields than its predecessor, DOA Songkhla 1. It produces an average fresh pod yield of 2,637.5 kg ha^{-1} and a dry pod yield of 868.8 kg ha^{-1}, with respective increases of 27% and 31% compared to DOA Songkhla 1. Additionally, DOA Songkhla 2 has a protein content of 18.20%, 3.22% higher than its predecessor (Suwanprasert *et al*., 2023). Expanding cultivation to new areas could improve productivity, particularly in regions with unpredictable weather, such as southern

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Thailand. The Central Plain of Thailand, primarily focused on rice farming, faces challenges including climate variability, soil degradation, and increasing land competition. Bambara groundnut is a promising alternative due to its low fertilizer requirements (Khan *et al*., 2024), drought tolerance, and nitrogen-fixing ability. These traits support food security and improve farmers' livelihoods. Additionally, the crop's nutritional value makes it a sustainable food option for both rural and urban communities. However, challenges such as poor soil drainage, acidic soils, and inconsistent yields pose challenges for adoption in the Central Plain. To encourage its growth, it is important to determine how well DOA Songkhla 2 can handle tough conditions in the Central Plain, such as clay soils and poor drainage. This study focused on growth parameters, yield components, and strategies for optimizing agronomic practices. The goal is to identify key growth characteristics and develop strategies to expand Bambara groundnut cultivation in Thailand sustainably.

Materials and methods

Location and experimental design

Experiments were conducted in the Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, from November 2023 to March 2024. A randomized complete block design (RCBD) with four replications was used. The treatments included two Bambara groundnut varieties: DOA Songkhla 1 (the check variety) and DOA Songkhla 2 (the newly released variety).

Soil preparation, planting and cultural practices

The soil was plowed twice in perpendicular directions, then leveled. Afterward, soil samples were collected for physical and chemical analysis. The experimental site was divided into eight plots, each measuring 4.5 × 6 meters, with a 70 cm alley left between the plots to facilitate field management. In each plot, seeds were spaced 50 cm apart between rows and 40 cm apart within rows, totaling 160 hills per plot. Three seeds were sown per hill, and replanting was completed within 14 days.

A sprinkler irrigation system was implemented to ensure a sufficient water supply. Weed control involved a combination of manual removal and chemical application. Specifically, alachlor, a pre-emergent herbicide, was applied at a rate of 3.75 liters per hectare after planting. Fertilization involved applying a mixed NPK fertilizer (15:15:15) at a rate of 188 kg ha-1, four weeks after planting. The fertilizer was sprinkled along the sides of the rows and covered at the base of the soil while it was moist. Iprodione was sprayed on the plants to prevent leaf blight disease.

Data collection

Data on root dry weight, stem dry weight, leaf dry weight, pod dry weight, and total dry weight were collected from four plants at 45, 55, 65, 75, 85, 95, and 105 days after planting (DAP), and from ten plants at the harvest stage. For each plot, ten grams of leaves were collected to measure the leaf area (LA) using the LI-3100C leaf area meter (LI-COR, Inc.), and the leaf area index (LAI) was subsequently calculated. Roots, stems, and leaves were dried in a hot-air oven at 80°C for 48 hours or until a constant dry weight was achieved. The dry weights of these samples were recorded to calculate the crop growth rate (CGR) and harvest index (HI).

Leaf area index (LAI) was calculated as follows:

$$LAI = LA/GA$$

where LA was the leaf area, and GA was the land area.

Crop growth rate (CGR) was calculated according to the equation below:

$$CGR = 1/GA \times [(W2- W1)/(T2-T1)]$$

where, GA was the land area, and W2 and W1 were the dry weights of the plant at times T2 and T1, respectively.

Harvest index (HI) calculations were determined using the formula:

$$\text{Harvest Index} = \text{Economic yield/Biological yield}$$

Data analysis

The data were analyzed using a randomized complete block design (RCBD), and differences among treatment means were evaluated using the least significant difference (LSD) method at a 0.05 probability level. Multiple regression analysis was used to identify the growth characteristics that contributed to yield. All statistical analyses were conducted using the Statistix 8 program (Statistix, 2003).

Results

Soil properties

Prior to planting, soil samples were collected from depths of 0–15 cm and 15–30 cm to analyze their chemical and physical properties. The results of this analysis are summarized in Table 1. The soil was acidic (pH 4.2–4.4) and non-saline, with electrical conductivity (EC) ranging from 0.20 to 0.27 mS/cm. The

organic matter (OM) content was moderate, ranging from 2.17% to 2.88%. Adequate levels of available phosphorus (P) and potassium (K) were detected, with concentrations of 41–42 mg/kg and 313–377 mg/kg, respectively. The total nitrogen content was approximately 0.14%. The experimental site was characterized by clay soil, with sand comprising 2.8–3.8%, silt 26.8–29.8%, and clay 64.4–67.4%.

Table 1. Soil properties of the experimental site

Physical and chemical properties	Depth 0-15 cm	Depth 15-30 cm
Soil texture	Clay	Clay
Sand (%)	3.8	2.8
Silt (%)	26.8	29.8
Clay (%)	64.4	67.4
pH	4.4	4.2
EC (dS/m)	0.20	0.27
OM (%)	2.81	2.17
Total N (%)	0.14	0.14
Available P (mg/kg)	41	42
Available K (mg/kg)	377	313

Development and growth characteristics

Two Bambara groundnut varieties were cultivated in the Central Plain of Thailand. The newly released variety, DOA Songkhla 2, matured in approximately 91 days, while the check variety, DOA Songkhla 1, took about 108 days to reach maturity (Figure 1). Growth pattern analysis revealed that both varieties exhibited similar plant height, canopy size, and leaf quantity between 45 and 65 days after planting. However, from 75 days after planting until harvest, DOA Songkhla 1 demonstrated greater leaf growth compared to DOA Songkhla 2.

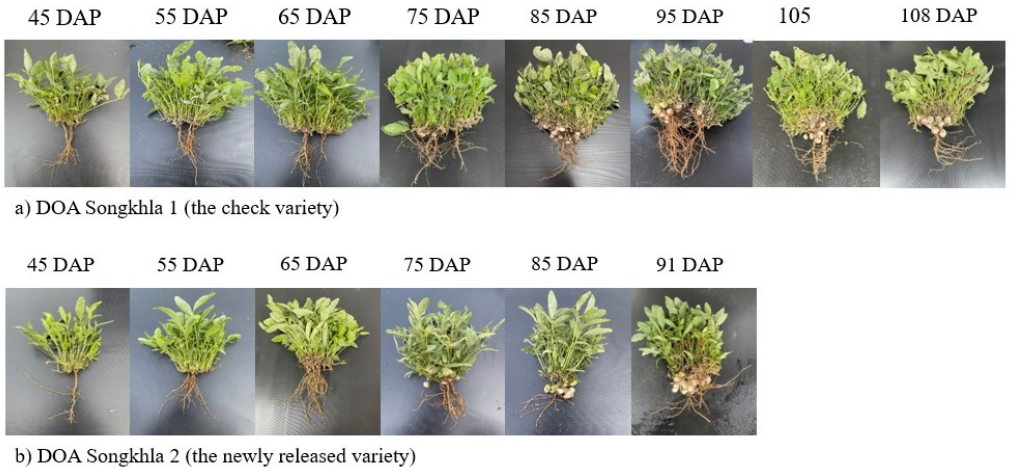

Figure 1. Development and growth characteristics of (a) DOA Songkhla 1 (the check variety) and (b) DOA Songkhla 2 (the newly released variety) as observed during cultivation in the Central Plain of Thailand

Table 2 indicated significant differences ($P \leq 0.05$ and $P \leq 0.01$) in the crop growth rate of the two varieties across all growth stages, except during the initial 45 days after planting. The crop growth rate increased over time. The superior growth rate of DOA Songkhla 1 suggests better adaptability to soil conditions. DOA Songkhla 1 achieved its peak growth rate (14.65 g m^{-2} d^{-1}) between 85 days after planting and harvest. In comparison, DOA Songkhla 2 peaked at 12.52 g m^{-2} d^{-1}. DOA Songkhla 2 exhibited a lower growth rate than DOA Songkhla 1, which corresponded to differences in leaf area indices (Figure 2).

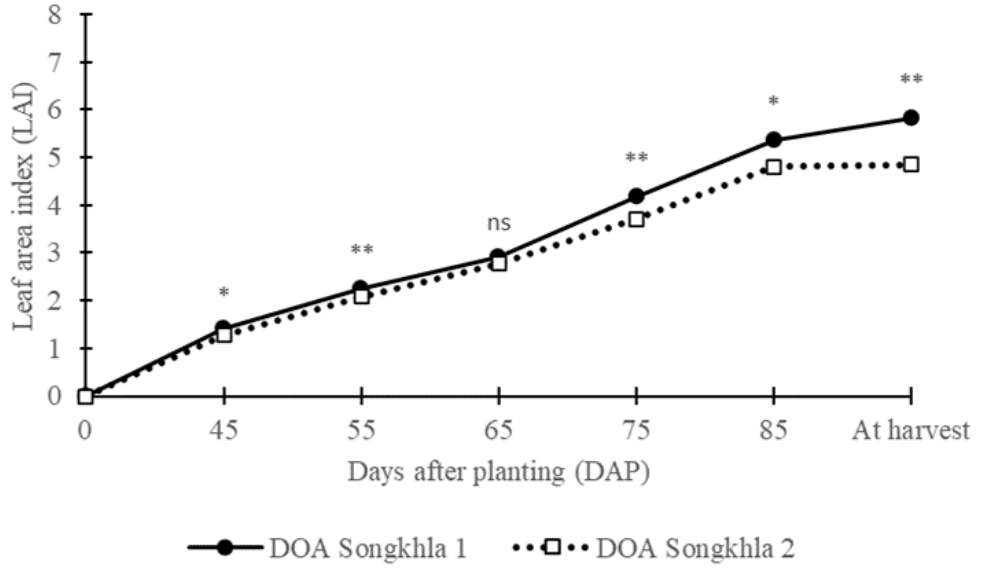

Figure 2. Presents the mean Leaf Area Index (LAI) values measured at 45, 55, 65, 75, and 85 days after planting (DAP), as well as at harvest, for two Bambara groundnut varieties cultivated in the Central Plain of Thailand

Table 2. Shows significant differences ($P \leq 0.05$ and $P \leq 0.01$) in the crop growth rate of the two varieties across all growth stages, except during the first 45 days after planting

Variety	CGR (g m^{-2} d^{-1})					
	PT to 45 DAP	45 to 55 DAP	55 to 65 DAP	65 to 75 DAP	75 to 85 DAP	85 to harvest
DOA Songkhla 1	0.08	1.32[a]	2.72[a]	3.67[a]	5.89[a]	14.65[a]
DOA Songkhla 2	0.07	0.53[b]	1.49[b]	2.26[b]	3.59[b]	12.52[b]
F-test	ns	*	*	**	*	*
C.V. (%)	19.71	27.87	16.69	8.83	14.03	5.13

PT = planting, DAP = days after planting, ns = non-significant, * and **= significantly different at $P \leq 0.05$ and significantly different at $P \leq 0.01$, respectively. Means within the same column followed by the different letters are significantly different by LSD.

The dry weights of stems, leaves, and roots increased progressively over time for both varieties, as shown in Table 3. At 45, 65 DAP, and harvest, DOA

Songkhla 1 exhibited significantly higher stem dry weights. No significant differences in stem dry weights were observed during other growth stages. There were negligible differences in leaf growth between the varieties at 45 and 55 DAP. After 65 DAP, DOA Songkhla 1 demonstrated more pronounced leaf development than DOA Songkhla 2. Similarly, significant differences in root dry weights ($P \leq 0.05$ and $P \leq 0.01$) were recorded at 45 DAP, 65 DAP, 75 DAP, and at harvest, with DOA Songkhla 1 exhibiting superior root development.

Overall, the dry weight distribution revealed that leaves contributed the largest proportion, followed by roots, while stems had comparatively lower dry weights. These findings highlighted the superior stem and leaf development of DOA Songkhla 1 compared to DOA Songkhla 2.

Table 3. Means for stem dry weight, leaf dry weight, and root dry weight were recorded at 45, 55, 65, 75, and 85 days after planting (DAP) until harvest for two Bambara groundnut varieties

Variety	Stem dry weight (kg ha^{-1})					
	45 DAP	55 DAP	65 DAP	75 DAP	85 DAP	At harvest
DOA Songkhla 1	75.75^{a}	57.25	94.06^{a}	151.75	259.56	539.16^{a}
DOA Songkhla 2	38.50^{b}	35.31	63.75^{b}	110.75	200.00	206.46^{b}
F-test	**	ns	*	ns	ns	**
C.V. (%)	8.82	19.78	11.20	16.49	22.03	4.96
	Leaf dry weight (kg ha^{-1})					
DOA Songkhla 1	89.25	72.63	256.69^{a}	288.50	555.88^{a}	818.25^{a}
DOA Songkhla 2	75.38	62.19	172.56^{b}	287.19	397.94^{b}	456.03^{b}
F-test	ns	ns	**	ns	**	*
C.V. (%)	23.94	1.71	4.97	15.41	5.42	21.93
	Root dry weight (kg ha^{-1})					
DOA Songkhla 1	16.13^{a}	32.63	96.75^{a}	272.56^{a}	486.31	845.00^{a}
DOA Songkhla 2	9.38^{b}	30.01	71.06^{b}	147.94^{b}	306.63	416.69^{b}
F-test	*	ns	*	**	ns	*
C.V. (%)	18.32	27.01	11.39	13.63	27.60	25.08

DAP = days after planting, ns = non-significant, * and **= significantly different at $P \leq 0.05$ and significantly different at $P \leq 0.01$, respectively. Means within the same column followed by the different letters are significantly different by LSD.

Pod yield and harvest index

Table 4. Fresh pod yield, dry pod yield, and harvest index of DOA Songkhla 1 and DOA Songkhla 2 grown in the Central Plain. DOA Songkhla 1 had a significantly higher dry pod yield and harvest index ($P \leq 0.05$), indicating better nutrient allocation efficiency. On average, the DOA Songkhla 1 variety produced 1,344.00 kg ha^{-1} of fresh pods and 331.86 kg ha^{-1} of dry pods. In contrast, the DOA Songkhla 2 variety yielded 1,162.60 kg ha^{-1} of fresh pods and 264.02 kg ha^{-1} of dry pods. Additionally, the harvest index of the DOA Songkhla 1 variety (0.28) was significantly higher ($P \leq 0.01$) than that of the DOA Songkhla 2 variety (0.18).

Table 4. Comparison of pod weights and harvest index between Bambara groundnut varieties

Variety	Fresh pod weight (kg ha^{-1})	Dry pod weight (kg ha^{-1})	Harvest index (HI)
DOA Songkhla 1	1,344.00^{a}	331.86^{a}	0.28a
DOA Songkhla 2	1,162.60^{b}	264.02^{b}	0.18b
F-test	*	*	**
C.V. (%)	5.49	3.96	5.79

* and **= significantly different at $P \leq 0.05$ and significantly different at $P \leq 0.01$, respectively. Means within the same column followed by the different letters are significantly different by LSD.

Relationship between growth characteristics and dry pod yield

A multiple regression analysis was performed to identify growth characteristics that influenced pod yield. The initial full-model analysis had indicated that only leaf dry weight was statistically significant, whereas LAI, stem dry weight, and CGR were not. Sequential fitting of the reduced model showed that leaf dry weight at harvest and at 85 DAP were the key growth characteristics that affected dry pod weight, explaining 44.4% and 42.3% of the variation, respectively (Table 5). These results demonstrated that leaf dry weight during the late reproductive stage had played a critical role in determining the yield of Bambara groundnut.

Table 5. Regression analysis of leaf dry weight at various stages and dry pod weight of Bambara groundnut varieties

Source	D.F.	S.S.	% S.S.	Probability
Leaf dry weight at harvest	1	4,443.1	44.4	< 0.01
Leaf dry weight at 85 DAP	1	4,240.8	42.3	< 0.05
Residual	5	1,341.0	13.3	
Total	7	10,024.8	100	

DAP = days after planting

Discussion

This study evaluated the adaptability, growth, and yield potential of the Bambara groundnut variety DOA Songkhla 2 in the Central Plain of Thailand, revealing a significant yield gap compared to its genetic potential. While Suwanprasert *et al*. (2023) reported a dry pod yield potential of 868.8 kg ha^{-1}, actual yields reached only 264.02 kg ha^{-1}, representing a 70% reduction from the expected yield. Regional studies also reported yields approximately 31% below potential (Wongwichaiwat *et al*., 2023), questioning the variety's suitability for the region and highlighting the need for further research on its adaptability.

The reduced yields were likely caused by unfavourable agronomic and environmental conditions, including clayey soils with poor drainage and aeration. Bambara groundnuts thrive in well-drained sandy soils, as waterlogging increases pod rot risk. Additionally, soil pH levels outside the 5.0–6.5 range hindered

nutrient uptake, exacerbating the yield gap. Sowing date also impacted yield, with early November identified as optimal (Khan *et al*., 2024), while planting at a later date produced lower yields.

Physiological traits such as the leaf area index (LAI) and crop growth rate (CGR) further explained yield variability. The LAI for DOA Songkhla 2 ranged from 3.71 to 4.85 between 75 days after planting (DAP) and harvest, surpassing the 3.23–3.59 range reported by Wongwichaiwat *et al*. (2023). This difference was attributed to greater leaf area and dry weight accumulation after 75 DAP. Similarly, CGR peaked between 85 DAP and harvest, later than previously reported, reflecting differences in leaf development and biomass accumulation.

Excessive leaf dry weight during the late reproductive stages was identified as a key factor affecting yield because nutrient diversion toward leaf biomass reduced pod development efficiency. Low harvest index (HI) values highlighted the need for improved nutrient allocation. Compared to HI values of 0.20–0.60 reported by Gao *et al*. (2020), the observed imbalance between leaf biomass and pod development highlights the need for optimizing nutrient partitioning.

In summary, this study identified a substantial yield gap for DOA Songkhla 2 in the Central Plain, which was driven by unsuitable soil conditions, suboptimal sowing dates, and inefficient biomass allocation. While leaf dry weight at 85 DAP was critical for yield, excessive accumulation reduced efficiency. These findings emphasize the need for improved agronomic practices and soil amendments to increase yields. Future research should optimize nutrient allocation and address soil limitations to maximize DOA Songkhla 2's yield potential.

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Investigation of genetic diversity of two elite rice cultivars using SSR based marker

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Sinumporn, S. (2024). Investigation of genetic diversity of two elite rice cultivars using SSR based marker.

Abstract Rice diversity plays an important role in breeding program. Microsatellite or SSR can be utilized to distinguish rice cultivar at specific loci. Polymorphism showed between different rice cultivars population are considered as useful SSR. Khao Dawk Mali 105 (KDML105) and IR64 are two elite rice cultivars and survey showed that 7 out of 27 SSR revealed polymorphism. Polymorphism information content (PIC) ranged from 0.0 (Monomorphic) – 0.5 (Polymorphic) with average PIC value was 0.13 per locus. RM551 RM5371 RM20224 RM424 RM3 RM17470 RM6480 possessed highest PIC value of 0.5. Total 34 single locus allele were amplified with average was 1.26 allele per locus. Due to two studied rice cultivars, maximum of 2 alleles were detected in two cultivars (RM551 RM5371 RM20224 RM424 RM3 RM17470 RM6480) and one allele were detected in remaining SSR. Preliminary study provided a useful SSR information for extension study with a greater number of rice cultivars population.

Keywords: Rice, Genetic diversity, Simple sequence repeat, Polymorphism

Introduction

Rice is an important cereal for half of world population (Jiang *et al*., 2023). Rice quality has continuously been improved for many years. Different traits of appeared in various aspect, for example morphology, biochemistry, shape appearance, etc. Rice diversity is an important factor for develop new cultivar. Different aspect can be merge and trace back via marker. In quantitative, Rice yield is considered as important clue for rice production. Fragrance also plays a crucial determining role in rice quality. Several rice varieties, of which belonged to indica, were classified as fragrance when grow in specific area, especially in South and Southeast Asian (Ootsuka *et al*., 2014). Favourite of Thai Hommali rice increase its demand in many places such as America and Europe, where rice is not staple diet. Other than Thai Hommali aromatic rice, Pakistani and Indian 'Basmati' also most favorites for fragrant trait. Basmati, mostly consume in Indian subcontinent and middle east, of which possess a fluffy cooked characteristic and displayed fragrance only when it is grown in the north-western foothills of the Himalayas (Mahajan *et al*., 2018). Thai Hommali rice usually coincide with its soft-cooked texture, is well-known for its popcorn-like flavor, of which attract consumer both local and international (Li *et al*., 2016). It is widely accepted that

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Thai Hommali rice contributes highest quality when it is grown in area of northeastern of Thailand called "Thung Kula Rong Hai" where harsh environment, traditional rice growing practice as well as and post-harvest storing conditions accumulate rice fragrance. Due to its low yield and limited growing area, of which specific area was recognized as Thung Kula Rong Hai Hommali rice, it is sold at higher price compared to non-fragrance harder-cooked rice. In fragrance rice, numerous compounds, reported more than two hundred, contribute to aroma, of which the presence of 2-acetyl-1-pyrroline (2AP) was a crucial composition, of which has been found in rice elsewhere but not root (Inpradit *et al*., 2023; Jiang *et al*., 2023; Rice Science Center, 2022). Previous study showed that *BADH2* role was fragrance decisive gene. Missing of eight nucleotides at exon 7 of *badh2* recessive allele triggered 2AP accumulation (Inpradit *et al*., 2023). In rice breeding, selection of fragrant line offspring using morphological markers was time consuming and laborious task, in which hefty assessed and discrepancy were easily involved (Bradbury *et al*., 2005). Moreover, measurement of 2AP was also high cost to detect and budget overwhelming procedure.

Classification of rice is useful to distinguish gene or locus or characteristic. Abundant of rice accession refer to diversity of genetic source for breeding project. However, parent selection for further study is a crucial step to determining achieve goal (Pathaichindachote *et al*., 2019). Conventional breeding exploits on appeared characteristics, e.g., height, length, color, etc., of which time and labour consume. Recent technique method that allowed shorter time consuming, and less laborious was PCR-based SSR marker, of which useful for rice selection with cost effective. Although molecular marker located distance from gene or QTLs responsible for the trait, multiple markers provided robust distinguish of individuals. With trustful pattern, consistency, high accuracy, uncomplicated result, and robust throughput have contributed as reasons for widespread utilization of molecular marker among rice breeding laboratories around the world (Rice Science Center, 2022, Aljumaili *et al*., 2018; Saengprajak, 2012; Shabir *et al*., 2017). At the same time, co-dominant segregation linkage and repetitive pattern, it was popular to distinguish allele, gene, or QTLs at particular loci (Shabir *et al*., 2017). Moreover, it is useful tool to identify individuals' genetics, purity content, conservation procedure as well as for breeding purpose using marker assisted selection (Aljumaili *et al*., 2018). Other than genetics, environment factors also play a role in fragrance determination. Geographical area, crop manipulation, climate conditions are contributed to different 2AP accumulation. Soil nutrient such as Mn also effects to 2AP addition (Li *et al*., 2016), also N nutrient content direct effect to 2AP content (Jiang *et al*., 2023).

In this study, polymorphism of reported molecular marker were assessed. Polymorphism difference information should be useful for further study related to rice selection. In this study two cultivars, KDML105 and IR64, were evaluated for alleles polymorphism at specific loci via selected SSR marker.

Materials and methods

Plant material

Two cultivars named KMDL105 and IR64 were used in this study. Rice was grown from seed in pot within Rajamangala University of Technology Isan. DNA extraction was employed using CTAB method (Doyle and Doyle, 1990). Agarose gel electrophoresis of extracted DNA were conducted to determine DNA quality. DNA were kept at 4 °C until further utilized.

PCR assay

PCR primers for DNA markers were retrieved from previous study and name of primers indicated in Table 1. The PCR was performed in a total of 25 µl volume. PCR cocktail mixture containing 10 ng DNA template, 25 pmole each of primers, 12.5 µl of 2X Master Mix DNA Polymerase Taq (GoTaq® Promega, USA). PCR cycling conditions were as follows; 95°C (5 min); then 35 cycles of 95°C (30 sec), 56°C (30 sec), 72°C (30 sec), and a final extension of 72°C (5 min). Electrophorized gel was visualized in a gel visual equipment for DNA bands detection. a 100-bp DNA ladder (ExcelBand™ 100 bp DNA Ladder, SMOBIO) was loaded and parallel run with PCR product.

Results

Genetic diversity determines distinguish rice cultivar. DNA marker or molecular marker provided useful genetic information and its related phenotype. Several DNA markers could be exploited for difference purposes. This genetic information is inherited from previous generation and are transferable for offspring.

Molecular marker polymorphism

In this study, two rice cultivars and 27 of reported microsatellite markers acquired from previous studies were investigated. Reviewed detail of markers was listed in table 1 where expected size, SSR motif, Marker Type, Approximate size, and References are shown. In total 27 SSR markers were test, 7 out of 27 showed polymorphisms (Table 2), while 20 SSR markers showed monomorphic (Table 3). Polymorphism information content (PIC) was calculated according to Serrote *et al*. (2020). PIC value ranged from 0.0 (Monomorphic) – 0.5 (Polymorphic) with average PIC value was 0.13 per SSR marker. RM551 RM5371 RM20224 RM424 RM3 RM17470 RM6480 possessed highest PIC value of 0.5. Allele number varies, in accordance with PIC. Total 34 single locus allele were amplified with average was 1.26 allele per SSR marker. Maximum of 2 alleles were detected in two cultivars (RM551 RM5371 RM20224 RM424 RM3 RM17470 RM6480) and one allele were detected in monomorphic SSR loci.

Table 1. Detail of retrieved SSR markers

Marker number	Marker name	Repeat Motif	Marker Type	Approximate size	References
1.	RM413	(AG)11	SSR	79	Pandita *et al*., 2023
2.	RM232	(CT)24	SSR	158	Palanog *et al*., 2014; Zhang *et al*., 2013
3.	RM72	(TAT)5C(ATT)15	SSR	166	Zhang *et al*., 2013
4.	RM209	(CT)18	SSR	134	Rana *et al*., 2009
5.	RM219	(CT)17	SSR	202	Biswas *et al*., 2013, Zhang *et al*., 2013
6.	RM224	(AAG)8(AG)13	SSR	157	Zhang *et al*., 2013
7.	RM551	(AG)18	SSR	192	Kim *et al*., 2014
8.	RM308	(AT)4-6-(GT)2T2(GT)7	SSR	132	Tang *et al*., 2009
9.	RM1155	(AG)13	SSR	148	Rahman *et al*., 2012
10.	RM5371	(TC)13	SSR	143	Rahman *et al*., 2012
11.	RM17483	(AGG)8	SSR	147	Tagle *et al*., 2016
12.	RM6266	(CTC)9	SSR	160	Rahman *et al*., 2012
13.	RM20224	(CT)25	SSR	194	Rahman *et al*., 2012
14.	RM223	(CT)25	SSR	165	Prathepha and Srisa-Ard, 2014
15.	RM3843	(GA)23	SSR	172	Tagle *et al*., 2016
16.	RM424	(CAT)9	SSR	239	Rahman *et al*., 2012
17.	RM241	(CT)31	SSR	138	Zhang *et al*., 2013
18	RM21	(GA)18	SSR	157	Cobelli *et al*., 2016
19.	RM3	(GA)2GG(GA)25	SSR	145	Zhang *et al*., 2013
20.	RM279	(GA)16	SSR	174	Rahman *et al*., 2012
21.	RM289	G11(GA)16	SSR	108	Rahman *et al*., 2012
22.	RM10713	(AGA)12	SSR	144	Rahman *et al*., 2012
23.	RM6909	(TTA)14	SSR	159	Tagle *et al*., 2016
24.	RM5503	(TC)27	SSR	200	Tagle *et al*., 2016

Table 1. Detail of retrieved SSR markers

Marker number	Marker name	Repeat Motif	Marker Type	Approximate size	References
25.	RM3534	(GA)12	SSR	129	Tagle *et al*., 2016
26.	RM17470	(CT)20	SSR	142	Tagle *et al*., 2016
27.	RM6480	(GCC)9	SSR	121	Tagle *et al*., 2016

Discussion

Two studied rice cultivars, KDML105 and IR64, exhibits different allele at particular locus. Total 27 SSR markers from previous studies, exhibited polymorphism in the reports, were retrieved. In this study 34 allele can be amplified, ranging from 1-2. PIC value ranged from 0.0 (Monomorphic) – 0.5 (Polymorphic) with average PIC value was 0.13 per SSR marker. In the other study, detection average of 8.4 allele per locus, ranging from 3 to 21 in 69 Argentine rice accessions (Giarrocco *et al.*, 2007), average of 9.5 allele per locus, ranging from 5 to 18 per locus in Thai and 9 exotic-rice cultivars using 20 SSR markers (Kanawapee *et al.*, 2011). At the same time, some report elucidates lower allele (Zhang *et al.*, 2011; Nachimuthu *et al.*, 2015). Due to both cultivars belonged to *Indica* (*Oryza sativa* L. ssp. *Indica*) and share similar aspects, therefore it is not uncommon that many loci exhibited no polymorphism. Together with limited cultivars in the study, lower allele detected and calculate PIC was presented.

In this study, two cultivars were clarified. Among 27 SSR markers 7 of them showed useful polymorphism. This crucial step for further study with more cultivars. In the next study chapter, Polymorphism appeared SSR will be used for clarifying more rice, whether landrace or commercial cultivars, and many useful characteristics might be distinguished via selected marker.

Table 2. SSR markers elucidated polymorphism

Marker number	Marker name	Number of alleles	PIC value
1	RM551	2	0.5
2	RM5371	2	0.5
3	RM20224	2	0.5
4	RM424	2	0.5
5	RM3	2	0.5
6	RM17470	2	0.5
7	RM6480	2	0.5

Table 3. SSR markers elucidated no polymorphism

Marker number	Marker name	Number of alleles	PIC value
1	RM413	1	0
2	RM232	1	0
3	RM72	1	0
4	RM209	1	0
5	RM219	1	0
6	RM224	1	0
7	RM308	1	0
8	RM1155	1	0
9	RM17483	1	0
10	RM6266	1	0
11	RM223	1	0
12	RM3843	1	0
13	RM241	1	0
14	RM21	1	0
15	RM279	1	0
16	RM289	1	0
17	RM10713	1	0
18	RM6909	1	0
19	RM5503	1	0
20	RM3534	1	0

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Effect of *Melastoma malabathricum* leaf powder particle size on the physicochemical properties and acceptability of beef meatballs

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Abstract The increasing consumption of meatballs accompanied by various serving and selling methods poses challenges related to product shelf life. The use of synthetic preservatives in meatballs can negatively impact consumers, making the use of natural ingredients like senduduk (*Melastoma malabathricum*) leaves essential. The physicochemical properties and panelist acceptance of beef meatballs with the addition of senduduk leaf powder of different particle sizes including 30, 40, and 50 mesh were evaluated. The senduduk leaf powder was added at 0.75% of the meat weight. Ingredients were included meat (400 g), tapioca flour (200 g), ice cubes (104 g), salt (12 g), pepper powder (4 g), garlic powder (4 g), and senduduk leaf powder according to the treatments. The observed variables included cooking yield, pH, moisture content, water-holding capacity, emulsion stability, tenderness, folding test, and panelist acceptance. It was not significantly differed in cooking yield, pH, and moisture content, except for T1, which had the highest moisture content. Water-holding capacity was not significantly differed, except for T1. Emulsion stability of meatballs with senduduk leaf powder (T1, T2, T3) were lower than T0. Overall, adding senduduk leaf powder improved tenderness. The addition of 50 mesh senduduk leaf powder (T3) enhanced the panelist acceptance. These findings indicated the potential of fine senduduk leaf powder to improve the physicochemical properties and acceptability of meatballs.

Keywords: Beef meatball, *Melastoma malabathricum*, Physico-chemical properties

Introduction

Meatballs are one of the most popular processed meat products in Indonesia and several Southeast Asian countries. The diversity in how meatballs are served and sold, along with their wide appeal among consumers, has expanded alongside modern culinary trends. This phenomenon is not only increased the demand for processed meat products but also contributed to higher consumption of animal protein among the population. However, meatballs, as a processed food, have a major drawback in that they are highly perishable due to microbial growth and oxidative changes during storage. Consequently, many meatball producers add synthetic preservatives and antioxidants to extend the product's shelf life and maintain quality. Unfortunately, the use of synthetic additives often raises in their potential negative impacts on consumer health (Liang, 2023; Aribisala and Sabiu, 2023).

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Therefore, the search for natural preservatives and antioxidants becomes highly relevant. Natural compounds, especially phytochemicals like terpenoids and polyphenols, have emerged as effective substitutes, offering antioxidant properties and safety (Río *et al.,* 2021). One promising natural ingredient is the senduduk (*Melastoma malabathricum*) leaf, which has long been recognized in traditional medicine for its pharmacological benefits (Zheng *et al.,* 2020). Several studies have shown that *M. malabathricum* leaves possess strong antioxidant, antimicrobial, and anti-inflammatory activities, largely attributed to their bioactive compounds such as flavonoids, tannins, and saponins (Idris *et al.,* 2022; Tiwari *et al.,* 2023). Although the pharmacological potential of this plant has been extensively used in food, particularly as an additive in powdered form, remains relatively under-researched.

The application of *M. malabathricum* leaf extract in food products is important due to its prominent antibacterial and antioxidant properties. This plant extract can function as a natural preservative, enhancing food safety and quality. The leaves extract of *M. malabathricum* has demonstrated significant antibacterial activity against various pathogens, including *Staphylococcus aureus* and *Escherichia coli*, which are common contaminants in food products (Tan *et al.,* 2024). The effectiveness of this extract is linked to its phytochemical constituents, such as flavonoids and tannins, which have been proven to inhibit microbial growth (Tiwari *et al.,* 2023). Additionally, the extract exhibits strong antioxidant properties due to its high total phenolic and flavonoid content, making it beneficial for preventing oxidation in food products (Idris *et al.,* 2022). *In vitro* studies were shown that the extract can effectively scavenge free radicals, which could potentially extend the shelf life of meat products (Idris *et al.,* 2022).

However, using M. *malabathricum* in powdered form has extensively studied, but the powdered leaves of *M. malabathricum* in food products is limited. This study aimed to evaluate the physicochemical properties of beef meatballs with the addition of the powdered leaves of *M. malabathricum*.

Materials and methods

Powder preparation

Leaves of *M. malabathricum* were collected from local shrubs in Central Bengkulu Regency, Indonesia. Mature leaves were selected for the study. After being thoroughly cleaned to remove any contaminants, the leaves were air-dried for 72 hours. They were then further dried in an oven at 60 ºC for 5 hours. Once fully dried, the leaves were ground into a fine powder and sieved through 50, 40, and 30-mesh screens. The powder from each mesh size was used for experimental analysis.

Meatballs preparation and treatments application

Bali beef femur was ground, pounded, and mixed with salt and one-third of the total ice. Another one-third portion of ice, *M. malabathricum* leaf powder (according to the treatments), and tapioca were then added, and the mixture was pounded for 2 minutes. Subsequently, the final one-third portion of ice, along with garlic and pepper, was added and pounded until smooth (approximately 3 minutes). The mixture was allowed to rest for 10 minutes, then shaped into round balls with a diameter of 5 cm and boiled in water until they floated. The meatballs were then drained and observed at 5 hours. The experiment was conducted using a completely randomized design, with each treatment replicated 4 times. The composition of each treatment is shown in Table 1.

Table 1. Composition of meatballs

Ingredients	T0	T1	T2	T3
Beef meat (g)	400	400	400	400
Tapioca (g)	200	200	200	200
Cubic ice (g)	104	104	104	104
Salt (g)	12	12	12	12
Garlic powder (g)	4	4	4	4
Pepper powder (g)	4	4	4	4
M. malabathricum leaf powder (g)	-	3	3	3

The composition is for 1 batch; T0: meatballs without *Melastoma malabathricum* leaf powder; T1: meatballs with the addition of *Melastoma malabathricum* leaf powder with a particle size of 30 mesh; T2: addition of *Melastoma malabathricum* leaf powder with a particle size of 40 mesh, T3: addition of *Melastoma malabathricum* leaf powder with a particle size of 50 mesh.

Cooking yield measurement

The cooking yield was determined by measuring the weight difference between the raw dough and the cooked meatballs, and then divided this difference by the weight of the dough.

pH determination

The pH of meatballs was determined by blending 1 gram of sample with 9 ml of distilled water until a homogeneous mixture was obtained. The sample-water mixture was then measured for pH using a calibrated pH meter (Savadkoohi *et al.*, 2014).

Moisture content (%) determination

The moisture content was assessed using the oven drying method described by Horwitz and Latimer (2005). Approximately 2 g of the sausage sample was dried in an oven at 105 °C until a stable weight was reached. The moisture content was calculated using the following formula.

$$\text{Moisture Content (\%)} = \frac{\text{initial weight} - \text{dried weight}}{\text{initial weight}} \text{x } 100$$

Water holding capacity (WHC) determination

The assessment of water holding capacity (WHC) was performed the method outlined by Jung and Joo (2013), with slight modifications. A 5 g portion of mashed sausage sample was placed in a centrifuge tube, followed by the addition of 10 ml of distilled water. The mixture was maintained at 30°C for 30 minutes. Then, it was centrifuged at 3000 rpm for 30 minutes. The liquid above the sediment was carefully discarded, and the tube was incubated again at 30°C for 10 minutes before the supernatant was removed. The WHC was then calculated using the following formula:

$$\text{WHC (\%)} = \frac{\text{Weight of sample without supernatant}}{\text{Weight of sample with water added}} \times 100$$

Emulsion stability

The procedure followed the method described by Yum *et al.* (2018). A sample weighing 7.5 g was placed into a centrifuge tube and heated in a water bath at 70°C for 30 minutes. The sample was then centrifuged at 1000 rpm for 10 minutes. The supernatant was carefully decanted and weighed. This liquid was subsequently dried in an oven at 105°C for 16 hours. The dried residue left behind was measured as the fat content. The water content was calculated by subtracting the fat weight from the total liquid weight.

$$\text{Released fluid (\%)} = \frac{\text{fluid weight}}{\text{sample weight}} \text{x } 100$$

Tenderness determination

The assessment of sausage tenderness was performed using a universal penetrometer, following the procedure outlined by Sumarmono (2012). First, the penetrometer was calibrated to ensure the needle indicator was set at zero. Then, weights were attached to the plunger head of the needle rod, with the total load (in grams), calculated by summing the weights of the attached items, including the plunger head and needle rod. The sample was carefully placed beneath the needle, after which the plunging lever was pressed for 10 seconds. The depth of needle penetration into the sample, measured in millimeters, was recorded from the indicator scale. The sausage's hardness was expressed in millimeters per gram per second.

Folding test

The fold test was followed the method described by Lanier (1992). A slice of sausage, 3 mm thick and 2.5 cm long, was taken from the center of the sample.

The slice was folded using the index finger and thumb, and the results were rated according to the following criteria: a score of 5 was given if no cracks appeared during folding, a score of 4 if there were no cracks when folded halfway, a score of 3 if slight cracking occurred gradually at the halfway fold, a score of 2 if cracks formed quickly at the halfway fold, and a score of 1 if the sausage cracked when pressure was applied by the thumb and index finger during folding.

Panelist's acceptability

The acceptability of the samples was evaluated through sensory analysis by 25 untrained panelists, who were students from the Animal Science Department at the Faculty of Agriculture, University of Bengkulu. Prior to the evaluation, the panelists received brief instructions. The assessment utilized a line scale ranging from 0 (strongly dislike) to 9 (strongly like), where panelists marked their preference along a 0-9 cm line. The distance from the 0-point was then measured to obtain the evaluation data. It followed a procedure described by Meilgaard *et al.* (1999)

Statistical analysis

The data were subjected to Analysis of Variance (ANOVA), followed by post-hoc comparison using Duncan's Multiple Range Test (DMRT) to identify significant differences between treatments, with a significance threshold set at 0.05.

Results

Cooking yield, pH, Moisture content, and water holding capacity

The result of cooking yield, pH value, moisture content, and water holding capacity are depicted at Table 2. Cooking yield is an important indicator in determining the quality of processed meat products, as it relates to the product's ability to retain liquid components during the cooking process. The results showed that the addition of *M. malabathricum* leaf powder affected the cooking yield of beef meatballs (Table 2). Treatment T1 (leaf powder with a particle size of 30 mesh) exhibited the highest cooking yield at 107.7 ± 0.10%, which was significantly different ($P<0.05$) compared to the control treatment (T0) at 107.1 ± 0.06%. The cooking yields for T2 (40 mesh) and T3 (50 mesh) were 107.6 ± 0.21% and 107.4 ± 0.23%, respectively, both showing values comparable to T1. Generally, the addition of M. malabathricum leaf powder increased meatball cooking yield. These results suggested that the addition of *M. malabathricum* leaf powder had the potential to enhance the cooking yield of meatballs, with the larger particle size in T1 providing the best results.

The pH value is a factor that influences microbial stability and the sensory characteristics of processed meat products. The results indicated that the pH values of meatballs across all treatments ranged from 6.12 to 6.21, with the control treatment (T0) was the highest pH value at 6.21 ± 0.18 (Table 2). Although there were varied in between treatments, the differences in pH values were not statistically significant (P>0.05). The addition of *M. malabathricum* leaf powder was not altered the pH of the product, which remained within the normal range for beef meatballs. This pH stability is found to be crucial in maintaining the product's quality and safety during storage.

Moisture content in processed meat products is affected the product's moisture, texture, and consumer acceptability. The highest moisture content was found in treatment T1 at 64.3 ± 0.01%, which was significantly different (P<0.05) compared to T0 (63.2 ± 0.00%), T2 (63.3 ± 0.00%), and T3 (63.1 ± 0.00%). (Table 2). These results indicated that the addition of *M. malabathricum* leaf powder with a particle size of 30 mesh (T1) increased the moisture content of meatballs, contributing to enhance the juiciness. The smaller particle size in T1 found to play a role in improving the meat fiber's ability to retain water during cooking, thereby preventing excessive water loss.

WHC is a crucial parameter in determining the texture quality of processed meat products, as it is directly related to the product's ability to retain water during cooking and storage. The results showed that treatment T1 had the highest WHC at 54.0 ± 0.01%, which was significantly higher (P<0.05) than T0 (51.2 ± 0.01%), T2 (50.8 ± 0.01%), and T3 (50.5 ± 0.01%) (Table 2). The addition of *M. malabathricum* leaf powder with a particle size of 30 mesh (T1) was increased the water holding capacity of the meatballs. This improvement is found to be better interaction between the meat fibers and the powder particles, which enhanced the meat matrix's ability to bind water molecules more efficiently. These results also suggested that larger powder particle sizes improved the structure of processed meat, thereby strengthening water retention during cooking.

Table 2. Cooking yield, pH value, moisture content, and WHC of meatballs with the addition of *M. malabathricum* leaf powder of different particle sizes

Variables	Treatments			
	T0	T1	T2	T3
Cooking yield (%)	107,10±0.06^{b}	107.70±0.10^{a}	107.60±0.21^{a}	107.40±0.23^{ab}
pH value	6.21±0.18	6.13±0.03	6.12±0.08	6.16±0.13
Moisture content	63.20±0.00^{b}	64.30±0.01^{a}	63.30±0.00^{b}	63.10±0.00^{b}
WHC (%)	51.20±0.01^{b}	54.00±0.01^{a}	50.80±0.01^{b}	50.50±0.01^{b}

Different superscript letters in the same row indicate a significant difference (P<0.05).

Emulsion stability, tenderness, folding test, and acceptability

Emulsion stability is one of the crucial parameters in processed meat products, as it reflected the emulsion's ability to retain water and fat phases during the heating process. The results showed a significant difference (P<0.05) in

emulsion stability between treatments. The control treatment (T0) had the highest percentage of released water (0.95 ± 0.00%), which was significantly higher than all treatments (P<0.05) with the addition of *M. malabathricum* leaf powder. T1, T2, and T3 released water percentages of 0.84 ± 0.01%, 0.84 ± 0.00%, and 0.82 ± 0.00%, respectively. These findings indicated that the addition of *M. malabathricum* leaf powder enhanced emulsion stability, with the T3 treatment (50 mesh) providing the best results. The smaller particle size used in T3 resulted in a more uniform distribution within the meat matrix, thereby strengthening the emulsion's ability to bind water and fat.

Tenderness is one of the sensory characteristics that directly affects consumer acceptability of meat products. The results showed that the tenderness of the meatballs was significantly influenced (P<0.05) by the addition of *M. malabathricum* leaf powder. Treatment T3 (50 mesh) yielded the highest tenderness value of 0.27 ± 0.02 mm/g/s, which was significantly higher (P<0.05) than other treatments. The tenderness values of T0 (control) and T1 (30 mesh) were the lowest, at 0.24 ± 0.07 mm/g/s and 0.24 ± 0.02 mm/g/s, respectively. T2 (40 mesh) showed better results than the control, with a tenderness value of 26 ± 0.02 mm/g/s. This difference suggested that the smaller particle size in T3 contributed to improve tenderness, possibly through its influenced on the meat protein structure and interaction with the water components in the product.

The folding test is a method used to assess the elasticity of meatballs. The test results revealed a significant difference between treatments (P<0.05), with the highest value observed in the T3 treatment (50 mesh) at 3.76 ± 0.76. The control treatment (T0) exhibited the lowest folding test value of 2.60 ± 0.69, while T1 and T2 had values of 3.26 ± 0.78 and 3.16 ± 0.88, respectively. These results indicated that the addition of *M. malabathricum* leaf powder improves the elasticity of meatballs, with the smaller particle size in T3 yielding the best results. This increased in elasticity may be attributed to better interaction between the meat fibers and the leaf powderto maintain the meat structure during cooking.

Table 3. Emulsion stability, tenderness, folding test, and acceptability of meatballs with the addition of *M. malabathricum* leaf powder of different particle sizes

Variables	Treatments			
	T0	T1	T2	T3
Emulsion stability (released water, %)	0,95±0,000^{a}	0,84±0,001^{b}	0,84±0,000^{b}	0,82±0,000^{b}
Tenderness (mm/g/s)	0,24±0,007^{c}	0,24±0,002^{c}	0,26±0,002^{b}	0,27±0,002^{a}
Folding test	2,60±0,69^{c}	3,26 ± 0,78^{b}	3,16 ± 0,88^{b}	3,76 ± 0,76^{a}
Acceptabililty	6,26±1,10^{b}	6,11±1,01^{b}	6,09±1,20^{b}	7,06±0,81^{a}

Different superscript letters in the same row indicate a significant difference (P<0.05).

Product acceptability is a key parameter in determining consumer acceptance of the final product. The results showed that treatment T3 (50 mesh) had the highest acceptability score of 7.06 ± 0.81, which was significantly higher ($P<0.05$) than the other treatments. The acceptability scored for T0 (control), T1 (30 mesh), and T2 (40 mesh) were 6.26 ± 1.10, 6.11 ± 1.01, and 6.09 ± 1.20, respectively. These findings suggested that the addition of *M. malabathricum* leaf powder with smaller particle sizes (T3) enhanceed consumer acceptability of the meatballs, due to its positive effects on texture, juiciness, and elasticity.

Discussion

Cooking yield

The addition of leaf powder with particle sizes ranging from 30 to 50 mesh improved the cooking yield due to enhance dispersibility and interaction with water during the cooking process. This size range allowed for optimal surface area exposure, facilitating better water and nutrient absorption. Leaf powder with particle sizes of 30 to 50 mesh demonstrated superior dispersibility in water, which was crucial for cooking applications. This property ensure that the powder integrates well with other ingredients, resulting in a more uniform mixture (Lai *et al*., 2024). *M. malabathricum* contains polysaccharides and fibers that can absorb and retain water, resulting in higher moisture content in the cooked meatballs.

The addition of *M. malabathricum* leaf powder enhanced the cooking yield of meatballs primarily due to the presence of polysaccharides and fibers in the leaf. These components possesd high water-binding capacities, allowing them to absorb and retain moisture (Gorlov *et al.,* 2015) during the cooking process. As a result, less water was lost leading to a higher cooking yield. Similar studies have demonstrated that the inclusion of dietary fibers, such as those derived from mushrooms, improved moisture retention in meat products, leading to increase the cooking yields (Tu *et al.,* 2021).

pH value

The addition of *M. malabathricum* leaf powder in meatball formulations aims to harness its bioactive potential, particularly the phenolic and flavonoid contents, which are known for their antioxidant and antimicrobial properties (Idris *et al.,* 2022; Tiwari *et al.,* 2023). However, despite the theoretical capacity of these compounds affected food quality parameters such as pH, it showed that adding 0.75% of the leaf powder based on meat weight with particle sizes ranging from 30-50 mesh was not significantly affected the pH of meatballs. This phenomenon is related to the concentration of the powder, its physical form, and interactions between the active compounds and the meat matrix.

Regarding concentration, the addition of 0.75% leaf powder in the meat formulation was relatively low that the phenolic and flavonoid compounds released into the meat matrix may not be sufficient to cause significant changes in

pH (Beya *et al.,* 2021). Phenolic compounds are typically acidic and can interact with the meat's buffering system, but at low concentrations, this effect is remained the undetectable (Martillanes *et al.,* 2017).

Using leaf powder as a food additive differs from leaf extracts in terms of the bioavailability of the active compounds. In powder form, the leaf cells remain intact or the majority of the cell walls are preserved, which can hinder the release of phenolics and flavonoids into the meat during processing (Padayachee *et al.,* 2013; Melini *et al.,* 2020). In contrast, leaf extracts are undergone bioactive compound isolation which are significantlyfound to impact on food product characteristics including pH (Tchabo *et al.,* 2022).

Moreover, meat is a complex food matrix with a strong natural buffering capacity. The meat's buffering system is involved in proteins, ions, and salts, and to maintain a stable pH range when small amounts of acidic or basic compounds are added (Schönfeldt and Hall, 2017). In this study, the relatively low amount of leaf powder was not sufficient to overcome the meat's natural buffering system, and the pH of the meatballs remained stable despite the addition of acidic phenolic compounds.

Moisture content

The differences in moisture content of meatballs with the addition of 0.75% *M. malabathricum* leaf powder of varying particle sizes can be explained by factors related to particle size and the physical interactions between the leaf powder and meat components. Larger leaf powder particles (30 mesh) tend to have a smaller surface area as compared to finer particles (40 and 50 mesh). Larger particles absorbed less water than smaller particles because they had a lower surface-to-volume ratio. Consequently, when larger particles (30 mesh) are added to the meatballs, more water remains available within the meat matrix since the particles did not absorb as much water as the finer particles. This results in higher moisture content in T1 (30 mesh) as compared to T2 and T3 (40 and 50 mesh), where smaller particles can absorb more water into their structure (Zain *et al.,* 2021; Padayachee *et al.,* 2013).

Additionally, larger *M. malabathricum* leaf powder particles (30 mesh) interacted differently with meat proteins as compared to smaller particles. Larger particles tended to form larger aggregates within the meat, which did not bind water as effectively as finer particles that distributed more evenly throughout the meat matrix and better absorb water. It leaded to less interference in water distribution by larger particles, resulting in higher moisture content in the meatballs (Kasote *et al.,* 2021).

M. malabathricum leaf powder contains dietary fiber, which influenced water absorption capacity. In finer particles (40 and 50 mesh), this fiber had more exposed and able to absorb water more readily as compared to larger particles. This is explained why T2 and T3 had lower moisture content as compared to T1, while the control (T0) was not significantly changed in moisture content due to

the absence of added fiber from the leaf powder (Fernandes *et al.,* 2023; Melini *et al.,* 2020).

Water holding capacity

The pattern observed between moisture content and water holding capacity (WHC) of the meatballs can be attributed to the relationship between the meat's ability to retain water and the distribution of water within the meat matrix. WHC refers to the meat's capacity to retain water during processes such as grinding, mixing, and cooking, which is heavily influenced by the physical interactions between meat components, particularly proteins, and water.

In meatballs with the addition of larger *M. malabathricum* leaf powder particles (T1, 30 mesh), is tended to absorb less water, leaving more water available within the meat matrix. This excess water increased the moisture content and simultaneously improved WHC because more water is retained by the meat matrix. In contrast, finer particles in T2 and T3 (40 and 50 mesh) are found to be more efficient at absorbing water, leaving less water in the meat, which resulted in lower WHC (Fernandes *et al.,* 2023; Shahidi and Yeo, 2016).

Meat proteins, particularly myofibrils, play a critical role in absorbing and retaining water. The interaction between *M. malabathricum* leaf powder and meat proteins are altered how proteins function in water retention. Larger particles may not disrupt the protein network as much as smaller particles, allowing the proteins in T1 to retain more water. Conversely, smaller particles might interfere with protein structure more significantly, reducing WHC in T2 and T3 (Shahidi and Yeo, 2016).

Additionally, *M. malabathricum* leaf powder contains dietary fiber, which can interact with water and influence WHC. In finer particles (40 and 50 mesh), the fiber may be more exposed and absorb water more efficiently, reducing the amount of water that can be retained by the meat matrix. Consequently, WHC decreased along with moisture content in T2 and T3, while in T1, the larger particles may absorb less water, allowing more water to remain in the meat, thereby increasing WHC (Melini *et al.,* 2020).

Emulsion stability

The improvement in emulsion stability observed in the study involving *M. malabathricum* leaf powder can be attributed to several factors related to the powder’s properties and particle size. Emulsion stability in processed meat products is important because it reflects the product's ability to retain water and fat during processing, especially under heat treatment. In this case, the addition of *M. malabathricum* leaf powder significantly enhanced the stability of the emulsion compared to the control.

The finer particle size in T3 (50 mesh) likely played a key role in enhancing emulsion stability. Smaller particles have a larger surface area, which can interact more effectively with water and fat molecules. This results in better distribution

within the meat matrix, strengthening the emulsion's ability to trap water and fat, reducing the amount of released water during heating (Fernandes *et al.,* 2023). The improvement seen in T3 indicates that finer particles can better integrate into the meat matrix, forming a more stable and homogenous emulsion compared to larger particles in T1 (30 mesh).

M. malabathricum is known to contain bioactive compounds such as polyphenols and fibers, which may also contribute to enhanced water and fat-binding capacity (Shahidi and Yeo, 2016). These components can interact with proteins and lipids in the meat, helping to form stronger emulsions by creating more stable networks that retain water and fat more effectively. This explains the lower water release in T1, T2, and especially T3 compared to the control (T0), which had no leaf powder and, therefore, fewer stabilizing agents in the emulsion.

The fiber content in *M. malabathricum* leaf powder may also aid in improving emulsion stability by enhancing the meat matrix's ability to hold water. Dietary fiber has water-holding capacity and can contribute to the formation of a gel-like network within the emulsion, which reduces the mobility of water and fat and prevents phase separation during cooking (Melini *et al.,* 2020). The more uniform distribution of smaller particles in T3 likely resulted in better interaction with the water and fat phases, explaining why T3 had the lowest percentage of water released.

Tenderness

The findings on the tenderness of meatballs with the addition of *M. malabathricum* leaf powder suggest that the smaller particle size of the powder (50 mesh, T3) significantly improved tenderness compared to the control and other treatments. Several factors contribute to this phenomenon. It most probably due to particle size and meat protein interaction. The finer powder in T3 (50 mesh) is likely to have a greater surface area, allowing it to more evenly distribute within the meat matrix. This finer distribution can improve the interaction between the powder's bioactive compounds and the meat proteins, resulting in a more uniform texture. The smaller particles may also facilitate better water retention, which contributes to a softer and more tender texture in the cooked product (Fernandes *et al.,* 2023). Finer particles can help prevent the formation of a dense or tough texture by disrupting the protein structure less compared to larger particles.

Water content plays a crucial role in meat tenderness. In this study, T3 likely retained more water due to the smaller particle size, which contributed to increased juiciness and a softer texture. The enhanced water retention allows the meat proteins to maintain a more gel-like structure during cooking, which prevents excessive hardening of the meatball matrix (Melini *et al.,* 2020). This relationship between water retention and tenderness is well documented in meat science, where increased moisture leads to a more tender bite.

M. malabathricum leaf powder contains phenolic compounds and dietary fiber, both of which can interact with the meat matrix. These compounds may also play a role in softening the meat texture by interacting with muscle proteins and

water, improving the overall tenderness (Shahidi and Yeo, 2016). The improved tenderness in T3 may be due to a more effective interaction between these compounds and the meat matrix at the finer particle size, leading to a softer final product.

Folding test

The increased elasticity of meatballs, as reflected by higher folding test scores, with smaller *M. malabathricum* powder particles (such as T3 with 50 mesh) can be attributed to several factors related to particle size, protein interaction, and water retention.

Smaller particles, like those in T3 (50 mesh), have a larger surface area, allowing them to integrate more thoroughly within the meat matrix. This results in better interaction between the bioactive compounds of the leaf powder and the myofibrillar proteins in the meat. These proteins (e.g., actin and myosin) are responsible for forming the gel-like structure that provides elasticity during cooking. With finer particles, the protein network becomes more cohesive, leading to greater elasticity in the final product (Flory *et al.,* 2023; Cheng *et al.,* 2021).

Finer particles are also more effective at retaining water, which plays a critical role in the elasticity of meat products. Water interacts with the protein network, allowing it to expand and become more flexible during cooking. This increased water retention enhances the gel-forming properties of the proteins, contributing to the higher folding test scores seen in T3 (Melini *et al.,* 2020; Gómez *et al.,* 2020).

Smaller particle sizes also allow for a more uniform distribution of the powder within the meat, creating a more homogenous matrix. This uniformity helps maintain the meat structure during cooking, leading to greater elasticity and flexibility. Larger particles, by contrast, may form aggregates that disrupt the even distribution, reducing elasticity (Feng *et al.,* 2018).

Overall, the improved elasticity in T3 can be explained by better interaction between the finer *M. malabathricum* particles and meat proteins, as well as enhanced water retention, which collectively strengthen the meatball's structure and elasticity.

Sensorial acceptability

The increased consumer acceptability in treatment T3 (50 mesh) compared to the other treatments can be attributed to the effects of smaller particle size on important sensory attributes like texture, juiciness, and elasticity. These attributes play a key role in determining overall product acceptability.

Smaller particles, as in T3, allow for a smoother and more refined texture, which consumers often associate with higher quality in meat products. A more uniform distribution of the fine powder in the meatball matrix reduces any graininess, making the texture more pleasant to the palate (Fiorentini *et al.,* 2020).

Finer particles also enhance water retention, leading to a juicier meatball. Juiciness is a critical factor for consumers when evaluating meat products, as it directly affects mouthfeel and overall satisfaction. Finer particles in T3 create a better interaction with the water in the meat matrix, contributing to a moist and juicy product, which is often favored by consumers (Garmyn, 2020).

The elasticity of the meatballs, which was higher in T3, also positively influences consumer perception. A product that is more elastic and cohesive feels higher in quality and more satisfying to chew. This textural improvement, resulting from better interaction between fine particles and meat proteins, likely contributed to the higher acceptability scores in T3 (Petrat-Melin and Dam, 2023).

These combined effects of texture, juiciness, and elasticity help explain why T3 achieved the highest acceptability score, demonstrating the importance of fine particle size in improving sensory attributes that drive consumer preferences for meat products.

The study demonstrated that the addition of *M. malabathricum* leaf powder to beef meatballs positively impacted their physicochemical properties and consumer acceptability. Specifically, meatballs containing the smallest particle size of 50 mesh (T3) exhibited the best results in terms of tenderness, elasticity, and overall sensory acceptability, likely due to the finer particle size facilitating better interaction with meat proteins and improving water retention. Although the addition of *M. malabathricum* leaf powder did not significantly affect the pH of the meatballs, it did enhance key attributes like moisture content, water-holding capacity, and emulsion stability. These findings suggested that using fine *M. malabathricum* leaf powder can improve the quality and acceptability of meatballs, offering potential as a natural additive for processed meat products to enhance texture, juiciness, and elasticity.

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In vitro propagation of Turmeric (*Curcuma longa* Linn.) using murashige and skoog (MS) media supplemented with varying concentrations of thidiazuron (TDZ)

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Abstract Turmeric (*Curcuma longa* Linn.) is a valuable spice and medicinal herb with growing demand worldwide. *In vitro* propagation techniques offer a promising opportunity for enhancing turmeric cultivation by providing a controlled environment for mass production of planting materials. Thidiazuron (TDZ), a plant growth regulator, has shown potential for improving in vitro propagation efficiency in various plant species. This study investigates the use of Thidiazuron (TDZ) as a supplement to Murashige and Skoog (MS) media for the *in vitro* propagation of turmeric. The optimum concentration of TDZ to facilitate efficient *in vitro* propagation of turmeric was determined. Results showed that supplementing the MS medium with TDZ at levels ranging from 1.0 to 2.5 mg/L of water can result in higher bud proliferation, optimal shoot fresh weight, and higher stem girth in the in vitro-grown turmeric. Moreover, adding TDZ in media ranging from 0.01 to 2.5 mg/L of water promotes longer shoot length, a higher number of leaves, and a greater leaf area in the in vitro-grown turmeric. However, higher TDZ concentrations led to fewer but longer roots. Hence, TDZ can facilitate shoot and root regeneration in turmeric during in vitro propagation, providing several advantages for commercial production.

Keywords: Root regeneration, Plant growth regulators, Explant inoculation, Bud initiation, In vitro shoot regeneration, Micropropagation

Introduction

Turmeric (*Curcuma longa* Linn.) is a highly valued plant known for its culinary uses and potential applications in the medicinal and cosmetic industries. Its rhizome, that contains volatile oil and Curcumin, has gained attention for its anti-inflammatory, antioxidant, and anticancer properties (Iqbal *et al.,* 2003; Sompamit *et al.,* 2009). However, the limited flowering and sterile triploid nature (2n = 3x = 63) of turmeric make its propagation challenging (Ravindram *et al.,* 2007). Traditional methods, such as rhizome division, are time-consuming, inefficient, and susceptible to rhizome rot diseases (Khader *et al.,* 1994; Shagufta *et al.,* 2009). To overcome these limitations and ensure a consistent supply of disease-free planting material, *in vitro* propagation techniques have been explored.

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Tissue culture methods offer a means to replicate turmeric plants rapidly, increasing multiplication rates and reducing disease risks (Shirgurkar *et al.,* 2001). This approach provides a steady supply of high-quality, true-to-type plantlets, ultimately enhancing crop yield potential.

Extensive research has been conducted on the in vitro propagation of turmeric and its related species. The various tissue or organ explants and different combinations and concentrations of plant growth regulators (PGRs) in the culture media has been investigated from 1978 (Nadgauda, 1978) to recent studies in 2020 (Bhabha Atomic Research Centre, 2020; Sinchana *et al.,* 2020). Previous studies had utilized PGRs for in vitro propagation that included 6-benzyl amino purine (BAP), 1-naphthalene acetic acid (NAA), and 6-furfurylaminopurine [kinetin (KT)] (Panda *et al.,* 2007). BAP is favored for shoot elongation and multiplication, while NAA stimulates rooting. Different combinations of PGRs, such as BAP and NAA or KT and BAP, have been explored (Ghosh *et al.,* 2013; Mohanty and Sahoo, 2010).

Recently, Thidiazuron (TDZ) has gained significant interest for its applications in plant cell and tissue culture. TDZ exhibits auxin and cytokinin-like actions despite its chemical dissimilarity to regular auxins and cytokinins. It has been found to induce or enhance various cell biological activities (Guo *et al.,* 2011). In the in vitro propagation of turmeric (*Curcuma longa* L.), TDZ effectively promoted shoot proliferation in turmeric cultures, achieving a maximum of 20 shoots per explant at a concentration of 1.0 mg/L TDZ (Azad *et al.,* 2014). On the other hand, TDZ, in comparison with kinetin and BA, TDZ performed better in terms of shoot induction, demonstrating superior performance. A maximum of 22.9 shoots per explant was observed at a concentration of 2.0 mg/L TDZ (Gangopadhyay *et al.,* 2016). Additionally, TDZ has been shown to enhance rooting in turmeric shoots in combination with IAA (indole-3-acetic acid) (Mukherjee *et al.,* 2016).

Despite the promising results of TDZ in promoting shoot proliferation and enhancing rooting, further research is necessary to optimize its concentration and determine the most effective dosage in the MS medium for efficient micropropagation of turmeric. The study aimed to contribute to the existing knowledge in this area and provide insights into the commercial production of disease-free turmeric planting material.

Materials and methods

Research design

The study employed the Completely Randomized Design (CRD) to examine the effect of varying concentrations of Thidiazuron (TDZ) as a supplement to the Murashige and Skoog (MS) media on *in vitro* propagation of turmeric (*Curcuma longa* Linn.). Descriptive statistics, such as mean, standard deviation, and other relevant measures, were calculated to summarize each parameter across treatment and control groups. Inferential statistics, such as analysis of variance (ANOVA),

were employed to identify significant differences between the treatment and control groups for each parameter. A post-hoc analysis utilizing Tukey's HSD (Honestly Significant Difference) test was conducted to identify specific differences among the treatment groups when significant differences were detected.

Preparation of culture media

The MS (Murashige and Skoog, 1962) medium was used as a base medium supplemented with vitamins and organics, sucrose, and coconut water. The MS media contains a combination of macronutrients, micronutrients, vitamins, and plant growth regulators. Plant growth regulators, specifically as 6-BAP, IAA, and varying levels of TDZ were incorporated into the proliferation medium for specific purposes throughout the experiment. These regulators were used in initiating culture, promoting multiplication, and facilitating shoot development. On the other hand, NAA and different concentrations of TDZ were included in the rooting media to encourage root growth. The concentration of TDZ in the MS medium varied based on the specific treatments employed in the study, allowing for different experimental conditions investigated.

After formulating the media composition, the pH was adjusted to 5.8±0.1, and agar was added as a solidifying agent. The prepared media were autoclaved at 121°C for 20 minutes to ensure sterility.

Preparation of explants and establishment of aseptic culture

Disease-free turmeric rhizomes were carefully collected from mature plants from the turmeric plantation of the College of Agriculture, Isabela State University, Echague, Isabela. The rhizomes were delicately uprooted from the soil and special care was taken to avoid damaging the rhizomes during the collection process. Once separated from the plants, the rhizomes were thoroughly washed with running tap water to remove any adhering debris and dirt particles. Following this, the explants were prepared by excising the apical meristems, located at the tips of the rhizomes. Careful trimming and cutting were done until the explants were approximately 5cm cubes.

Surface sterilization was performed on the explants to establish a sterile environment. Initially, the explants were washed with a soapy water solution containing 20% hypochlorite and were rinsed multiple times with tap water to eliminate any remaining soap residue. Subsequently, the explants were immersed in a 20% hypochlorite solution for 20 minutes to ensure thorough sterilization. The explants were then washed three times with sterile water to eliminate any traces of the disinfectant.

In vitro establishment, inoculation of explants and bud initiation

The sterilized explants were carefully removed from the container and transferred to a sterilized steel dish. Further dissection was performed to reduce the size of each explant. Specifically, the shoot tip was carefully dissected, measuring approximately 1.0 x 0.5 cm. The dissected shoot tips were inoculated individually into a separate bud proliferation medium. The cultures were then maintained for 30 days inside a culture growth room. The growth room was maintained at a constant temperature of 23±2°C, and the lighting conditions were provided by daylight fluorescent illumination at an intensity of 120 µmol m-2 sec-1. The photoperiod followed a 16-hour light and 8-hour dark cycle.

In vitro bud multiplication

A sub-culturing technique was employed to achieve the multiplication of buds. The first subculture (S1) was initiated 30 days after the culture initiation (S0). Subsequent sub-culturing was carried out up to the third subculture (S3), with a 30-day interval between each sub-culturing.

The sub-culturing process was done by meticulously excising the buds that had developed from the initial culture in a sterile manner to ensure the preservation of the meristems. These excised buds were transferred into a separate, freshly prepared proliferation media. Clusters of buds were carefully trimmed before each sub-culturing step to optimize growth and development.

In vitro shoot regeneration

Following the completion of the third subculture (S3), the propagules, which had undergone threerounds of sub-culturing, were individually excised. These excised propagules were then transferred individually to freshly prepared proliferation media which had a composition similar to the mdeia used for bud initiation and multiplication.

The transferred propagules were allowed to develop as an individual plantlet for 30 days. During this time, they were carefully maintained in a culture growth room set at a temperature of 23±2°C and illuminated through daylight fluorescent lights that delivers an intensity of 120 µmol m-2 sec-1. The lighting followed a 16-hour photoperiod, providing the shoots with a suitable light-dark cycle.

In vitro root elongation and proliferation

After the successful shoot regeneration, individual *in vitro* shoots were carefully transferred to a separate rooting medium that had been solidified using agar. The rooting medium was composed of MS salts, along with the addition of vitamins, organics, sucrose, and coconut water. Notably, NAA replaced the previously used IAA and 6-BAP in this medium. However, TDZ was still included with varying concentrations to fulfill the experimental requirements.

The transferred *in vitro* shoots were allowed to establish roots by being placed in the solidified rooting medium. The cultures were then placed in the culture growth room and maintained for 30 days. The growth room maintained a constant temperature of 23±2°C, with daylight fluorescent illumination providing an intensity of 120 µmol m-2 sec-1. The lighting followed a 16-hour photoperiod, ensuring an appropriate light-dark cycle for the rooted shoots.

Disposal of spent media and plant specimen

After each transfer of plant materials into freshly prepared culture media, the culture bottles containing the spent media were subjected to immediate sterilization through autoclaving at 121°C for 60 minutes. This autoclaving process is a standard protocol observed at the Plant Tissue Culture Laboratory of the Isabela State University at Echague campus where the experiment was conducted. Once autoclaving is complete, the spent media and the used plant specimens were disposed of using standard waste disposal methods.

Data management and experimental layout

The study was laid out following the Completely Randomized Design (CRD) with three replications (Figure 2). Each treatment consisted of 16 culture bottles. The treatments were as follows:

T_0 – MS Media with no TDZ (Control)
T_1 – MS Media supplemented with 0.01 mg of TDZ/L of water
T_2 – MS Media supplemented with 0.1 mg of TDZ/L of water
T_3 – MS Media supplemented with 0.5 mg of TDZ/L of water
T_4 – MS Media supplemented with 1.0 mg of TDZ/L of water
T_5 – MS Media supplemented with 1.5 mg of TDZ/L of water
T_6 – MS Media supplemented with 2.0 mg of TDZ/L of water
T_7 – MS Media supplemented with 2.5 mg of TDZ/L of water
T_8 – MS Media supplemented with 3.0 mg of TDZ/L of water

All data were verified as to accuracy and completeness. The one-way Analysis of Variance (ANOVA) was used to determine the effect of the treatments used in the study. In cases where the ANOVA yielded significant results, the Tukey's Honest Significant Difference (HSD) test was employed to identify the variations among the means of different treatments. All data analyses were conducted using the Statistical Tool for Agricultural Research (STAR 2.0.1).

Results

In vitro growth performance of turmeric (Curcuma longa Linn.) as affected by varying concentrations of TDZ

The study investigated the effect of varying levels of TDZ on the *in vitro* propagation of turmeric. During bud proliferation, higher numbers of buds are

obtained in S1 and S3 when the culture media is supplemented with TDZ in the range of 1.0 to 2.5 mg/L of water. In S2, a TDZ concentration of 2.5 mg/L induces higher bud proliferation. These findings suggested optimal bud proliferation can be achieved by supplementing the MS medium with 1.0 to 2.5 mg/L of TDZ.

Table 1. *In vitro* growth performance of turmeric (*Curcuma longa* Linn.) as affected by varying concentrations of TDZ

Treatments	Number of Buds Emerged 30 Days after Bud Induction and 30 days thereafter			Shoot Proliferation taken 30 Days after Shoot Induction				
	S1	S2	S3	Stem Length (mm)	Stem Girth (mm)	Number of Leaves	Leaf Area (mm^2)	Shoot Fresh Weight (g)
T_0 – MS Medium with no TDZ (Control)	3.25^{c}	4.25^{e}	4.23^{d}	70.55^{c}	0.38^{c}	3.10^{b}	320.63^{d}	0.75^{d}
T_1 – MS Medium Supplemented with 0.01 mg of TDZ/L of water	3.21^{c}	4.30^{de}	4.27^{d}	87.23^{b}	0.40^{c}	3.70^{ab}	445.37^{cd}	0.95^{c}
T_2 – MS Medium Supplemented with 0.1 mg of TDZ/L of water	3.60^{c}	4.32^{de}	4.73^{c}	87.46^{a}	0.42^{c}	3.70^{ab}	449.73^{cd}	0.96^{c}
T_3 – MS Medium Supplemented with 0.5 mg of TDZ/L of water	4.05^{b}	4.40^{d}	5.53^{b}	88.90^{a}	0.47^{bc}	3.83^{a}	449.37^{cd}	1.01^{bc}
T_4 – MS Medium Supplemented with 1.0 mg of TDZ/L of water	5.03^{a}	5.06^{c}	6.73^{a}	88.47^{a}	0.47^{bc}	3.83^{a}	538.57^{bc}	1.06^{ab}
T_5 – MS Medium Supplemented with 1.5 mg of TDZ/L of water	5.13^{a}	5.05^{c}	6.73^{a}	88.47^{a}	0.57^{ab}	3.85^{a}	583.07^{bc}	1.07^{ab}
T_6 – MS Medium Supplemented with 2.0 mg of TDZ/L of water	5.18^{a}	5.76^{b}	6.83^{a}	88.83^{a}	0.57^{ab}	3.90^{a}	677.03^{ab}	1.09^{a}
T_7 – MS Medium Supplemented with2.5 mg of TDZ/L of water	5.26^{a}	6.32^{a}	6.83^{a}	88.63^{a}	0.58^{a}	4.05^{a}	761.27^{a}	1.11^{a}
Grand Mean	**4.34**	**4.93**	**5.74**	**86.07**	**0.38**	**3.75**	**528.13**	**1.00**
ANOVA	**	**	**	*	**	**	**	**

Regarding shoot growth parameters, the study demonstrated that the optimal shoot fresh weight is observed when the MS medium is supplemented with 1.0-2.5 mg/L of TDZ. Additionally, higher stem girth is obtained when 1.5-2.5 mg/L of TDZ is added to the medium. Longer shoot length and a higher number of leaves are observed when TDZ is added in media ranging from 0.01-2.5 mg/L. These results indicated that varying amounts of TDZ enhanced the growth performance of turmeric during in vitro culture. Furthermore, *in vitro*-grown turmeric exhibited a greater leaf area when the TDZ concentration was between 2.0 and 2.5 mg/L, suggesting that higher TDZ concentrations promote leaf growth during micropropagation.

However, in terms of root growth, an inverse relationship is observed between the amount of TDZ and the number and fresh weight of roots produced. Lower TDZ concentrations (0.01 mg/L) in the MS medium lead to more roots and heavier fresh roots. Conversely, higher TDZ concentrations promoted longer root growth. These findings indicated that lower TDZ concentrations (0.01 mg/L) facilitated root regeneration, resulting in a higher number and weight of roots, while higher TDZ concentrations encouraged longer root growth.

Discussion

The study demonstrated the effect of TDZ *in vitro* bud proliferation of turmeric. The result showed that higher concentrations of TDZ, specifically 2.5 mg/L (T7) and 2.0 mg/L (T6), consistently led to the highest bud emergence across all subculture stages. It suggested that a concentration of 2.5 mg/L of TDZ is particularly effective in promoting bud initiation and development. The data demonstrated a dose-response relationship between TDZ concentration and bud emergence. As the TDZ concentration increased, there was increased in the number of buds. The concentration of TDZ appeared significantly impact the proliferation of buds, indicating the importance of carefully optimizing the concentration in tissue culture protocols.

Findings showed that T7 (2.5 mg/L) consistently outperformed other treatments, and T6 (2.0 mg/L) ranked second across all subculture stages. It showed that particular TDZ doses effectively stimulated and induce bud development. The control group (T0) consistently exhibited the lowest bud emergence, emphasizing the necessity of TDZ supplementation for optimal results. This reinforced the notion that TDZ plays a crucial role in stimulating bud formation, and the absence of TDZ (or lower concentrations) resulted in a significantly lower number of buds.

The increasing trend in bud emergence from S1 to S3 indicated a cumulative effect of TDZ on the proliferation of buds with successive subcultures. This temporal pattern suggested that the effects of TDZ on bud emergence may become more pronounced over time.

The study investigated the effects of varying thidiazuron (TDZ) concentrations on shoot regeneration and elongation in turmeric meriplants. The stem length exhibited a clear dose-dependent relationship with TDZ concentration.

The treatment with 0.5 mg TDZ/L (T3) produced the longest stems, emphasizing the stimulatory effect of TDZ on stem elongation. Interestingly, the treatments with higher TDZ concentrations (T6 and T7) were closely followed, indicating that while an optimal concentration promotes elongation, excessively high concentrations may not further enhance this trait.

Similarly, stem girth was significantly influenced by TDZ, with T7 (2.5 mg TDZ/L) displaying the highest girth. It suggested that TDZ influenced stem length and contributed to the development of thicker stems. In terms of the number of leaves, the study revealed that T7 had the highest mean number of leaves, indicating a positive correlation between TDZ concentration and leaf production. This result showed implications for plants, which are found to be crucial factors in turmeric crops' overall health and productivity.

With regards to leaf area, T7 displayed the highest mean leaf area, affirming the role of TDZ in promoting more considerable leaf development. In terms of shoot fresh weight, T7 again emerged as the treatment with the highest mean weight. This result implies that TDZ is pivotal in enhancing overall biomass production in turmeric meriplants.

The results indicated that the optimal shoot fresh weight is achieved when the MS medium is supplemented with 1.0-2.5 mg/L of TDZ, while a higher stem girth is observed when 1.5-2.5 mg/L of TDZ is added to the medium. Longer shoot length and a higher number of leaves are observed when TDZ is added in concentrations ranging from 0.01-2.5 mg/L. Additionally, a larger leaf area is observed in in vitro-grown turmeric when the TDZ concentration is between 2.0-2.5 mg/L. These findings suggested that using varying amounts of TDZ enhances the growth performance of turmeric during *in vitro* propagation, compared to the absence of TDZ. Thus, TDZ promotes shoot regeneration and elongation in the micropropagation process of turmeric.

However, in terms of the number and fresh weight of the roots, an inverse relationship was revealed. Specifically, lower TDZ concentrations (MS Medium supplemented with 0.01 mg/L of TDZ) result in more roots and heavier fresh roots. Conversely, longer root length is observed with higher TDZ concentrations in the MS medium. This observation could be attributed to the fact that plantlets with fewer total roots exhibit longer individual roots. Overall, the findings suggest that shoot and root regeneration are facilitated by lower TDZ concentrations, specifically at 0.01 mg/L in the MS medium.

The study showed that different levels of thidiazuron (TDZ) significantly affected the growth of turmeric plants in controlled environments. Higher TDZ concentrations, especially at 2.5 mg/L and 2.0 mg/L, consistently result in more buds, indicating their effectiveness in promoting bud development. The study highlighted a clear connection between TDZ concentration and the number of buds, emphasizing the need for precise control in tissue culture practices. Treatment with 2.5 mg/L TDZ is consistently performed the best, emphasizing its efficacy in stimulating both bud and shoot growth. Overall, the findings suggest specific TDZ concentrations optimize various growth aspects in turmeric, showcasing its role in improving overall plant development during in vitro propagation. Additionally,

the study demonstrated a relationship between TDZ levels and root characteristics, indicating the influence of TDZ on both shoot and root regeneration.

The findings of the study aligned with previous research conducted on the effect of TDZ on the *in vitro* propagation of turmeric and other plant species. TDZ has been extensively studied for its efficacy in promoting shoot and root regeneration and bud formation in various plant species. It is a widely recognized plant growth regulator commonly utilized in tissue culture techniques to enhance axillary bud proliferation and facilitate shoot organogenesis and morphogenesis (Parmar and Jasrai, 2015; Guo *et al.,* 2011). However, the optimal concentrations of TDZ can vary depending on the specific plant species.

For instance, research focused on turmeric micropropagation revealed that using 1.0 mg/L TDZ resulted in an average of 3 shoots per explant (Suminar *et al.,* 2019). In the case of Curcuma caesia Roxb., the highest mean number of shoots/buds was achieved with a TDZ concentration of 0.1 mg/L (Fong and Sani, 2019). Similarly, in *Curcuma zedoaria* Roscoe, the most effective shoot multiplication occurred in an MS medium supplemented with 1.5 mg/L TDZ, leading to an average of 5.3 ± 0.24 shoots per explant during the regeneration of in vitro plantlets from explants (Hussain *et al.,* 2022).

Similar favorable effects of TDZ have been observed in other plant species, such as *Zingiber officinale* Rosc. Var. Rubrum (Karyanti *et al.,* 2021; Zhang *et al.,* 2011; Lo-apirukkul *et al.,* 2012; Verma and Bansal, 2014), *Capsicum annuum* L (Siddique and Anis (2006)., *Artemisia annua* L. (Lualon *et al.,* 2008)), *Casuarina cunninghamiana* Miq. (Shen *et al.,* 2010) and *Jatropha curcas* (Zhang *et al.,* 2013). Furthermore, TDZ has shown efficacy in inducing morphogenesis in plant *in vitro* propagation, as observed in *Echinacea purpurea* L. (Jones *et al.,* 2007; Guo *et al.,* 2011), African violet (Mithila *et al.,* 2003), *Vitex trifolia* L (Ahmed and Anis, 2012), *Curcuma caesia* Roxb. (Fong and Sani, 2019), *Musa* sp. (Ratnasari *et al.,* 2016), almond "Beldi" ecotypes (Kodad *et al.,* 2021), *Alstroemeria aurantiaca* cv. 'Rosita' (Hutchinson *et al.,* 2014).

In addition, the effectiveness of TDZ in inducing shoot multiplication and bud formation has been observed in various plant species, such as *Aloe vera* (L.) Burm. f. (Lavakumaran and Seran (2014), tea plant (Singh and Hazarika, 2020), *Tulipa edulis* (Zhu *et al.,* 2014), *Myrtus communis* L (Şana *et al.,* 2013), *Lilium monodelphum* M. Bieb, var. Armenum (Azeri and Öztürk, 2021), *R. decorum* (Wu *et al.,* 2023), *Uraria picta* (Parmar and Jasrai, 2015), *Astragalus cariensis* Boiss (Erişen *et al.,* 2011), *Etlingera coccinea* (Jualang *et al.,* 2015), *Bauhinia tomentosa* (Naz *et al.,* 2021), *Hydrangea quercifolia* Bartr (Ledbetter and Preece, 2004)., *Ocimum basilicum* (Siddique and Anis, 2007) , and others.

Studies are also shown that pre-treatment with TDZ can enhance multiple shoot formation in certain plant species before in vitro propagation. Researchers such as Mok and Mok (2001), Lata *et al.* (2002), Prathanturarug *et al.* (2005), Guo *et al.* (2011), Jahan *et al.* (2011), and Grąbkowska *et al.* (2014), have observed that TDZ pre-treatment increases the chances of tissues responding to other inductive stimuli and improves the efficiency of in vitro propagation.

TDZ, a synthetic cytokinin, is widely used in plant tissue culture to promote shoot regeneration and in vitro multiplication (Kaminek and Kastner, 2002). It mimics the natural plant hormone cytokinin, which regulates plant cell division and differentiation (Zeng *et al.,* 2018). TDZ interacts with cytokinin receptors in plant cells, triggering biochemical reactions that activate cell division genes and promote the formation of new plant tissue (Hussein *et al.,* 2011; Gaj, 2004; Ziv, 1991; Jones *et al.,* 2007).

Moreover, TDZ increases endogenous cytokinin levels and inhibits cytokinin-degrading enzymes, enhancing cell division and proliferation (Gaj, 2004; Zeng *et al.,* 2018). Its mode of action also involves inhibiting adenylate isopentenyltransferase (IPT), the enzyme responsible for endogenous cytokinin biosynthesis, leading to the accumulation of cytokinin precursors. These precursors can be converted into active cytokinins, triggering adventitious bud formation and shoot regeneration (Osugi *et al.,* 2017).

Furthermore, TDZ affects shoot elongation by regulating gene expression in cell expansion and differentiation. It upregulates genes associated with cell wall component synthesis and auxin/gibberellin signaling pathways, resulting in longer and more robust shoots (Jiang *et al.,* 2018; Li *et al.,* 2018; Yang *et al.,* 2018).

However, TDZ can inhibit root proliferation and elongation in some plant species (Cruz de Carvalho *et al.,* 2000; Kozak, 2010). High concentrations of TDZ in the culture medium can impede root development, while low concentrations may initially enhance root growth but inhibit it at higher concentrations (Fong and Sani, 2019; Lata *et al.,* 2002).

The rooting process with TDZ is complex, and its mechanism involves the modulation of auxin-cytokinin balance and the production of endogenous auxins. TDZ is often used with other rooting hormones, such as IBA or NAA, to improve rooting. However, the optimal concentration and duration of TDZ treatment depend on the plant species and tissue culture protocol. Excessive TDZ treatment can hinder rooting or result in shoot proliferation instead (Erland *et al.,* 2020).

Hence, TDZ effectively promotes shoot regeneration, bud formation, and in vitro multiplication by mimicking cytokinins and stimulating cell division. It also influences shoot elongation through gene regulation. However, its impact on root proliferation varies depending on the concentration and species, and careful optimization of TDZ treatment is necessary to achieve successful in vitro rooting in plant micropropagation.

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