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Influence of Thermal Processing on the Physicochemical Properties, Stability and Antioxidant Activities of Jasmin Rice Milk containing a Co-Encapsulated Powder of Black Rice and Green Tea

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Abstract

This study investigated the influence of various thermal processing methods on the physicochemical properties, total phenolic compounds (TPC), and antioxidant activities of Jasmin rice milk (JRM) enriched with a co-encapsulated powder of black rice and green tea (CEP). Higher concentrations of CEP in JRM enhanced TPC and antioxidant activities, particularly against DPPH and ABTS free radicals. However, preliminary acceptances including color, aroma, and overall appearance diminished at higher CEP levels. Optimal results were observed with a 2% CEP concentration. Thermal treatments at 121 °C for 35 min and 142 °C for 6 sec significantly altered viscosity, pH, and sedimentation stability. The principal component analysis revealed that samples treated at 63°C for 30 min and 72°C for 15 sec exhibited higher characteristics of lightness and viscosity, particularly in terms of TPC values and antioxidant activities. This research provides valuable insights into optimizing the heat treatment process for the application of JRM with CEP, highlighting the balance between processing effects and product quality.

Keywords: Jasmin Rice Milk; Co-Encapsulated Powder; Pasteurization; Ultra-High Temperature; In-Container Sterilization

1. Introduction

There is currently a growing trend towards the consumption of plant-based beverages, driven by the increasing popularity of vegetarianism and veganism. This trend has been driven by the desire to promote animal welfare, human health and environmental sustainability, leading to the development and utilization of animal-free food substitutes (Escobar-Sáez *et al.*, 2022). Rice milk is a plant-based product without lactose, allergenic milk protein and low cholesterol. Rice milk is also an alternative product that maintains nutrition similar to dairy milk and receives acceptance from consumers who are interested in plant-based products (Malyala *et al.*, 2018). Various technologies have been applied to the research and development of rice milk in recent years. Plengsaengsri *et al.* (2019) applied the response surface methodology to investigate the optimum conditions for producing commercial rice milk. The results indicated that the nutritional value of rice milk was comparable to that of cow's milk, which contained 86.85 % of water, 5.84 % of carbohydrates, 3.28 % of fat, 3.14 % of protein, 0.45 % of fiber and 0.44 % of ash. Silva *et al.* (2023) observed that white, black and red rice had possible to produce plant-based milk. The nutritional value of rice milk has been found to be related to the variety of rice. The rice milk made from black rice exhibited higher levels of phenolic compounds (77.03 mg GAE/100 g) and antioxidant activity (21.30 g/g DPPH• and 10.35 µM trolox/g in ABTS•+). Additionally, Thai Jasmine rice (*Oryza sativa.* L.cv. KDML105) is the most common rice variety utilized for rice milk production in Thailand due to its rich nutritional composition, which includes significant amounts of vitamins A, C, and D, as well as iron (Wasan *et al.*, 2022). Thai Jasmine rice was characterized by an intense floral fragrance and sweetness (Soontrunnarudrungsri *et al.*, 2014). Hence, they are suitable raw materials to produce rice milk which offers notable nutritional content, health advantages and appealing sensory characteristics to consumers.

Thermal processing, often known as heat treatment, is the technique employed in the production of plant-based milk. The main goal of this process is to enhance the shelf life of the product by eliminating pathogenic and spoilage microorganisms. Although thermal processing offers benefits in terms of extending the shelf life, it is important to recognize that there are some adverse effects, including the degradation of heat-sensitive chemicals and the physicochemical properties of the product (Aydar *et al.*, 2020; Romulo, 2022; Sethi *et al.*, 2016). Therefore, the effect of thermal process on physiochemical, nutritional, and sensory properties should be considered when developing plant-based milk. Pasteurization and ultra-high temperature are usually used for plant-based beverages to endorse product quality and safety during storage (Munekata *et al.*, 2020; Poliseli-Scopel *et al.*, 2012). Kwok *et al.* (2000) studied the effect of thermal processing on sensory quality changes in soymilk. During heating with time increasing, the sensory quality including color and flavor in soymilk deteriorated. Kadam *et al.* (2015) investigated the impact of temperature and composition on the thermo-physical properties of rice-milk during its processing in a helical coil heat exchanger. The study demonstrated that there was an increase in fouling thickness when the fluid moved to the outlet pipe, and the investigation indicated that the rate of fouling thickness increased over time. The presence of milk fouling at the outlet results in an 11°C temperature drop, which appears to be an unfavorable result.

Plant-based product markets have been developed in accordance with the demand to enhance consumers' health by encapsulating targeted bioactive compounds into innovative products (McClements, 2020; Ozdal *et al.*, 2020). However, there are few literatures on supplying bioactive compounds into plant-based milk for enhancement of bioactivity to promote health. The encapsulation process is a remarkable technique to enhance the efficiency of bioactive compound delivery in foods and drinks. Applications for encapsulation techniques can protect bioactive compounds in food products from moisture, extreme conditions and heat during processing for enhancing their stability and maintaining viability (Alu'datt *et al.*, 2022; Gibbs *et al.*, 1999). Several studies have been conducted on the development of plant-based milk as well as loading bioactive agents into preformed milk, such as the research of Waghmare (2020) that applied the nanoencapsulation of catechin and epigallocatechin gallate and liposomal beta-carotene incorporated with products. Furthermore, co-encapsulation of bioactive compounds is an emerging field for the development of functional food products at present. Co-encapsulation is an applied method for enhancing the bioactivity of target-specific health through the synergistic relationships of two or more compounds (Chawda *et al.*, 2017; Fleming *et al.*, 2021; Liu *et al.*, 2022). From our previous study, the co-extract, a combination of black rice and green tea extract, illustrated high antioxidant and anti-inflammatory potential (unpublished work). The co-extract was encapsulated by double emulsion (water in oil in water) encapsulation and freeze-drying methods for producing co-encapsulated powder to use in the matrix of plant-based milk, especially Jasmin rice milk (JRM) in this research.

The goal of the research is to develop JRM incorporated with co-encapsulated powders of black rice and green tea extract (CEP). This study investigates the effect of different thermal processes (i.e. including low temperature with long time pasteurization (LTLT), high temperature with short time pasteurization (HTST), ultra-high temperature treatment (UHT), and in-container on the physiochemical characteristics, total phenolic content and antioxidant activities of JRM contained CEP.

2. Materials and Methods

2.1 Materials

Jasmine rice (*Oryza sativa*. L. cv. KDML105) was purchased from the retail in Reading, UK. Black rice variety (*Oryza sativa* L. var *Leum Phua*) was harvested in 2018, Chiang Mai, Thailand. Green tea (*Camellia sinensis* var. *sinensis*; Oolong No.17) was harvested in 2018, Chiang Rai, Thailand. Alpha-amylase, Alcalase and Flavozyme enzymes were sourced from Novozymes A/S, Denmark. Reagents for total phenolic compounds and antioxidant activities assay were purchased from RCI Labscan Co., Ltd. (Bangkok, Thailand).

2.2 Overall experimental design

Figure 1 illustrates the schematic diagram of the overall experimental design. The initial step was the preparation of co-encapsulated powder from black rice (BR) and green tea (GT). Both BR and GT were extracted using pulse electric field-assisted water extraction under conditions under our previous study (Salee *et al.*, 2022). After

that, the co-extraction solutions of BR and GT were produced according to the procedure outlined in Section 2.3.

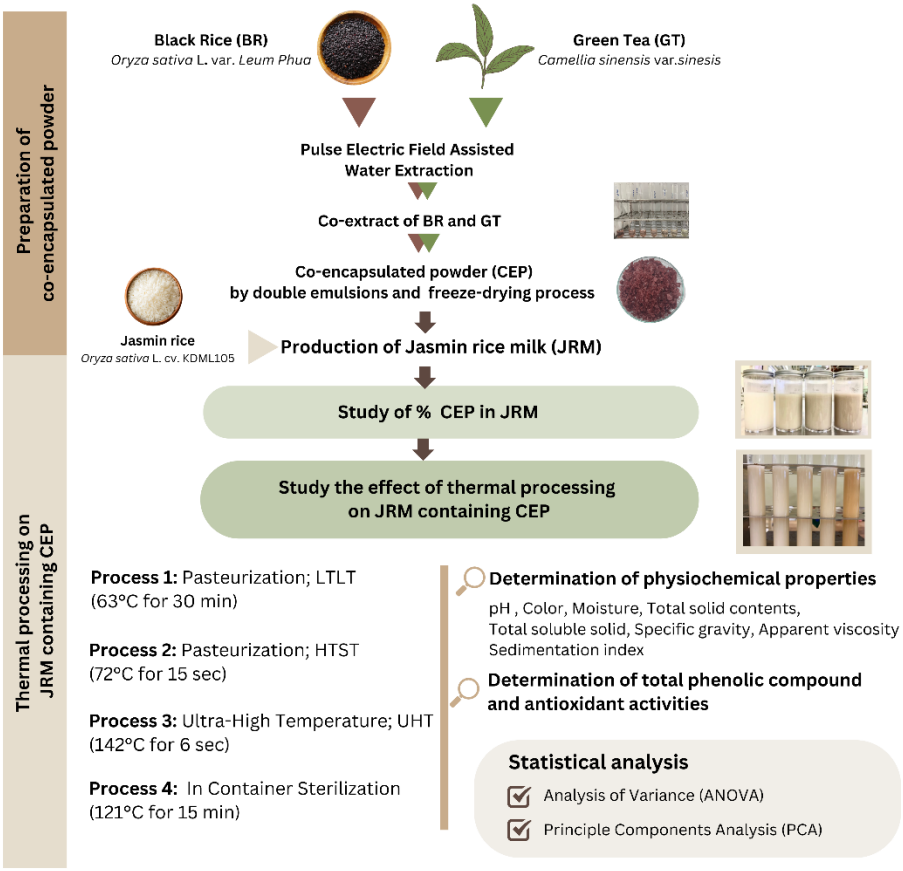


Figure 1 Schematic diagram of the experimental design

The co-extract solution was encapsulated and freeze-dried to produce CEP followed Section 2.4. The JRM was produced from Thai Jasmin rice and incorporated with CEP as described in Section 2.5. The suitable concentration of CEP in JRM was examined before the study of thermal processing. The CEP in JRM varied from 1-3%. The appropriate concentration of CEP in JRM was selected based on an assessment of its properties, TPC, antioxidant activities and preliminary acceptance. Finally, JRM containing CEP was subjected to various heating conditions to evaluate the effect on physiochemical properties, total phenolic compounds, and antioxidant activity (DPPH and ABTS assay).

2.3 Preparation of co-extract of BR and GT

BR and GT were extracted by pulse electric field-assisted water extraction (PEF). The extraction process was carried out in a PEF machine that was developed by Rajamangala University of Technology Lanna, Chiang Mai, Thailand. The conditions of PEF were operated following our previous study (Salee *et al.*, 2022): BRG/water: 0.5 g/mL; GT/water: 0.14 g/mL under 5 kV/cm of pulse intensity and 3,000 pulses of pulse number. Subsequently, the mixtures of extracts were shaken using an electrical shaker (Unimax 2010; Heidolph) at 150 rpm for 6 h. The mixtures were filtrated using Whatman® No. 1 filter paper (Merck, Germany) and then freeze-

dried. The co-extract was combined with BR and GT in a 1:2 ratio (mg/mL) in a 1 µM citric acid aqueous solution at pH~6. The mixture was then stirred for 30 min at 25°C using a water bath. Finally, the co-extract solution was filtered and freeze-dried for further encapsulation.

2.4 Preparation of co-encapsulated powder

The co-encapsulated powder was prepared by double emulsions (water-in-oil-in-water) using a two-step emulsification method, with some modifications based on previous research (Cenobio-Galindo *et al.*, 2019; Kanha *et al.*, 2020). Primary water-in-oil emulsions were prepared by mixing the co-extract, lipophilic emulsifier (lecithin) and oil phase (rice bran oil), allowing the mixture to stir at 10,000 rpm for 5 min. Finally, water-in-oil-in-water emulsions were produced by adding the previous water-in-oil emulsions to the hydrophilic emulsifiers formed with chitosan-carboxymethylcellulose solution. And finally, emulsions were solidified by mixing with a 35% maltodextrin solution until homogeneous and product were dried through the freeze-drying process.

2.5 Preparation of JRM with CEP

The process of JRM containing CEP production is briefly shown in Figure 2. The rice grain was ground to make rice flour. A mixture of rice flour (240 g) and potable water (1,760 mL)was boiled for gelatinization using a food processor (Thermomix TM6, Wuppertal, Germany) at 75 °C for 20 min. Then, the slurry was cooled down to 60 °C.

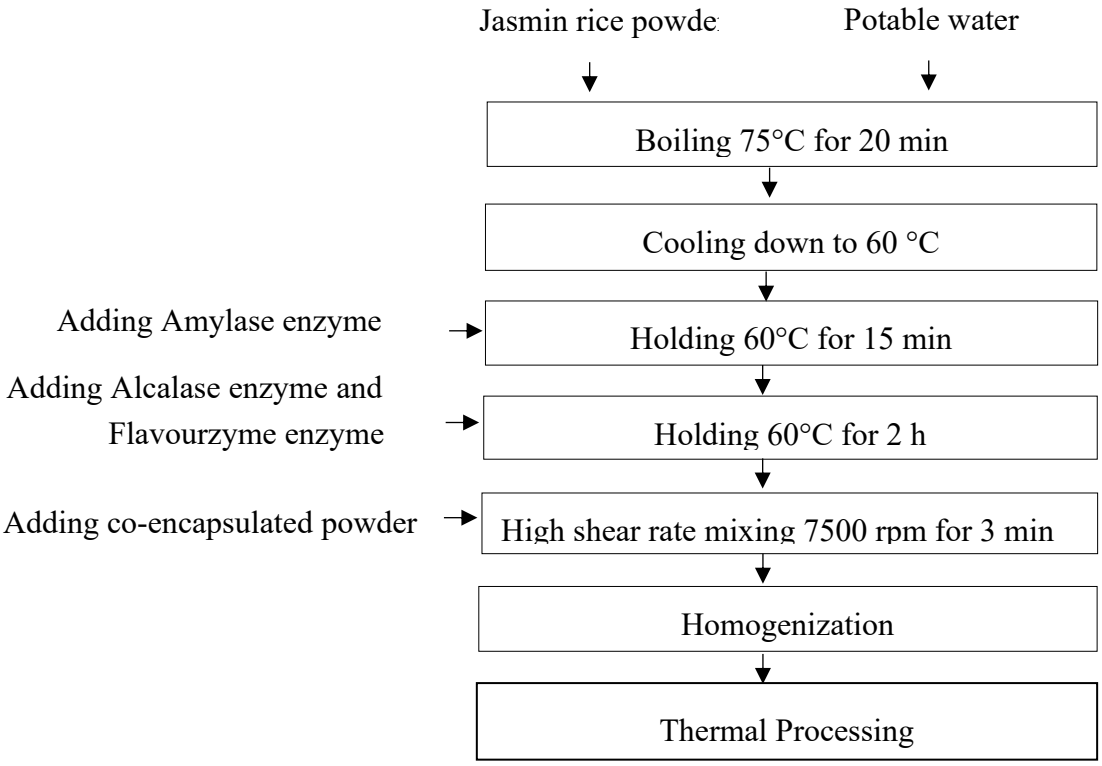


Figure 2 Production of JRM containing the CEP

In each production batch of plain JRM, a two-step enzymatic process was employed, utilizing Alpha-Amylase to hydrolyze starch (Amini *et al.*, 2019; Banyavongsa *et al.*, 2019) and a combination of Alcalase and Flavourzyme for exopeptidase and endo-peptidase activities (Hunsakul *et al.*, 2022; Tran *et al.*, 2021). Alpha-Amylase of 150 µL was added and heated at 60 °C for 15 min. Subsequently, 1 g of Alcalase and 1 g of Flavozyme were added and heated to 60 °C for 2 h. Then the slurry was heated at 95 °C for 10 min for enzymes inactivation (Guowei *et al.*, 2016). Plain JRM was produced. CEP was added to the plain JRM and followed by mixing through a high-shear mixer (Silverson L4RT High Speed Mixer, Silverson Machines Inc., USA) at 7,500 rpm for 3 min. Finally, JRM with CEP was homogenized through a two-stage high-pressure homogenizer (Type Panda, SN. 3753, GEA, Niro Soavi, Germany).

2.6 Thermal processing of the JRM with CEP

After the preparation of JRM containing CEP. Samples were then subjected to various thermal processing conditions as follows. After heat treatment, samples were transferred into sterile tubes and stored at 4 °C for further analysis within 3 days.

2.6.1 Pasteurization

Two types of pasteurization were applied to the samples, including low temperature with long time (LTLT) and high temperature with short time (HTST). Standard processing conditions for pasteurization were followed as recommended by the dairy industry (Lindsay *et al.*, 2021). For LTLT, samples in the sterilized container were subjected to a water bath at 63 °C for 30 min and subsequently cooled through cold water. For HTST process, samples were heated to 72 °C for 15 sec and then cooled down to 4 °C using a tubular heat exchanger (HTST/UHT system FT74XTS, Armfield Inc., UK).

2.6.2 Ultra-high temperature (UHT)

Samples were treated at a standard UHT condition (i.e. 142 °C for 6 sec) and then cooled down to 4 °C using a tubular heat exchanger (HTST/UHT system FT74XTS, Armfield Inc., UK) using a tubular heat exchanger at 142 °C for 6 sec and then cooled down to 4 °C.

2.6.3 In-container sterilization

Condition of in-container sterilization followed the sterilized soymilk process in batch production (Khodke *et al.*, 2014). The sample was filled in tin can and sterilized in a steam-air retort (Lagarde, Holmach Limited, UK) at 121 °C till achieving F0 value of 12 by the end of heating process. After processing to achieve the F0 value, samples were rapidly cooled to below 50°C by spraying water and then cooled in chilled water after being taken out of the retort. The samples were then dried and stored.

2.7. Determination of physiochemical properties

2.7.1 pH

The pH of sample was measured using a digital pH meter (Orion Star, Model A111, Thermo Scientific) in triplicate. The pH meter probe was inserted into the sample and the stable reading was the final pH value.

2.7.2 Color

The color of sample was measured using the color parameters L*, a*, and b*. A colorimeter (Chroma Meter CR-400, Konica Minolta, Inc., Japan) was used to measure the color parameters.

2.7.3 Moisture and total solid contents

The samples weighting from 0.9 to 1.0 g were transferred into the moisture analyzer (Hallmark Mechatronics, Model-Sartorius, Germany) at 105°C to measure moisture contents. And the total solid content was calculated using the following formula:

$$\% \text{ Solid Contents} = 100 - \% \text{ Moisture Contents} \quad (1)$$

2.7.4 Total soluble solid (TSS)

A droplet of 0.5 mL sample was placed on the sample slot of the digital refractometer HANNA model HI 96801 (HANNA Instruments Inc., USA) and the TSS was expressed in ˚Brix.

2.7.5 Specific gravity

The specific gravity was measured by using a specific gravity cup (50 mL). Firstly, the specific gravity cup was filled with distilled water and weighed. Then, the sample was filled to the same level in a similar specific gravity cup and weighed. The specific gravity of the sample was calculated by dividing the sample weight by the weight of distilled water.

2.7.6 Apparent viscosity

The apparent viscosity of sample was measured by Brookfield viscometer (Model DV-E, Brookfield Engineering Laboratories, Inc., USA) with spindle LV-1 (No. 61) at sample volume of 200 ml. The results are presented in millipascal- sec (mPa.s).

2.7.7 Sedimentation index (SI)

The sedimentation index was according to modifications method (Abedi *et al.*, 2014). Approximately 20 ml of sample was poured into a test tube. Test tubes were then covered and placed at room temperature for 24 h. The total height of the sample (Ht) and the height of the sedimentation (Hs) in the test tube were measured. The sedimentation index was calculated by the following formula:

$$\text{Sedimentation Index (SI)} = (Hs / Ht) \times 100\% \quad (2)$$

2.8 Total phenolic compound (TPC) and antioxidant activities

2.8.1 Sample preparation

The 10 mL of sample were centrifuged at 10,000 x g for 10 min and the supernatant was collected for analysis. The collected supernatant was kept at -20°C for future analysis.

2.8.2 TPC assay

The TPC was determined according to a modified Folin-Ciocalteu method of Apostolidis *et al.* (2011). Sample (1 mL) was added to a test tube with 1 mL of distilled water then 0.5 mL of Folin-Ciocalteu's reagent (1:10) was added. The mixture was left to stand for 30 min. After 5 min, 1 mL of 5% Na_2CO_3 was added and left to stand in the dark for 60 min. Absorbance was measured at 725 nm. The absorbance values were converted to TPC and reported in mg GAE/mL.

2.8.3 DPPH radical scavenging ability assay

The DPPH radical scavenging ability assay was determined according to a modified method of Long *et al.* (2021). Briefly, 1 mL of sample was mixed with 4 mL of 0.33 mmol/L DPPH solution (using distilled water as the solvent) and then left in the dark for 30 min. The absorbance at 517 nm was then measured and the DPPH radical scavenging rate was calculated, which expressed in mg GAE/mL.

2.8.4 ABTS radical scavenging ability

The ABTS radical scavenging ability assay was determined according to a modified method of Long *et al.* (2021). Briefly, 1 mL of sample was added to 2.5 mL of ABTS detection solution, shaken and then stored for 30 min in the dark at room temperature. The absorbance value at 734 nm was measured and the ABTS radical scavenging ability was calculated, which expressed in mg GAE/mL.

2.9 Preliminary acceptances test

The preliminary acceptability of samples was evaluated by five PhD students from the Department of Food and Nutritional Sciences at the University of Reading, UK, who had backgrounds in consuming plant-based milk. The samples were assessed for preliminary acceptance, including color, aroma, and overall appearance, aiming to guide the selection of the optimal percentage of CEP in JRM. The percentage of preliminary acceptability was calculated as (number of accepting participants/5) x 100%. Each sample (10 mL) was tempered to 25°C, coded with random three-digit codes, and presented in plastic cups to participants for independent observations of randomized samples.

2.10 Statistical analysis

SPSS (Version 17, SPSS Inc., Chicago, USA) was used to perform an analysis of variance (ANOVA) and to determine differences between samples using the Duncan Multiple Range Test (DMRT). The significance level was defined at $p \leq 0.05$. Each measurement was performed with at least three replicates. The standard deviation represented the uncertainty associated with each result.

2.11 Principal components analysis (PCA)

PCA was conducted to demonstrate the relationship between the different thermal processing and physicochemical properties, TPC and antioxidant activities of the samples. PCA was performed by SPSS (Version 17, SPSS Inc., Chicago, USA).

3. Results & Discussion

3.1 Concentration of CEP in JRM

The concentration of CEP in JRM at 1, 2 and 3 % w/v were investigated with a focus on its physicochemical properties, TPC, antioxidant activities and preliminary acceptance. The result is shown in Table 1.

Table 1 Physiochemical properties, TPC, antioxidant activities and preliminary acceptance of JRM at varying concentrations of CEP

	Concentration of CEP (%w/v of JRM)			
	Control	**1%**	**2%**	**3%**
Physiochemical properties				
pH	7.60 ± 0.09^{a}	7.50 ± 0.05^{b}	7.37 ± 0.02^{c}	7.05 ± 0.01^{d}
TSS (°Brix)	8.97 ± 0.06^{c}	9.17 ± 0.12^{c}	9.77 ± 0.12^{b}	10.33 ± 0.15^{a}
L*	53.95 ± 0.08^{a}	53.27 ± 0.50^{ab}	52.46 ± 0.12^{b}	51.20 ± 0.79^{c}
a*	-1.15 ± 0.01^{d}	-0.85 ± 0.02^{c}	-0.14 ± 0.01^{b}	0.46 ± 0.01^{a}
b*	-4.22 ± 0.03^{d}	0.83 ± 0.02^{c}	1.57 ± 0.02^{a}	1.41 ± 0.01^{b}
TPC (mg GAE/mL)	0.499 ± 0.064^{c}	1.055 ± 0.066^{b}	1.409 ± 0.095^{a}	1.543 ± 0.113^{a}
Antioxidant activities				
DPPH (mg GAE/mL)	0.113 ± 0.010^{d}	0.167 ± 0.015^{c}	0.203 ± 0.001^{b}	0.240 ± 0.008^{a}
ABTS (mg GAE/mL)	0.189 ± 0.017^{d}	0.259 ± 0.033^{b}	0.298 ± 0.040^{ab}	0.331 ± 0.046^{a}
% Preliminary acceptance				
Color	100 %	100 %	80 %	40 %
Aroma	100 %	100 %	80 %	60 %
Overall appearance	100 %	80 %	80 %	40 %

Note: (1) Values expressed mean ± SD of triplicate measurement.
(2) The superscript letters (a-d) in the same column indicate significant differences at $p \leq 0.05$, while ns indicates no significant difference at $p > 0.05$.
(3) mg GAE/mL: milligram of garlic acid equivalents per milliliter of sample
(4) Control: JRM without CEP

According to Table 1, the pH of the JRM exhibited a significant decrease as the concentration of co-encapsulated powder increased. Specifically, the pH declined from 7.50 observed at a CEP concentration of 1%, to 7.05 when the concentration of CEP was increased to 3%. This decline of pH can be attributed to the presence of lactic acid within the co-encapsulated powder, which was employed in dissolving chitosan during the preparation of hydrophilic emulsifiers as part of the encapsulation process. Consequently, when CEP was dissolved in JRM the presence of acidic protons contributed from lactic acid resulted in the pH decrease. TSS of JRM was found to be associated with the concentration of CEP. The control had a TSS of 8.97%, while JRM containing CEP exhibited TSS values ranging from approximately 9.17% to 10.33%. The increase in TSS was influenced by the quantity of maltodextrin present in CEP, as maltodextrin is derived from starch through partial hydrolysis and exhibits water-soluble properties (Takeiti *et al.*, 2010). The appearance of CEP and JRM with CEP in sterile bottle various CEP 0, 1, 2 and 3 % can be observed in Figure 3(a) and (b), respectively. The characteristic of CEP illustrated that the purple freeze-dried powder resembled a fractured glass-like structure of varying sizes. Additionally, the color of CEP was represented by the values L*=27.03, a*=13.08 and b*=5.72. The moisture content was determined to be 1.89%. The overall appearance of JRM with varying

amounts of CEP was noticeably different. The color of JRM that did not contain CEP appeared to be as white as boiled rice. The color of JRM samples containing CEP differed significantly from the color of control samples, which changed to a light grayish brown. The color attributes of milk samples with CEP at concentrations ranging from 1% to 3% exhibited variations with L* (brightness) values between 51.20 and 53.27, a* (- green to + red) values between from -0.85 to 0.46 and b* (–blue to + yellow) values between from 0.83 to 1.57. The L* of JRM decreased with an increase in the concentration of CEP, which can be attributed to the dispersion of powder particles. Figure 3(b) illustrates that the reduction in lightness is correlated with the increase in turbidity of JRM. The a* value of the samples displayed a significant increase with an increase in the concentration of CEP. The highest a* values were observed when CEP was at a concentration of 3% in JRM, with a value of 0.46. The observed red hue in the sample's color is likely influenced by the presence of CEP, which contains anthocyanins extracted from black rice. And the highest b* values were observed when CEP was at a concentration of 2% in JRM with a value of 1.57.

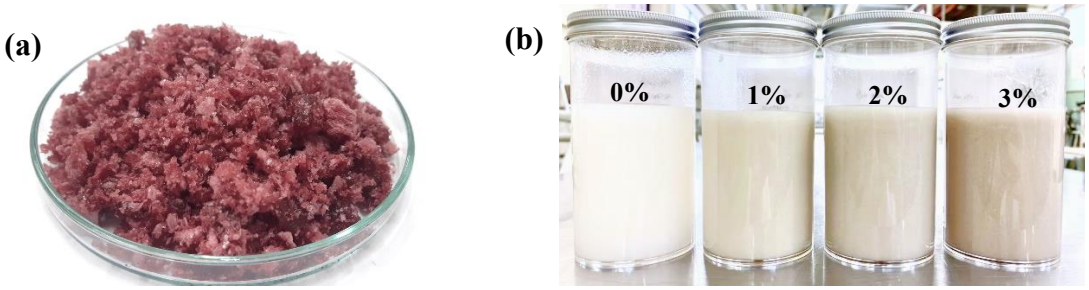


Figure 3 (a) The appearance of CEP and (b) Photograph of JRM containing CEP in sterile bottles

The significant difference in TPC was evident among the various concentrations of CEP. CEP of JRM with 2 and 3% showed notably higher amounts (1.409 and 1.543 mg GAE/mL, respectively). The addition of CEP to JRM led to an increase in TPC. This effect can be attributed to the presence of the main bioactive compounds within CEP, including anthocyanins and catechins. The TPC of JRM with CEP found a similar amount of red and black rice-based milk (120 GAE/100 mL, proximately) (Silva *et al.*, 2023). Sirirat *et al.* (2018) investigated the TPC of Khao Dawk Mali 105, Red Hawm, Jasmin, and Hawm Nil brown rice milk which showed values of 252.86, 250.71, and 256.43 mg GAE/L, respectively. These values were relatively lower in comparison to JRM containing CEP. Silva *et al.* (2023) described that it is necessary to perform the antioxidant activity test with multiple methods for indicating the antioxidant capacity of the product. Therefore, antioxidant activity against $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radicals were determined at varying concentrations of CEP in JRM. Following the same trend as the TPC, the antioxidant activities of JRM with CEP can be seen in Table 1. As the quantity of CEP in JRM increased, a significant enhancement in antioxidant activity against DPPH• and $ABTS^{\bullet+}$ free radicals were observed. The highest level of DPPH activity was observed at 3% of CEP, measuring at 0.240 mg GAE/mL. Furthermore, the high level of ABTS activity was identified at CEP concentrations of 2% and 3% with values of 0.298 and 0.331 mg GAE/mL,

respectively. Therefore, the findings of this study demonstrate that JRM supplemented with CEP exhibits significant TPC and antioxidant activities. These properties enable the neutralization of free radicals within the body, thereby potentially offering health benefits to humans when consumed in appropriate quantities. Additionally, JRM with CEP was suggested to develop a support-good health product.

Table 1 also showed that the preliminary acceptance evaluated by panelists were generally acceptable, which supported the choice of CEP concentrations for further research. The color and aroma acceptance of JRM with CEP exhibited a decline as the content of CEP increased from 1% to 3%. The highest color and aroma acceptance were observed in JRM with 1% CEP. The overall appearance of JRM with 1% and 2% CEP was shown to be higher compared to JRM containing 3% CEP.

In this study, the suitable CEP concentration was determined based on preliminary acceptance as well as the potential health benefits regarding TPC and antioxidant activities. Even though JRM with 1% CEP received the highest value in all preliminary acceptances, it exhibited relatively lower values of TPC, and antioxidant activities compared to 2% and 3% CEP. On the other hand, JRM with 3% CEP displayed high TPC and antioxidant activities. However, it exhibited the lowest color and overall acceptability, below 40%. The overall results indicate that 2% CEP in JRM exhibited high TPC and ABTS activity, closely resembling the values observed with 3% CEP. Additionally, the preliminary acceptance for JRM containing 2% CEP was valued at 80%. As a result, the 2% CEP concentration was chosen for further investigation to study the effect of thermal processing on the physicochemical properties and antioxidant activities of JRM.

3.2 Effect of heating conditions on JRM containing CEP

3.2.1 Physiochemical properties

The composition of JRM with CEP may be influenced by various heating conditions including changes in protein, carbohydrate, fat and phenolic compounds. Table 2 presents the physiochemical properties of JRM with 2% CEP when treated under different heating conditions.

Table 2 Effect of various heating conditions on the physicochemical properties of JRM with 2% CEP.

Physiochemical Properties	Heating conditions				
	Control	Process 1: LTLT	Process 2: HTST	Process 3: UHT	Process 4: In-container sterilization
pH	7.615 ± 0.005^{a}	7.516 ± 0.007^{b}	7.498 ± 0.009^{d}	7.506 ± 0.007^{c}	6.798 ± 0.007^{e}
Color					
L*	53.32 ± 0.17^{a}	50.57 ± 0.14^{c}	52.52 ± 0.10^{b}	47.79 ± 0.58^{d}	42.58 ± 0.29^{e}
a*	-0.15 ± 0.02^{d}	-0.36 ± 0.04^{b}	-0.45 ± 0.02^{c}	-0.43 ± 0.03^{c}	-1.13 ± 0.02^{d}
b*	1.67 ± 0.02^{e}	2.13 ± 0.02^{d}	2.35 ± 0.08^{c}	2.73 ± 0.08^{b}	5.72 ± 0.09^{a}
Moisture content (MC; %) ^{ns}	87.54 ± 0.19	87.68 ± 0.19	87.67 ± 0.24	87.57 ± 0.17	87.53 ± 0.34
Total solid content	12.46 ± 0.19	12.31 ± 0.19	12.32 ± 0.24	12.42 ± 0.25	12.43 ± 0.17

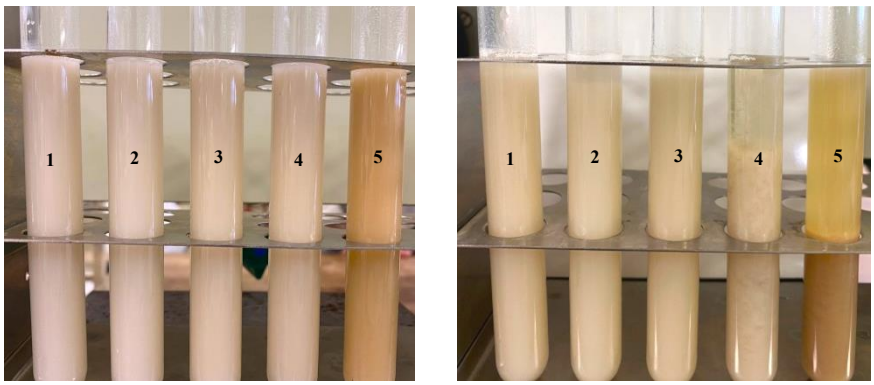
Physiochemical Properties	Heating conditions				
	Control	Process 1: LTLT	Process 2: HTST	Process 3: UHT	Process 4: In-container sterilization
(TS; %) ns					
Total soluble solid (TSS; °Brix)	9.70 ± 0.10^{c}	9.76 ± 0.05^{c}	10.19 ± 0.29^{b}	10.29 ± 0.21^{b}	11.67 ± 0.52^{a}
Density (kg/m^{3}) ns	104.02 ± 0.24	104.22 ± 0.65	104.07 ± 0.33	104.08 ± 0.31	104.16 ± 0.48
Viscosity (mPa.s)	19.54 ± 2.92^{a}	17.21 ± 2.53^{a}	19.20 ± 2.87^{a}	8.69 ± 0.10^{b}	5.89 ± 0.55^{c}
Sedimentation Index (SI; %)	4.76 ± 0.43^{a}	4.63 ± 0.52^{a}	7.28 ± 0.69^{b}	24.39 ± 0.52^{c}	50.33 ± 1.08^{d}

Note: (1) Values expressed mean ± SD of triplicate measurement.
(2) The superscript letters (a-e) in the same column indicate significant differences at $p \leq 0.05$, while ns indicates no significant difference at $p > 0.05$.
(3) Control: JRM with 2% CEP before heat treatment.

The heating led to a decrease in pH or made the JRM with CEP more acidic. Typically, the duration and intensity of heating can have an influence on the pH of foods. However, this impact can differ based on the properties, chemical composition and additive of food (Reineke *et al.*, 2011). The pH values of sample after heat treatment ranged from 7.52 (LTLT) to 6.79 (in-container sterilization). The results indicated that the long duration and intensity of heating at 121oC for 35 min resulted in an extremely decreased pH value compared to other conditions. The change in pH value when the sample was treated by the heating process could be affected by the thermal hydrolysis reactions of the protein in JRM with CEP (De Wit, 2009). Rutherfurd (2010) presented that free amino and carboxyl groups can be released during hydrolysis, leading to an increase in acidity. Our results were similar to Reineke *et al.* (2011), who found that the observed pH decreased by around 0.9 when temperature increased from 20°C to 130 °C. This was a minor influence from hydrolysis in the protein fractions of milk.

The color expressed as L*, a* and b* values of JRM with CEP through different heating conditions is shown in Table 2. The color appearance is also shown in Figure 4 (a). The L*, a* and b* values of the samples changed significantly when treated with heat processing. The L* value of the sample after pasteurization (LTLT and HTST) relatively decreased compared to the sample after UHT and in-container sterilization ($p<0.05$). The decrease in L* appearance in the dark brown sample was an influence of the Maillard reaction between free amino acids and reducing sugar at high temperature (Plengsaengsri *et al.*, 2019). The samples exhibited a notable decrease in the a* value following heat treatment, with a reduction in the level of the red hue observed in the samples. The red hue of CEP was more significantly reduced by heating processes of longer duration and higher intensity due to the degradation of anthocyanin pigments present in the CEP (Patras *et al.*, 2010). The results clearly indicate that the shift in the a* value of the sample through process of in-container sterilizing (-0.98) was higher than LTLT processing (-0.21). The b* value of the samples displayed an increase when treated with heat, resulting in a noticeable increase in the hue of the yellow color found in the samples. The change in the b* value could be impacted by the Maillard reaction or non-enzymatic browning that occurred during

heating due to the availability of sugar and amino in JRM (Buckholz *et al.*, 1980). This result was similar to an increase in b* value of tapioca starch during infrared heat treatment, depending on the different temperatures (Uraives *et al.*, 2019).



(a) **(b)**

Figure 4 Appearance of JRM containing 2% CEP (a) after heat treatment and (b) after storage at room temperature for 24 h. (1) Control (untreated sample), (2) LTLT, (3) HTST, (4) UHT and (5) In-container sterilization.

The results showed that the moisture content and total solid content of the sample under heating conditions were not significantly different ($p>0.05$). The moisture content and total solid content of samples ranged from 87.53% to 87.68% and from 12.31% to 12.46%, respectively. The results indicated that the TSS was significantly different in the heating treatment of temperature and time. The TSS of the sample through LTLT processing was not significantly different from the untreated sample. Additionally, the TSS of the sample through HTST and UHT processing was not significantly different. The TSS of the sample through in-container sterilization was highest, with a value of 11.67 °Brix. The increasing temperature and heating time caused the increased breaking of long chains of carbohydrate compounds into soluble sugar compounds (Astuti *et al.*, 2018; Ghnimi *et al.*, 2008) and the solubility of proteins in the dissolved compounds (Pelegrine *et al.*, 2005) in JRM as shown by TSS value.

The density of sample under heating conditions were not significantly different, and the values varied from 104.02 to 104.22 kg/m^3. The viscosity of sample through LTLT and HTST processing was not significantly different from the untreated sample. Additionally, the heating under temperatures greater than 100 °C by UHT and in-container sterilization processing led to a significant decrease in the viscosity of the sample to 8.69 and 5.89 mPa.s ($p<0.05$). The results demonstrated that increasing heating time would cause a higher-level breakdown of the starch molecules after gelatinization and resulted in a lower viscosity product (Abu-Jdayil *et al.*, 2004; Prawta *et al.*, 2010).

The sedimentation index (SI) was defined as the measure of sample instability or stability of particles in the JRM with CEP after being placed at room temperature for a duration of 24 h. The SI of the sample through LTLT processing was not significantly different from the untreated sample. Furthermore, the heating under HTST, UHT and in-container sterilization processing led to a significant increase in

the SI of the sample to 7.28, 24.39 and 50.33, respectively. The change in SI is presented clearly by the appearance of the sample in Figure 4 (b). An extreme increase in SI was observed with higher heating temperatures during UHT and in-container sterilizing processes, resulting in more particle sedimentation settled at the bottom of the sample. The protein denaturation and the gravitational overall particle size distribution were the main causes of particle sedimentation after storage (Souza *et al.*, 2023). The enhancement of storage stability can be achieved through many processing techniques, including homogenization to reduce droplet size, as well as the addition of functional and colloidal components (Nitisuk *et al.*, 2018).

3.2.2 Total phenolic compound and antioxidant activities

Table 3 presents the total phenolic compound and antioxidant activities of JRM with 2% CEP when treated under different heating conditions.

Table 3 Effect of various heating conditions on the total phenolic compound and antioxidant activities of JRM containing 2% CEP.

	Heating Conditions				
	Control	**Process 1: LTLT**	**Process 2: HTST**	**Process 3: UHT**	**Process 4: In-container sterilization**
TPC (mg GAE/mL)	1.527±0.164^{a}	1.341±0.009^{b}	1.275±0.156^{c}	1.116±0.004^{d}	1.059±0.053^{e}
Antioxidant activities					
DPPH (mg GAE/mL)	0.193±0.005^{a}	0.151±0.003^{b}	0.133±0.002^{c}	0.129±0.002^{c}	0.115±0.004^{d}
ABTS (mg GAE/mL)	0.266±0.033^{a}	0.209±0.025^{b}	0.144±0.149^{c}	0.113±0.009^{cd}	0.104±0.007^{d}

Note: (1) Values expressed mean ± SD of triplicate measurement.
(2) The superscript letters (a-d) in the same column indicate significant differences at $p \leq 0.05$
(3) mg GAE/mL: milligram of garlic acid equivalents per milliliter of sample
(4) Control: JRM with 2% CEP before heat treatment.

JRM with CEP exhibited a notable presence of various phenolic compounds, including p-coumaric acid, ferulic acid, and vanillic acid (Yamuangmorn *et al.*, 2020). Additionally, CEP was shown to include anthocyanin, catechin, and catechin derivatives. The TPC of the untreated sample was 1.527 mg GAE/mL and the TPC of the sample through various heating conditions was in the range of 1.059 - 1.341 mg GAE/mL (Table 3). There was a significant difference in the TPC in the different heating conditions. It was evidently observed that the TPC in the sample decreased when the heating time and temperature were increased. The loss of TPC in JRM with CEP during the heating process may be due to the degradation or decomposition of phenolic compounds (Jirattanarangsri, 2018). In this study, the black rice and green tea extracts were encapsulated within a carrier material known as CEP. However, it was noted that the homogenization process resulted in the destruction of some wall materials present in the CEP. During thermal processing, it is possible for some phenolic compounds to be released and encounter high levels of thermal energy, resulting in their degradation.

The antioxidant activities of the sample were determined to be against $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radicals. Results in Table 3 indicate that thermal processing could lead to a decrease in both antioxidant activities (DPPH and ABTS). The DPPH and ABTS activities of the sample through various heating conditions were in the

range of 0.115–0.11 and 0.104–0.209 mg GAE/mL, respectively. The HTST and UHT processes resulted in non-significant DPPH and ABTS activities. It was found that sterilizing the sample in a container at 121 °C for 35 min greatly decreased the DPPH and ABTS activities by about 40% and 60%, respectively. The ability to against $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radicals of the sample followed the same trend of decreasing TPC. According to Zapata *et al.* (2022), this change occurred because high temperatures deteriorate the structure of the phenolic compounds, which means they lose their antioxidant properties, following first-order kinetics like in the degradation of phenolic compounds. In addition to the temperature effect, changes in pH can also impact the antioxidant activities. The pH level displays an influence on the degradation process of significant chemicals, the stability of phenolic compounds, and their capacity for antioxidant activity (Krungkri *et al.*, 2019).

Overall, it was observed that the pasteurization process (LTLT; 63 °C for 30 min) encouraged the preservation of JRM with CEP because it was effective in maintaining the phenolic compounds and antioxidant activities when compared to an untreated sample. Nevertheless, the sample produced through the pasteurization process exhibited a shorter shelf life in comparison to the sterilization process conducted at temperatures exceeding 100 °C. The present study also proposed the application of the UHT process to prolong shelf life and enable room storage. However, it is essential to consider the physicochemical properties associated with this technique.

3.3 PCA of thermal processing effects on JRM containing CEP

Figure 5 presents the applied PCA, which was done using all measured variables exhibiting significant correlations in JRM with CEP subjected to various heating conditions. The observed variability was explained by two principal components, accounting for 95.11% of the total variance in the dataset. The green circle represents all the responses including physiochemical properties, TPC and antioxidant activities, while the dark blue triangle is the heating conditions. The resultant biplot exhibits different categories. One group was located on PC1(+) and marked by a pink circle, which represents two pasteurization conditions (LTLT and HTST). This group exhibited higher values of TPC, antioxidant activities, L* and viscosity. The result demonstrated an association with the findings presented in Tables 2 and 3, as well as with the general effect of heating conditions below 100°C on food characteristics. Another group was generated by the UHT processing, notably positioned on PC2(+) and visibly identified by a blue circle. This group was characterized by density, pH and a*. The in-container sterilization was assessed based on the measurements of total soluble solids (TSS) and the b* value on PC2(-), which was indicated by a yellow circle.

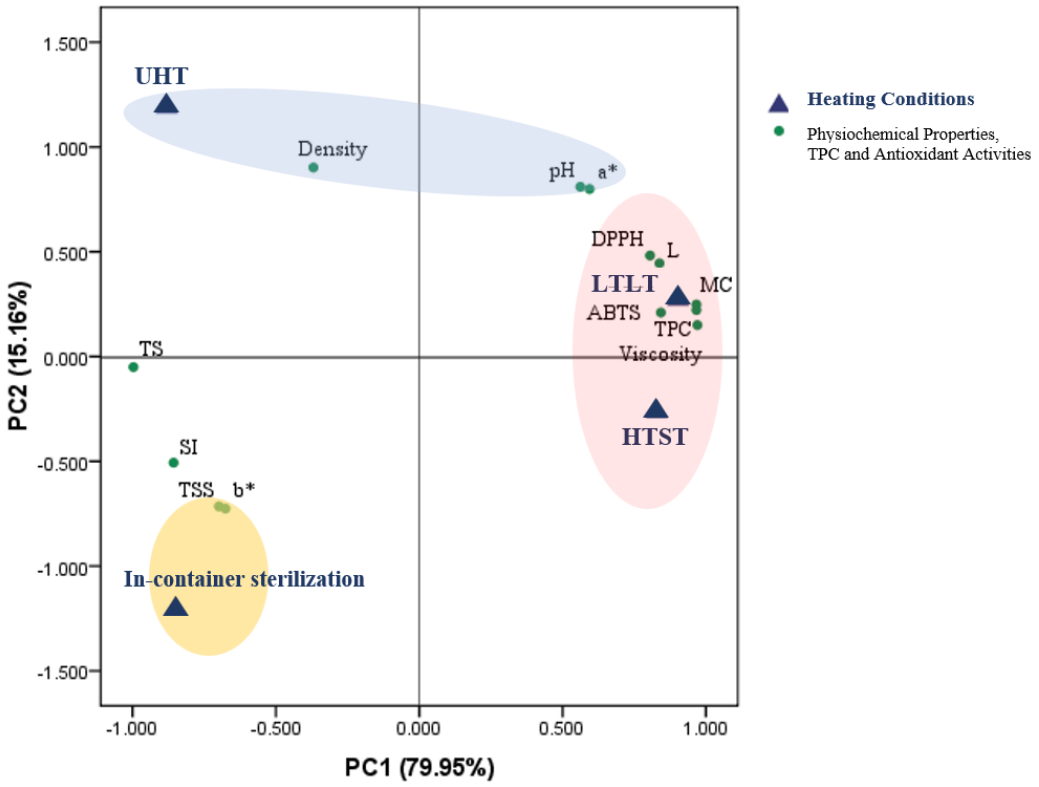


Figure 5 PCA based on physiochemical properties, TPC and antioxidant activities of JRM containing 2% CEP processed at different heating conditions including (1) LTLT (63 °C for 30 min.), (2) HTST (72 °C for 15 sec), (3) UHT (142 °C for 6 sec) and (4) In-container sterilization (121 °C for 35 min).

The distinct clusters indicate significant impacts of heating condition on the physiochemical properties, TPC and antioxidant activities of JRM containing CEP. PCA results indicated that higher TPC and antioxidant activities were observed in the samples following the LTLT and HTST processes. This suggests that producing JRM with CEP in the future could help maintain its beneficial health properties during processing

4. Conclusion

The suitable CEP concentration in JRM was determined based on preliminary acceptance and potential health benefits regarding TPC and antioxidant activities. The 2% CEP concentration was chosen for investigation into the impact of thermal processing. The color appearance of JRM with CEP changed significantly when treated with heat processing. Pasteurization reduced the L* value, while high temperatures over 100°C greatly decreased it. The TPC of the sample decreased with heating time and temperature, possibly due to the degradation of phenolic compounds. All heating conditions led to a decrease against DPPH and ABTS free radicals. The PCA method showed a significant correlation between duration and intensity of heating and physiochemical properties, TPC, and antioxidant activities of sample. LTLT and HTST resulted in higher TPC and antioxidant activities, suggesting the great potential of using CEP to produce functional JRM in the industry.

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