

## The effect of homogenization and sterilization on the stability and nutritional evaluation of Vietnamese purple rice milk supplemented with sesame, soybean and water caltrop

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### Article history:

Received: 23 July 2020

Received in revised form: 22 August 2020

Accepted: 14 October 2020

Available Online: 29 October 2020

### Keywords:

Purple rice,

Homogenization,

Sterilization,

Nutritional evaluation,

Stability

### DOI:

[https://doi.org/10.26656/fr.2017.4\(6\).379](https://doi.org/10.26656/fr.2017.4(6).379)

### Abstract

Lactose intolerance in human beings is the inability to digest significant amounts of lactose due to a shortage of the lactase enzyme. Rice milk is made as an alternative milk for people with limitation of milk consumption. The effect of (i) homogenization pressure (10-30 MPa) and time (5-20 mins); (ii) and sterilization conditions (3-11 mins at 121.1°C) on the quality of purple rice milk were investigated. To understand the quality of rice milk, the Nutrition Facts was established and the percentage daily value was calculated. The study of homogenization with pressure and time showed that the lowest creaming index was achieved at pressure 20 MPa and duration time of 10 to 15 mins, owing to the stable emulsions that were homogenized in the presence of additives. Sterilizing at 121.1°C for 7 mins could provide an acceptable microorganism compared to others. The results indicated that the percentage of calories from the macronutrients in this formula was within the AMDRs standard, with the percentage of calories ranges from 60.23, 15.26 and 24.5% of carbohydrate, protein and lipid, respectively. Furthermore, it was observed that the chemical components of the product were not significantly different during storage while the total microbial counts and lipid oxidation performed as peroxide value were lower than the maximum allowed limit after four months of storage at ambient temperature (28±2°C).

## 1. Introduction

Milk and other dairy products are a major source of calcium and it is essential for the growth and repair of the bones. Lactose intolerance in human beings is the inability to digest significant amounts of lactose, the predominant sugar of milk. This inability results from a shortage of the enzyme, lactase, which is normally produced by the human cells that can then be absorbed into the bloodstream. Therefore, rice milk which is free from lactose maintains the nutritive value similar to dairy milk may be accepted by the consumers (Padma *et al.*, 2018). Rice milk is considered the best hypoallergenic form of milk. Moreover, purple rice (*Oryza sativa* L.) is a special cereal which is cultivated in Vietnam and some other Asian countries. This rice contains high anthocyanin which is not only the common natural colour but also has potential applications such as the antioxidant component and many other health benefits (Hu *et al.*, 2003). Recent studies on rice milk products have been carried out (Thuy *et al.*, 2015; Banyavongsa *et al.*, 2019), especially the study of Banyavongsa *et al.*

(2019) has formulated an appropriate formula between the purple rice, sesame, soybean and water caltrop milk. Soy milk, sesame milk and water caltrop milk, the water extract of soybean, sesame and water caltrop, offers a promising performance as a carrier of probiotics (Shimakawa *et al.*, 2003). Furthermore, it is enriched in nutritive elements like proteins, unsaturated fatty acids, free amino acids and polypeptides, while containing only a small amount of saturated fatty acid and it lacks cholesterol or lactose (Yang and Li, 2010). Homogenization is a suitable process for the processing of rice milk. Previous research works have demonstrated that fat particle size, dispersion and temperature had significant effects on the stability of foods containing high-fat content such as milk, yoghurt and cheese (Shaker *et al.*, 2000). The homogenization for reducing fat globule size is required before heat treatment to retain the emulsion stability. Besides, processing temperature also has a significant effect on the stability of rice milk. Furthermore, the previous research work demonstrated that more aggregates of fat globule were observed at higher heating temperatures (Simuang *et al.*, 2004).

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From the literature described above, this research was aimed to investigate the effect of homogenizing pressure and time and sterilizing condition on the stability of rice milk. Moreover, the aim of this study also was to investigate the changes in the chemical composition during the storage and the establishment of Nutrition Facts of rice milk.

## 2. Materials and methods

### 2.1 Materials

Rice (ST Purple rice) (Figure 1) collected from Soc Trang province (Viet Nam), soybean (MTD 760 variety) supplied from Department of Genetics and Plant Breeding, College of Agriculture, Can Tho University, sesame and water caltrop were bought in the local market.



Figure 1. The purple rice

### 2.2 Preparation of rice milk supplemented with soybean, sesame and water caltrop milk

Before processing, purple rice was cleaned several times to remove dust, dirt and to reduce the chance of contamination, then blended into a puree. The ratio between rice and water is 1:7, and gelatinized at 85°C for 30 mins; the puree was liquefied by 0.149% v/w  $\alpha$ -amylase at 80°C for 57 mins. The puree was saccharified by 0.143% v/w glucoamylase at 60°C for 40.94 mins. The suspension was filtered through 2 layer fabric (Banyavongsa *et al.*, 2019). Soybean (MTD 760 variety) extracted by the simple traditional method of milk extraction (Ikya *et al.*, 2013). Sesame milk prepared by using 12% (w/v) initial concentration of seeds (Fitrotin *et al.*, 2015). Water caltrop milk was prepared as described by Banyavongsa *et al.* (2019). After being obtained, all kinds of milk were mixed into a formulation. The ratio between rice milk, soybean milk, sesame milk and water caltrop milk were 70%, 10%, 8% and 12%, respectively (Banyavongsa *et al.*, 2019).

### 2.3 Homogenization of purple rice milk

The purple rice milk was kept overnight at 4°C. Before all treatments, the milk was warmed to approximately 20°C. The milk was homogenized at 10, 15, 20, 25 and 30 MPa for 5, 10, 15, 20 mins using a

homogenizer (GJB-4-40, China) at an inlet temperature of 30±1°C. The viscosity of purple rice milk measured using a dynamic viscometer (Brookfield DV-E, USA). The creaming index was analyzed as described by Bylund (2003).

### 2.4 Sterilization of purple rice milk

After selected homogenization conditions, purple rice milk was sterilized at 121.1°C for 3, 5, 7, 9 and 11 mins. The F-value was calculated as describes by Holdsworth (1985). Total viable counts were determined on Plate Count Agar medium (AOAC, 2005). The plates were incubated at 35°C for 48 hrs. The sterilized rice milk product was analyzed for the nutritional quality and stored at 28±2°C for 4 months.

### 2.5 Physical and chemical analysis

Moisture, protein, fat, crude fibre content and peroxide value were determined by AOAC (2005) methods. The total carbohydrate content was determined according to the method of McCready (1971) and Dubois *et al.* (1956). The determination of calcium, sodium and potassium was carried out by flame photometry, then followed by the procedure of Arunkumar *et al.* (2015). The cholesterol content was determined according to the method of Dinh *et al.* (2008). Vitamin C was determined by Indophenol method (Zvaigzne *et al.*, 2009). Total carotenoid content was determined according to the method of Parrish and Bauernfeind (1977).

### 2.6 Calculation of total calories and percentage of the daily value (%DV)

Total calories of the milk were calculated by the formula as follows: total calories (kcal) = fat (g) x 9 (kcal/g) + protein (g) x 4 (kcal/g) + total carbohydrate (g) x 4 (kcal/g). The %DV for a nutrient is calculated by dividing the amount of a nutrient in a serving size by its daily value, then multiplying that number by 100 (Thomson and Manore, 2017).

### 2.7 Statistical analysis

Data analyses were carried out using STATGRAPHICS Centurion XV.I (U.S.A.). Values were expressed as percentage and mean±SD. The significance/non-significance of results was determined using one-way ANOVA.

## 3. Results and discussion

### 3.1 Effect of homogenization on the stability and viscosity of rice milk

The creaming index of the non-homogenized

Table 1. The creaming index of purple rice milk at different pressure and time of homogenization

Time (min)	Pressure (MPa)	Creaming index (%)	Viscosity (MPa.s <sup>-1</sup> )	Time (min)	Pressure (MPa)	Creaming index (%)	Viscosity (MPa.s <sup>-1</sup> )
5	10	7.30 <sup>a</sup>	1.82	15	10	4.68 <sup>d</sup>	1.86
5	15	7.42 <sup>a</sup>	1.83	15	15	2.35 <sup>fg</sup>	1.86
5	20	6.61 <sup>b</sup>	1.86	15	20	2.20 <sup>gh</sup>	1.88
5	25	3.23 <sup>e</sup>	1.9	15	25	2.01 <sup>hi</sup>	1.92
10	10	5.42 <sup>c</sup>	1.84	20	10	2.48 <sup>f</sup>	1.9
10	15	4.87 <sup>d</sup>	1.86	20	15	2.15 <sup>ghi</sup>	1.91
10	20	2.13 <sup>ghi</sup>	1.86	20	20	2.05 <sup>hi</sup>	1.92
10	25	2.06 <sup>hi</sup>	1.92	20	25	1.94 <sup>i</sup>	1.96

Values with different superscript letters within the column indicate a significant difference ( $P < 0.05$ ). The creaming index (%) and viscosity control purple rice milk were 48.153% and 1.8 MPa.s<sup>-1</sup>, respectively

(control sample) and homogenized samples at different pressures and time were significantly different ( $p < 0.05$ ) (Table 1). The highest creaming index was found for the control sample (48.15%). As the pressure and processing time increased, the creaming index decreased, indicating a high efficiency from the homogenization process. The homogenization with pressure 5, 10, 15 and 20 MPa in different times indicated that the creaming index decreasing remarkably from 7.29 to 3.23%, 5.42 to 2.06%, 4.68 to 2.01% and 2.48 to 1.94%, respectively. Considered as the result of viscosity, the homogenized samples were slightly more viscous than the non-homogenization rice milk because it is consistent with the presence of flocculated droplets. The better condition for homogenization as creaming stability was found in homogenized rice milk at the pressure above 20 MPa and time above 10 minute, properly due to the higher viscosity in these samples and low in the creaming index. Non-homogenization rice milk is more prone to creaming than homogenized rice milk because of its larger globule size. Homogenization, through the application of pressure, is able to reduce the milk fat globule size and prevents fat separation from the milk (Sfakianakis and Tzia, 2014) due to pumping milk at high pressure through a valve. The process which breaks fat globules to smaller particles (about 3.5 to 1  $\mu\text{m}$ ) because the homogenization process does not sufficiency causes an increase in the separated area (Massoud *et al.*, 2014). The better condition for homogenization as creaming stability was found in homogenized rice milk at the pressure above 20 MPa and time above 10 minute, properly due to the higher viscosity in these samples and low in the creaming index. The effect of homogenization on the change of the rice milk in the physical properties results from an increase in milk fat dispersion degree and the subsequent absorption of milk protein on the inter-phase surface. The level of protein adsorbed on the surface of fat globules and their interactions with plasma proteins play an essential role in the stabilization of homogenized milk. Al-Hilphy *et al.* (2012) found that

the creaming index was reduced with increasing time of homogenization with ultrasonic treatments at the power of 338 and 430 W. Ultra-high pressure homogenization (UHPH) also effected on the physico-chemical characteristics of tiger nuts' milk (Codina-Torrella *et al.*, 2017), the final product presented the highest colloidal stability after treatment, principally against creaming rate due to the reduction in particle size and the new particle interactions.

### 3.2 Effect of sterilization of quality and the shelf-life of rice milk

Steam sterilization is non-toxic, inexpensive, rapidly microbial sporadic and rapidly heat and penetrates fabrics (Gaillard *et al.*, 2005). As far as the sterilization is concerned, the combination of temperature and time of the lethal effect on microorganism and the chemical substance present is influenced by sterilization. The sterilization was selected based on the basis of  $F \geq F_0$ . Depending on the pH value and target microorganisms, rice milk is standardized with  $\text{pH} > 4.6$ . So, the target microorganism for sterilization values is 3 (Weemaes, 1997). Thus, the results in Table 2 showed that the product could be sterilized at temperature 121°C for 5 and 7 mins (corresponding with the F-value were 4.8 and 6.84, respectively). Sterilization condition (121°C for 7 mins) was adopted in this case due to the sensory characteristics of the product.

Table 2. Sterilization regimes (F-value)

Time (min)	Temperature (°C)	F-value
3	121	2.93
5	121	4.8
7	121	6.84
9	121	8.79
11	121	10.74

### 3.3 The nutritional values, energy-yielding and energy balance

The proportion of energy provide from the main nutrients in purple rice milk was presented in Table 3.

Rice is always perceived as a rich carbohydrate source in diet and similarly, it is observed that rice milk contains more sugar than normal cow's milk. Rice milk is lactose-free making itself a perfect alternative for patients suffering from the lactose intolerance (Lomer *et al.*, 2008). Acceptable Macronutrient Distribution Ranges for Adults (as a percentage of Calories) are as follows: protein: 10-35%, fat: 20-35%, carbohydrate: 45-65%. A balance of the macronutrients can help ensure adequate intakes of micronutrients as well (Thompson and Manore, 2017). The percentage of calories from the macronutrients in this formula was within the AMDRs standard, ranging from 60.23, 15.26 and 24.5% of carbohydrate, protein and lipid, respectively. A diet which is balanced in the macronutrient distribution is recommended for the malnutrition and over intake because unbalanced nutrients profile causes an increased risk of problem health such as chronic disease, diabetes, cancer and various other health complications (Shannon and Rodriguez, 2014). The Nutrition Facts of the purple rice milk was established (Figure 2). As in the General Guide to Calories (based on a 2,000 calorie diet): 40 Calories is low, 100 Calories is moderate and 400 Calories or more is high. The calculation of energy in this product was moderated accounting for 159.98 calories. The percentage of the daily value (%DV) shown in the Nutrition Facts label is the guide to the nutrients in one serving of food and met the energy requirement of alternative milk for people who have lactose allergy from cow's milk. The rice milk was free of lactose and cholesterol but it had the sweetness from starch and energy  $\approx 96.36$  calories (29.2 cal/100 mL) for serving size. The calculated %DV of vitamin A is 0.06%, it means that the content of this component in the product is less ( $\leq 5\%$ ). Some of the components are identified as the limit, such as fat, saturated fat, trans fat, cholesterol, or sodium. The calculated %DV of sodium (0.04%) (less than 5%). However, the %DV of carbohydrate and fat were estimated at 8.03% and 6.7% (between 5 and 20%), indicating a good nutritional product. The high amounts of these components (%DV $>20\%$ ) may increase the risk of certain chronic diseases. Health experts recommend that we keep our intake of saturated fat, trans fat and cholesterol as low as possible as part of a nutritionally balanced diet. However, the product contained a moderate amount of fiber (%DV is 13.12%), calcium (5.95%) and iron content (17.27%). With the energy and nutritional value provided, this product is usually to be able to partly meet the energy requirements for healthy food. In addition, considering that the price of rice milk was lower than cow's milk, it is a good choice for people who are lactose intolerant or cow's milk allergic.

Table 3. Macronutrient content, yielding-energy and percentage of energy providing from 100 mL of purple rice milk

Nutrients	Content (g)	Energy-yielding nutrients (Calories)	Percentage of energy providing from nutrients (%)
Carbohydrate	7.3	29.2	60.23
Protein	1.85	7.4	15.26
Lipid	1.32	11.88	24.5
Total		48.48	100

Nutrition Facts		
1 serving per container Serving size 330 mL		
Amount per serving		
Calories	159.99	
Calories from fat 39.2		
% Daily Value*		
Total fat	4.36 g	6.70%
Transfat	0 g	
Cholesterol	0 mg	0%
Sodium	0.99 mg	0.04%
Total Carbohydrate	24.09 g	8.03%
Fiber	3.28 g	13.12%
Protein	6.11 g	
Vitamin A	0.59 mcg	0.06%
Calcium	65.47 mg	5.95%
Vitamin C	1.2 mg	2.00%
Iron	2.42 mg	17.27%
Potassium	158.36 mg	4.52%
*The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2000 calories a day is used for general nutrition advice.		

Figure 2. Nutrition facts of purple rice milk

### 3.4 Microbiological analysis

The microbiological analysis of rice milk during storage are given in Table 4, the absence of coliform bacteria was found while the number of total viable count (TVC) of the milk was detected in the 3<sup>rd</sup> and 4<sup>th</sup> month accounting for  $1.5 \times 10^2$  and  $1.8 \times 10^2$  CFU/mL, respectively. These values were below the limit of acceptable counts for dairy milk. Jay (2008) suggested the anaerobic plate count of milk from healthy dairy products should be less than  $10^3$  CFU/mL. According to Onweluzo *et al.* (2009), the TVC in vegetable milk was less than the limit of acceptable level for milk products. It could be observed that the rice milk products could be stored at ambient temperature ( $30 \pm 2^\circ\text{C}$ ) for 4 months.

Table 4. Microbiological analysis of rice milk during storage

Storage (month)	Total viable count (CFU/mL)	Coliform (CFU/mL)
1	ND	ND
2	ND	ND
3	$1.5 \times 10^2$	ND
4	$1.8 \times 10^2$	ND

ND, not detected.

### 3.5 Chemical analysis of purple rice milk during storage

The results indicated that the chemical compositions of purple rice milk including protein, fat and

carbohydrate contents were not significantly affected by the storage while anthocyanin of the product was a significant change during storage (Table 5). Brodziak *et al.* (2017) illustrated a significant effect of temperature and storage time on the content of fat, lactose and dry matter, while only storage time was unaffected the protein content. Omer and Eltinay (2009) reported that storage milk at room temperature were significant changes in pH, acidity, lactose, total solid but insignificant changes in protein when storage for 42 days. The anthocyanin compound showed a gradual decrease from 2.04% to 1.21% in the fourth month of storage due to the anthocyanin being more sensitive to degradation and easily affected by several parameters. Sivamaruthi *et al.* (2016) reported about the degradation of this compound quickly (about 90%) by sterilization like heat and sonication. In addition, light is an essential factor required for the biosynthesis of pigments, it stimulates the anthocyanin denaturation faster than oxygen due to the UV protective nature of anthocyanin (Furtado *et al.*, 1993). Thus, storing the anthocyanin-rich food materials at room temperature that contacts directly to the light resulted in decreasing anthocyanin compounds.

### 3.6 The peroxide value of sterilized milk

Sterilized milk is designed to extend the shelf-life of milk that can be stored at ambient temperature for a long period. In this work after four months of storage, the peroxide value increased slightly from 0.148 to 0.198 meq/kg fat. The rise in peroxide value indicated in Figure 3, peroxide values slightly increased due to the breakdown of fatty acids into oxidation products. Lipid oxidation is known to be increased by several factors including heat, light, radiation, moisture and storage (Savage *et al.*, 2002). The sample had a lower peroxide value when compared to the standard in milk should be less than 0.6 meq/kg fat (Codex Alimentarius International Food Standards, 2011). The lower peroxide value indicated a longer period of storage. The results of this study agreement to Ajmal *et al.* (2018) assessed the non-effect of peroxide value on UHT milk during storage period at 30, 60 and 90 days. Similarly, Baladhiya *et al.* (2018) reported the peroxide value of in-container sterilization of *halwa* did not affect on sensory attributes of the product after 90 days of storage.

## 4. Conclusion

The sufficiency of homogenization as creaming stability was found in homogenized rice milk after homogenized at a pressure above 20 MPa for 15 mins. Comparing among commercial sterilizing conditions of study, heating at 121.1°C for 7 mins provided the acceptable mixed purple rice milk. The percentage of calories from the macronutrients was within the AMDRs standard. The nutrition compositions of the rice milk indicated unchanged after four months of storage. In addition, the present study demonstrated that purple rice milk could be considered as suitable economical substitutes for cow's milk and an ideal nutritional supplement for lactose intolerant population. The future works can be applied at a larger scale in order to fully utilize the available materials in Vietnam and create a new healthy nutritious food product.

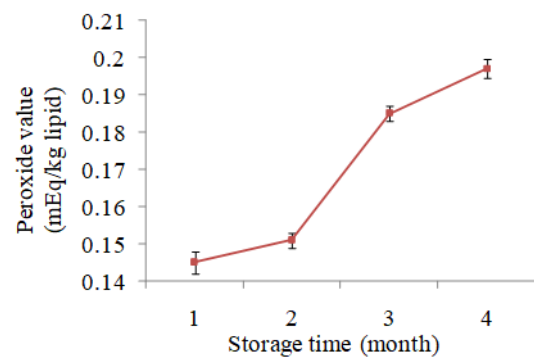


Figure 3. Effect of storage time on peroxide value of sterilized milk

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

We would like to express our gratitude to all those who gave us the possibility to complete this research. We would like to thank VLIR Network Vietnam Project for their support our study.

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Table 5. Chemical properties of rice milk during storage

Storage (month)	Chemical compositions			
	Protein (%)	Carbohydrate (%)	Lipid (%)	Anthocyanin (mg/L)
1	1.46±0.06	6.22±0.20	1.25±0.04	2.17±0.08
2	1.47±0.05	6.22±0.87	1.26±0.02	2.04±0.37
3	1.46±0.03	6.55±0.42	1.27±0.13	1.66±0.08
4	1.46±0.02	6.22±0.05	1.26±0.01	1.21±0.29

Values were expressed as mean±SD

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