

## Effects of Sterilization Times on Physicochemical Properties of Canned Gruel Produced from Red Jasmine Brown Rice Fortified with D-glucosamine

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### Abstract

Glucosamine prepared from crustacean wastes is widely used for inflammatory bowel disease and osteoarthritis (OA) treatment. In the present study, glucosamine prepared from blue swimming crab shells (*Portunus pelagicus*), a by-product from crab processing, was added into red jasmine brown rice gruel to be a new food item for elderly. Effects of different sterilization times and physicochemical properties of canned gruel prepared from red jasmine brown rice incorporated with glucosamine were studied. Canned Gruels, in presence of D-glucosamine (1.13% w/v or 1,500 mg/can (307x113 cm)) were sterilized at 121°C for different sterilization times (0, 5, 10, 20, 30, 40, 60, 90 and 120 min). The results showed that glucosamine was reduced by 33.7% during sterilization for 10 min. Glucosamine content was then gradually decreased after 30 min of sterilization. A pH value of canned gruel was decreased from 5.84±0.03 to 3.81±0.01 with increasing time of sterilization (p<0.05). Nevertheless, the increasing in turbidity and lowering  $L^*$ ,  $a^*$  and  $b^*$  of canned gruel were detected as longer time of sterilization. The highest viscosity of gruel milk was found at 40 min of sterilization (12.75±0.41 cps) (p<0.05). Thereafter, a slight decrease in viscosity was noticeable. With increasing sterilization times, lower hardness and greater stickiness of canned gruel were obtained. These results suggest that canned gruel prepared from red jasmine brown rice supplemented with glucosamine can be applied as new food product for elderly or health concerning consumers, particularly for bone and joint problems.

**Keywords:** glucosamine, physicochemical properties, red brown jasmine rice, sterilization, canned gruel

### 1. Introduction

Crab industries generate large amounts of crab biowaste during processing [1], approximately 40-50% of total weight of crustaceans [2]. The waste is sold to feed mills at a low price. However, this waste can be used to produce value-added products because it is rich in chitin [3] and glucosamine can be derived from chitin [4]. Glucosamine (C<sub>6</sub>H<sub>13</sub>NO<sub>5</sub>) is an amino monosaccharide that has biological effect to the body [5]. Glucosamine has a potential to prevent joint structure change in patients with osteoarthritis [6]. It was also reported having anti-inflammatory [7], antibacterial [8], anti-cancer effects [9] and antioxidants [10]. At present, the

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number and proportion of older people increases rapidly. Thai older people (aged 60 or older) expected to increase from 10.5% in the year 2005 to 15.7% by 2030 [11]. Population ageing is associated with an increased risk of disease burden and disability [12]. These people are disabled and dependent on others for acquiring, preparing their food [13]. Thus, food for elderly should have essential nutrients such as vitamins, minerals from natural food sources [14], high fiber [15], antioxidant and anti-inflammatory phytochemicals [16]. So, the canned gruel produced from red jasmine brown rice fortified with D-glucosamine is an interesting alternative for product development and product value added due to red jasmine brown rice is especially rich in anthocyanin pigments, phytochemicals, protein and vitamins [17]. Rice gruel is easy to eat with soft texture and digestible with high nutrition [18]. Addition of glucosamine in food products is promised to help consumer who has health concerns on osteoarthritis or bone and joint disease. So, the canned gruel fortified with glucosamine can be a new food product for elderly or health concerning consumers. However, stability of glucosamine in canned gruel during heat treatment, particularly sterilization has not been reported. Sterilization level in terms of time treatment may also affecting some physicochemical properties of canned gruel. Therefore, the aim of this study was to study effects of sterilization times on physicochemical properties of canned gruel produced from red jasmine brown rice fortified with D-glucosamine.

## **2. Materials and Methods**

### **2.1 Materials**

Red jasmine brown rice (*Mali Gomain Surin*) was used for canned gruel production. Red jasmine brown rice obtained from Surin province, Thailand was stored in vacuum polyethylene bags at room temperature until the experimentation. Blue swimming crab shell (*Portunus pelagicus*) from fresh crab with average size of 6-7 crab/kg was obtained from Viya crab product Co., LTD, Suratthani, Thailand. The crab shell was washed thoroughly with tap water and dried in hot air oven at 100°C for 4 h. Dried crab shell was milled using a hammer mill prior to sift through a 0.75 mm sieve. The milled crab shell was packed in polyethylene bag and kept at 4°C until used for glucosamine preparation.

### **2.2 Preparation of glucosamine**

Glucosamine from blue swimming crab shell was isolated according to the method of Sibi *et al.* [2] with some modifications. The milled crab shell was deproteinized with 3.5 M NaOH with a ratio of 1:9 (w/v) at 65°C and stirred using overhead stirrer for 2 h. The suspension was cooled to room temperature prior to filtration with Whatman No. 1 filter paper. The residue was washed with distilled water with a ratio of 1:50 (w/v) for 3 times. Then, crab shell suspension was neutralized using 0.1 M HCl and washed with distilled water. After filtration, deproteinized crab shell was demineralized by soaking into 1 M HCl with a ratio of 1:15 (w/v) and stirred at room temperature for 18 h. The demineralized sample was washed with distilled water, neutralized and subjected to soak with 0.35% sodium hypochlorite, washed with distilled water, dried in hot air oven at 60°C for 4 h. The dried sample was then hydrolyzed with 30% (w/v) HCl with a ratio of 1:19 (w/v) at 100°C for 30 min, followed by decolorization with activated charcoal and filtration. The filtrate was concentrated by evaporation at 45°C prior to crystallization with 95% ethanol with a ratio of 1:9 (w/v). After centrifugation at 5,000×g for 15 min, the glucosamine crystals were washed in 99% ethanol, dried in hot air oven and referred to as 'glucosamine'.

### **2.3 Production of canned red jasmine brown rice gruel fortified with D-glucosamine**

Canned gruel production was performed according to the method of Chumniwikri [18] with some modifications. Red jasmine brown rice were rinsed by water with a ratio of 1:9 (w/v) for 2 min and drained for 15 min. Then, rice samples (20 g) were put into lacquer cans (307x 113) prior to

fill with 150 ml of salt-glucosamine mixture, containing water, 0.20% (w/v) NaCl and 1.13% (w/v) glucosamine (1,500 mg/can), exhaust at 85°C and seam immediately. The canned gruel was sterilized by water spray overpressure retort (KM-P95SS, KM grandpack Co., Ltd. Bangkok, Thailand) at 121°C for different sterilization times (0, 5, 10, 20, 30, 40, 60, 90 and 120 min) at 2 bar pressure. After sterilization, canned red jasmine brown rice gruel fortified with D-glucosamine (CRBG) was cooled immediately, cleaned and stored at room temperature.

#### **2.4 Determination of glucosamine content**

Glucosamine content of CRBG samples was determined according to the method of Wu *et al.* [19]. The CRBG was ground for 1 min using a blender prior to centrifugation at 10,000 g at 25°C for 20 min. Supernatant (2 ml) was collected and mixed with 1 ml of 0.8% ninhydrin and 2 ml of 0.2 M phosphate buffer (pH 6.0) in a test tube. The mixture was then heated in a boiling water bath for 10 min. The solution was briefly cooled and the absorbance was measured at 570 nm using a spectrophotometer (V-1200, Shanghai Mapada Instruments Co., Ltd. China). D-glucosamine were used as the standards. The relative glucosamine content of the samples was calculated compared to that obtained at the highest glucosamine content.

#### **2.5 pH value analysis**

20g of CRBG were mixed with 100 ml of distilled water. The mixture was filtered through Whatman No. 4 filter paper. The pH value of the filtrate was measured at room temperature using a pH meter (FE-20, Mettler-Toledo Instruments Co., Ltd., Zurich, Switzerland).

#### **2.6 Color measurement**

Color of CRBG was measured according to the method of Chumniwikri and Peuchkamut [18] with a slight modification. CRBG was drained through a strainer. The drained water, referred as 'gruel milk' was collected and subjected to measurement of color by the Colour Quest XE colorimeter (Hunter Lab., Hunter Assoc. Laboratory, VA, USA) The results were expressed in CIE LAB system as  $L^*a^*b^*$ .  $L^*$  indicates the lightness of the color measured from black ( $L^*=0$ ) to white ( $L^*=100$ ),  $a^*$  determines the color in the red ( $a^*>0$ ) and green ( $a^*<0$ ) dimension, and  $b^*$  determines the color in the yellow ( $b^*>0$ ) and blue ( $b^*<0$ ) dimension. Three replicates were carried out for each sample.

#### **2.7 Turbidity analysis**

CRBG was drained through a strainer. The gruel milk was collected and subjected to turbidity analysis by using Turbidimeter (Lovibond, TurbiCheck, Dortmund, Germany). The result was expressed in nephelometric turbidity units (NTU).

#### **2.8 Viscosity measurement**

Viscosity measurement of gruel milk from CRBG was carried out using a Brookfield viscometer (Brookfield Engineering Inc, Model DV-III, USA) equipped with spindle No.18 at 200 rpm. Rotational speed of the spindle measures the range of a DV-III in centipoises. The viscosity was determined at room temperature and repeated three times for each sample.

#### **2.9 Texture profile analysis (TPA)**

Texture profile of CRBG was analyzed according to the method of Chumniwikri and Peuchkamut [18] with a slight modification. CRBG was drained through a strainer. The drained rice was collected and used for texture profile analysis using a back extrusion test. The compressed distance was 50% strain at a speed 1.0 mm/sec. TPA was performed using a texture analyzer (TA-Xt.plus, Stable Micro System, UK). Texture parameters consisting of hardness and stickiness were derived from the instrument software.

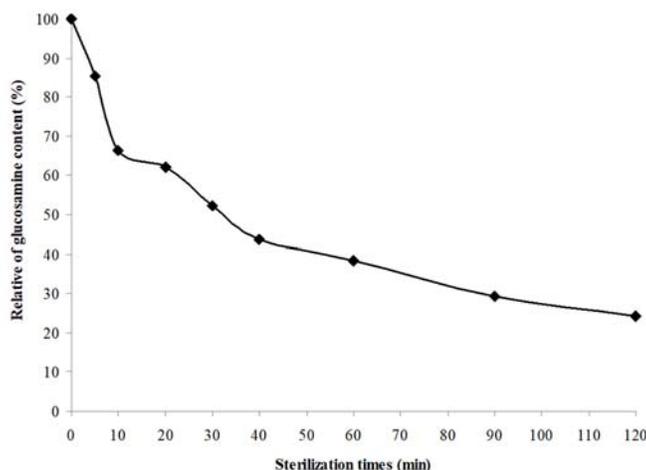
### 2.10 Statistical analysis

All data were subjected to analysis of variance (ANOVA) and differences between means were evaluated by the Duncan's Multiple Range Test. SPSS Statistic Program (Version 16.0) was used for data analysis.

## 3. Results and Discussion

### 3.1 Stability of glucosamine in CRBG as affected by sterilization times

Canned gruel produced from red brown jasmine rice fortified with glucosamine (CRBG) was sterilized at 121°C for different sterilization times. Stability of glucosamine in CRBG was monitored as relative glucosamine content in CRBG at different time of sterilization as shown in Figure 1. The results showed that glucosamine was dramatically reduced by 33.7% during sterilization for 10 min. A drastic decrease in glucosamine content was noticeable with increasing sterilization times to 30 min ( $p < 0.05$ ). Glucosamine content in CRBG was then gradually decreased to be about 30% at 120 min of sterilization. As a result, glucosamine from blue swimming crab, which was added in CRBG, had low stability during sterilization. However, Al-Hamidi *et al.* [20] reported that maximum temperatures ( $T_{max}$ ) of D-glucosamine was at 190-194 °C. Although CRBG was sterilized at 121°C, low stability of glucosamine might be related to pH lowered by glucosamine HCl addition. At acidic condition and high temperature treatment, glucosamine could be degraded or disintegrated to be other derivatives of glucosamine [20]



**Figure 1.** Relative glucosamine content (%) in canned gruel produced from red brown jasmine rice fortified with glucosamine (CRBG) sterilized at different times.

### 3.2 pH of CRBG after sterilization

Table 1. shows pH of CRBG sterilized at different times. As glucosamine used in CRBG was in an acidic form of glucosamine HCl, the pH of salt-glucosamine mixture for CRBG was observed at 5.84. After sterilization, the pH of CRBG decreased from 5.69 to 3.81 with increasing sterilization time from 5 to 120 min ( $p < 0.05$ ). The result suggested that glucosamine HCl might be dissociated at higher level of heat treatment. Xavier [21] also reported that hydrogen chloride liberated in the probe at elevated temperature.

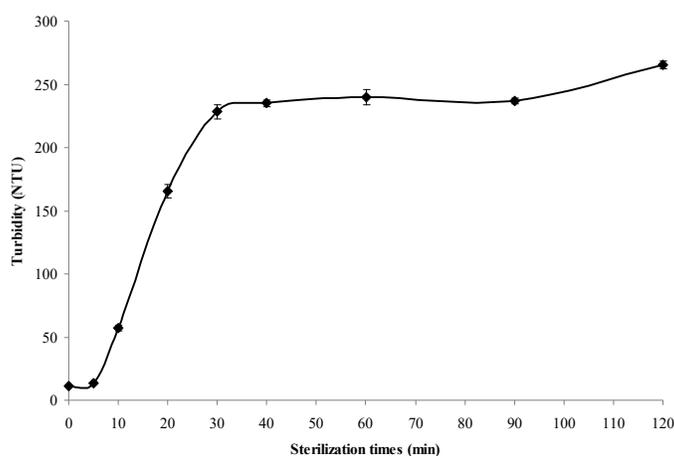
**Table 1.** pH of canned gruel produced from red brown jasmine rice fortified with glucosamine (CRBG) sterilized at different times

Sterilization times (min)	pH
0	5.84±0.03 <sup>i</sup>
5	5.69±0.04 <sup>h</sup>
10	5.54±0.12 <sup>g</sup>
20	5.28±0.01 <sup>f</sup>
30	4.79±0.02 <sup>e</sup>
40	4.49±0.03 <sup>d</sup>
60	4.31±0.02 <sup>c</sup>
90	4.20±0.03 <sup>b</sup>
120	3.81±0.01 <sup>a</sup>

Values are express as mean ± standard deviation (n = 3). Different superscript letters in the same column indicate significant differences (p<0.05).

### 3.3 Turbidity of CRBG

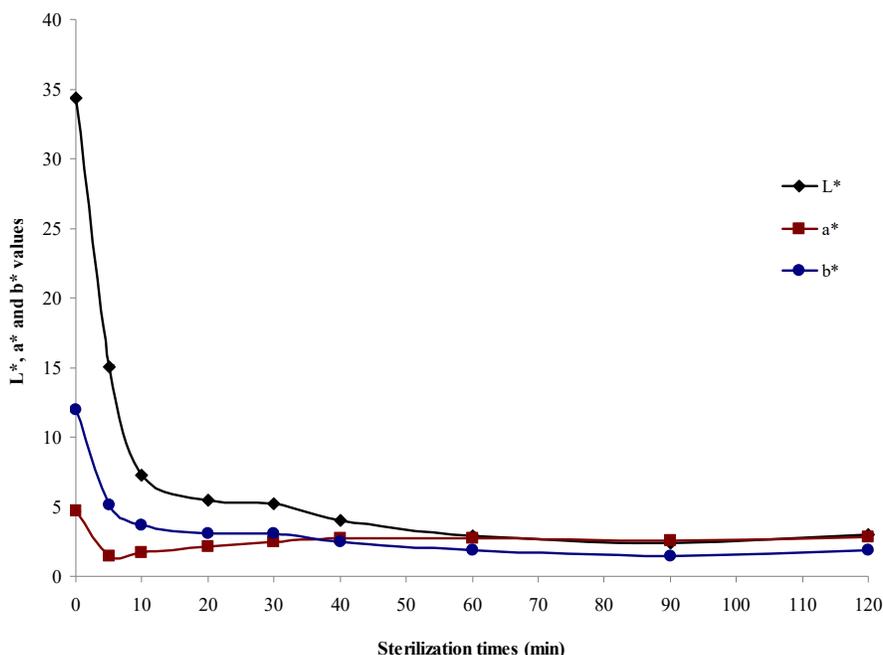
Turbidity expressed as nephelometric turbidity units (NTU) of CRBG sterilized at different times is depicted in Figure 2. Turbidity of CRBG was noticeably increased from 10 NTU to 230 NTU when sterilization time increased from 5 min to 30 min. Then, turbidity had not significantly changed when CRBG was sterilized at 30 min to 90 min (p>0.05). A slight increase in turbidity of CRBG was observed after sterilization at 120 min. With longer sterilization time, rice could greater absorb water and swell, compared to at shorter sterilization time [22]. Then, starch molecules in rice were disrupted and dissolved in water, affecting higher turbidity of CRBG. The result also indicated that CRBG was completely cooked after 30 min of sterilization.



**Figure 2.** Turbidity (NTU) of canned gruel produced from red brown jasmine rice fortified with glucosamine (CRBG) sterilized at different times.

### 3.4 Color of CRBG

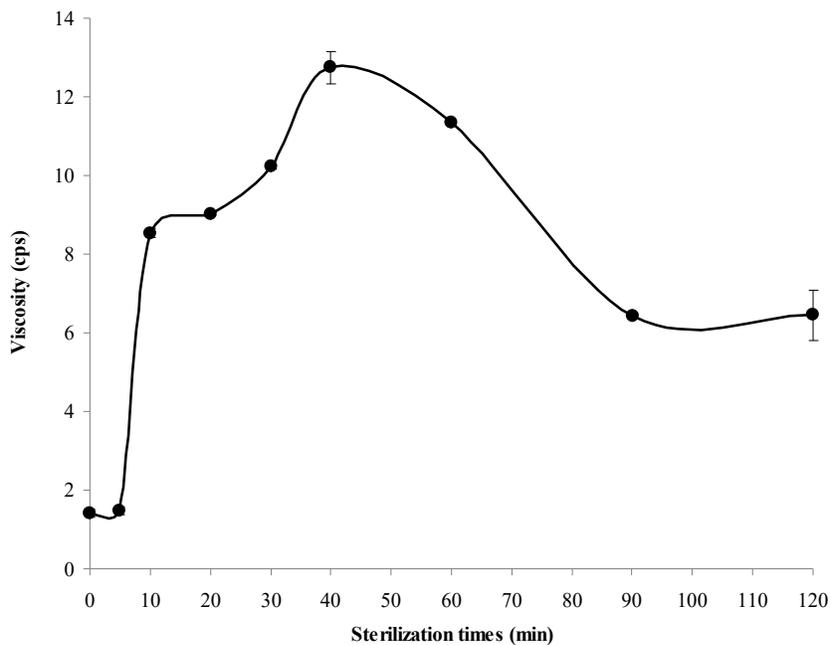
Color values of lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) of CRBG sterilized at different times are shown in Figure 3. The result showed that a sharp decreased in  $L^*$ ,  $a^*$  and  $b^*$  values of CRBG were observed at 10 min of sterilization. A slight decreased in  $L^*$ ,  $a^*$  and  $b^*$  values were detected until 40 min of sterilization. The changes in colors of CRBG was not noticeable after 60 min of sterilization ( $p>0.05$ ). As a result, the color of gruel milk from CRBG was changed from reddish pink to light brown. Thitipramote *et al.* [23] found that the major pigments in the bran layer of red jasmine brown rice are proanthocyanidins (3.168 mg CE/mg extract). Proanthocyanidins are water soluble pigment which can leach into cooking water [24]. Finocchiaro *et al.* [24] reported the major pigments (anthocyanins and proanthocyanidins) of red rice with antioxidant activities could be solubilized in cooking water.



**Figure 3.** Colors expressed as  $L^*$  (lightness),  $a^*$  (redness) and  $b^*$  (yellowness) of canned gruel produced from red brown jasmine rice fortified with glucosamine (CRBG) sterilized at different times.

### 3.5 Changes of viscosity

The viscosity of gruel milk from CRBG sterilized at different times is shown in Figure 4. The result revealed that sterilization time affected on viscosity of CRBG. The viscosity increased with increasing sterilization time from 5 to 40 min. The highest viscosity of gruel milk was found at 40 min of sterilization ( $12.75\pm 0.41$  cps) ( $p<0.05$ ). Then, sharp decrease in viscosity was noticeable. The results indicated that increasing sterilization times caused starch granules swell and absorb more water. Thus, with longer sterilization time, decreasing in free available water in the system and more viscosity took place in rice gruel [25].



**Figure 4.** Viscosity (cps) of canned gruel produced from red brown jasmine rice fortified with glucosamine (CRBG) sterilized at different times.

**Table 2.** Hardness and stickiness of canned gruel produced from red brown jasmine rice fortified with glucosamine (CRBG) sterilized at different times.

Sterilization times (min)	Hardness (g.f)	Stickiness (g.f)
0	13077.81±14.81 <sup>a</sup>	4.12±0.05 <sup>a</sup>
5	610.86±9.48 <sup>ab</sup>	9.54±0.41 <sup>b</sup>
10	483.79±0.35 <sup>bc</sup>	45.21±0.64 <sup>c</sup>
20	465.78±3.29 <sup>cd</sup>	42.38±1.31 <sup>cd</sup>
30	469.77±3.89 <sup>cd</sup>	41.68±1.66 <sup>cd</sup>
40	466.12±1.83 <sup>d</sup>	40.58±1.58 <sup>dc</sup>
60	457.24±1.95 <sup>e</sup>	42.94±0.91 <sup>ef</sup>
90	451.68±0.88 <sup>f</sup>	44.39±1.10 <sup>fg</sup>
120	445.32±1.81 <sup>g</sup>	46.45±1.18 <sup>g</sup>

Values are express as mean ± standard deviation (n = 3). Different superscript letters in the same column indicate significant differences (p<0.05).

### 3.6 Textural properties of CRBG

Textural properties of CRBG are presented in term of hardness and stickiness (Table 2). Generally, hardness of CRBG decreased with increasing sterilization times, whereas stickiness increased. This result may be due to better gelatinization for longer heating time. Tester [26] reported that during cooking, rice granules absorb water and swell greater than the uncooked rice, resulting in the granule expansion. Amylose was then leached that caused decreasing in hardness and increasing in stickiness of the gruel [26].

## 4. Conclusions

Sterilization times showed significant effects on the physicochemical properties of CRBG. Lost of glucosamine, reduction of pH value, changes in color, turbidity and viscosity were noticeable. Textural properties of CRBG were also affected by time of heat treatment during sterilization. With longer sterilization time, the rice gruel tended to be better gelatinization. The results also showed that CRBG was fully cooked at 40 min of sterilization. Therefore, canned gruel incorporated with glucosamine could be made by typical canning process and can be an alternative product for elderly or health concerning consumers.

## 5. Acknowledgements

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