Effect of Heating Temperature on Selected Properties and Shelf-life of Black Grass Jelly in Sugar Syrup in Retort Pouches

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Abstract

Black grass jelly in sugar syrup (BGJSS) is a popular dessert for quenching thirst during the summer in Thailand. However, BGJSS has a very short shelf life due to its low acidity and high moisture content; its typical storage life is less than a week in a refrigerator. This study investigated the production of BGJSS in retortable pouches in order to increase its shelf life. Sterilization in a steam-air retort at 115°C required a process time of 52.30 min with F_0 values of 5.166, while sterilization at 121°C required a process time of 38.30 min with F_0 values of 6.919. Physicochemical, microbiological, and sensory characteristics of the products were determined during storage for four months at ambient condition. Total number of microorganisms, yeasts and molds, coliform bacteria, *Escherichia coli* and *Clostridium botulinum* did not exceed food safety standards throughout storage period. Overall consumer acceptability of BGJSS in retortable pouches was not significantly different from control samples, both after the production and 4-month storage period.

ผลของอุณหภูมิในการให้ความร้อนต่อสมบัติบางประการและอายุการเก็บรักษาผลิตภัณฑ์เฉาก๊วยพร้อมดื่มในบรรจุภัณฑ์อ่อนตัว

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บทคัดย่อ

น้ำเฉาก๊วย (เจลเฉาก๊วยในน้ำเชื่อม) เป็นของหวานที่นิยมบริโภคในฤดูร้อนของประเทศไทย แต่น้ำเฉาก๊วยมีอายุการเก็บรักษาสั้นมาก เนื่องจากเป็นอาหารที่มีความเป็นกรดต่ำและมีความชื้นสูง โดยมีอายุการเก็บรักษาโดยทั่วไปน้อยกว่าหนึ่งสัปดาห์ในตู้เย็น การศึกษาครั้งนี้จึงมุ่งพัฒนาผลิตภัณฑ์น้ำเฉาก๊วยในบรรจุภัณฑ์อ่อนตัวที่มีอายุการเก็บรักษายาวนานขึ้น โดยทำการสเตอริไรส์น้ำเฉาก๊วยที่อุณหภูมิ 115 องศาเซลเซียส ซึ่งใช้เวลาในการสเตอริไลซ์ 52.30 นาที มีค่า F_0 เท่ากับ 5.166 และที่อุณหภูมิ 121 องศาเซลเซียส ซึ่งใช้เวลาในการสเตอริไลซ์ 38.30 นาที มีค่า F_0 เท่ากับ 6.919 จากการศึกษาสมบัติทางเคมีกายภาพ จุลชีววิทยา และประสาทสัมผัสของผลิตภัณฑ์ที่เก็บรักษาไว้เป็นเวลา 4 เดือน พบว่า จำนวนจุลินทรีย์ทั้งหมด ยีสต์และรา โคลิฟอร์ม เชื้ออี. โคไล และเชื้อคลอสทริเดียม โบทูลินัม ไม่เกินค่ามาตรฐานความปลอดภัยของอาหารตลอดระยะเวลาการเก็บรักษา ผลิตภัณฑ์ที่พัฒนาขึ้นได้รับการยอมรับของผู้บริโภคโดยรวมไม่แตกต่างจากตัวอย่างควบคุม ทั้งหลังการผลิตและการเก็บรักษาที่อุณหภูมิห้องเป็นเวลา 4 เดือน

1. Introduction

Black grass jelly (BGJ), or *Chao Kuay*, is a jelly-like product obtained from a liquid extract of *Mesona procumbens* Hemsley leaves. The extracted polysaccharides can synergistically form a thermoreversible gel with other polysaccharides, such as starch, agar and carrageenan. Black grass jelly in sugar syrup (BGJSS) is a popular dessert in Thailand, especially in the summer. However, BGJSS is a low-acid food with high moisture content, which has a shelf life less than a week in a refrigerator. Both spoilage and pathogenic bacteria can possibly grow under this condition. A thermal process with sealed containers is thus recommended to prevent their growth on foods [1]. Sterilization is a common thermal process used in food industry with the main objective of extending shelf life of food products and to make food safe for human consumption by destroying all pathogenic and most spoilage microorganisms [2]. Retort pouches have been widely recognized as one of the best alternatives to metal cans for producing thermally processed shelf stable foods [3]. The retortable pouch is a flexible laminated pouch that can withstand thermal processing temperatures and combines the advantages of metal can and plastic packages [4]. Retort pouches have several advantages, such as being lightweight and cheaper, shelf stability, lower storage space requirements, and ease of opening and preparation. It can also reduce heat exposure during sterilization, resulting in improved quality and packaging economy [5].

The main aim of the present study was to develop a shelf-stable BGJSS product using a retort pouch processing technique, as well as to evaluate the changes in quality attributes during storage.

2. Material and Methods

2.1 BGJ Preparation

The dried black grass (*Mesona procumbens* Hemsl) leaves were procured from a local market in Kamphaeng Phet province, Thailand. Then, 100 g of dried leaves were blanched in boiling water for 10 minutes, followed by blanching in 0.25% (w/v) sodium carbonate solution for 6 h before the filtering through a filter cloth. Afterward, the filtrate was mixed with 3% (w/v) of potato flour as a gelling agent [6]. All mixtures were poured on the stainless steel plates before cooling to a temperature of 25°C and subsequently stored in a refrigerator (~4°C).

2.2 Packaging Material

Prefabricated 15 x 20 cm multilayer laminated retortable pouches consisting of 12 μm polyester/ 15 μm nylon/9 μm aluminum foil/80 μm casted polypropylene (total thickness 116 μm) with 300 g capacity were used as the packaging material. BGJ was cut into 1 x 1 cm cubes. Each report pouch was filled with 80 g of BGJ and 120 g of 40 °Brix syrup.

2.3 Thermal Processing of BGJSS in Retort Pouch

A pilot-scale 45 × 50 × 50 cm capacity steam-air retort system (091-A, Serial number 313, FMC Food Tech, Belgium) was used for the experiment. The retort used compressed air for over-riding pressure and a high-pressure water-circulating pump for pressurized cooling [5]. The temperature of product was continuously recorded during heat processing through copper-constantan thermocouples, which were fixed at the geometric center of the pouches and connected to the Ellab CTF 9008 data recorder (Ellab A/S, Roedovre, Denmark). The retort temperature was set at 115 and 121.1°C with the pressure of 1.5 bar throughout heating and cooling cycles. The processing was carried out in order to achieve required F_0 value with a maximum temperature of 121.1 °C. After attaining required F_0 value, the product temperature was reduced to 50 - 55 °C with pressurized cooling (compressed air and water) within 4 - 5 minutes. The cooled pouches were wiped dry and examined for any visual defects.

2.4 Physicochemical Analysis of BGJSS

The BGJSS was blended before measurement and evaluated for physicochemical parameters including color, pH value and total soluble solids content. The color was measured in *L*a*b** space by HunterLab (ColorFlex, HunterLab, USA). The total soluble solids content was determined by hand refractometer (Atago, Japan). All measurements were performed in triplicate each month during the 4-month storage period.

2.5 Microbiological Analysis of BGJSS

The BGJSS was blended before measurement. Total viable count, coliform, *E. coli, Clostridium botulinum*, and yeast and mold were determined according to the Food and Drug Administration's Bacteriological Analytical Manual [7]. Two replicate tests were carried out each month during the 4-month storage period.

2.6 Sensory Evaluation of BGJSS

The sensory characteristics of samples sterilized at 115 and 121^{O}C as well as those of the commercial BGJSS samples (control sample) were determined using a 9-point hedonic scale [8]. Fifty semi-trained panelists who are familiar with BGJSS consumption were selected. The samples were packed into plastic cups with lids and kept refrigerated before serving. Samples were served with random three-digit code numbers in a random order at a temperature of 5 - 9°C. The mean hedonic scores of samples were rated by preference in comparison with the commercial BGJSS for color, odor, taste, texture and overall acceptability.

2.7 Statistical Analysis

Completely randomized design (CRD) was used for physicochemical and microbiological analyses in shelf-life study. Randomized complete block design (RCBD) was used for sensory evaluation of BGJSS after production at different temperatures compared with the commercial BGJSS. All data were calculated into means with standard deviations (means ± SD). Analysis of variance (ANOVA) was carried out using SPSS for Windows, version 11.5 (SPSS Inc., USA). Determination of significant differences among treatment means was performed by Duncan's new multiple range test (DMRT) at a 95% confidence interval.

3. Results and Discussion

3.1 Thermal Processing of BGJSS in Retort Pouch

Sterilization is a thermal process that is used to destroy vegetative cells and spores of both pathogen and spoilage microorganisms. *Clostridium botulinum* is most important pathogen in the sterilization process. It exists widely in environment and produces heat-resistant spores. In the absence of oxygen, e.g. in canned or pouched foods, the spore can germinate and excrete a lethal botulinum toxin [1]. The determining factor in selecting temperature and time for sterilization is pH of the food. The BGJSS product is a low-acid food with pH value in range of 6.30 - 7.92, which is susceptible to contamination by *C. botulinum*.

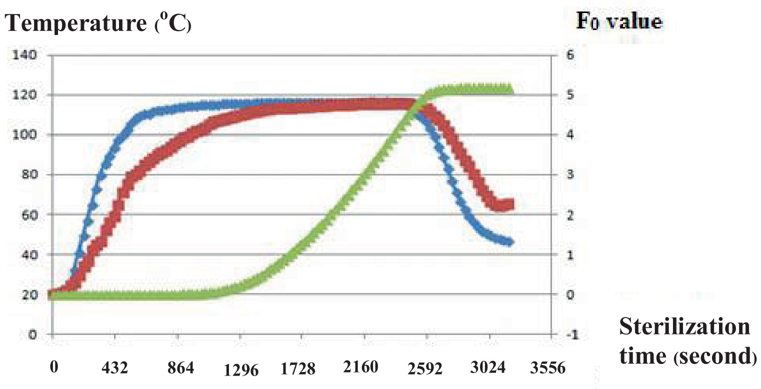
It is thus necessary to use retort processing to inactivate the microorganisms that may be present throughout entire food production process [1].

In Thailand, production of foods in hermetically sealed containers is regulated under the Notification of the Ministry of Public Health (No. 349) B.E. 2556 (2013) [9], which specifies that low-acid food (pH greater than 4.6) should be sterilized by a process equivalent to the lethality of 121°C (F_0) for at least 3 min to obtain a 10^{12} log cycles reduction (12-D process) of *C. botulinum* spores. This research determined that a F_0 value of more than 4 min should be obtained in order to ensure safety of consumers throughout shelf life at room temperature.

The purpose of heat penetration study is to evaluate heating and cooling behavior of the product/package combinations in a specific retort system for establishment of a safe thermal process. The heat penetration characteristics and F_0 value of BGJ in BGJSS in retort pouches are shown in Figure 1.

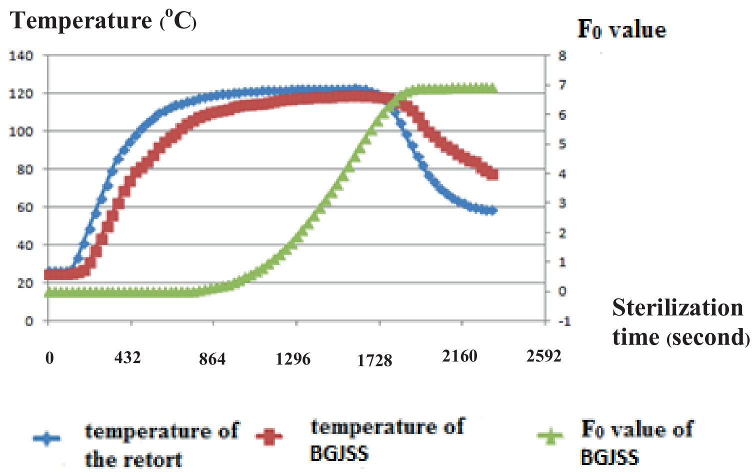
BGJSS temperature slowly increase due to low thermal conductivity and multilayer laminated retort pouches (polyester, nylon and polypropylene). The F_0 value increased as temperature of the retort and internal product temperature increased. At the end of heating process, the F_0 value for sterilization at 115°C was 5.166 min with a total process time of 52.30 min, while the F_0 value for sterilization at 121°C was 6.919 min with a total process time of 38.30 min. After heat processing, the pouches were cooled rapidly by circulating water. This sudden cooling prevented over-cooking and growth of surviving thermophiles.

BGJSS sterilization temperature was similar to the sterilization temperature of other foods, which is inversed to the time of sterilization with the equal or similar to the F_0 value. Tribuzi et al. [10] studied processing of chopped mussel meat in retort pouches at different retort temperature with same F_0 value of 7 min and found that sterilization at 110°C required a longer process time (91.9 min) compared to that using 121°C (17.9 min) [10]. Similarly, Sreenath et al. [11] studied processing of Indian mackerel canned in brine at different retort temperatures with same F_0 value of 8 min and found that sterilization at 115°C required a longer total processing time (60 min) compared to that with processing at 121.1°C (32.7 min) [11]. Therefore, the F_0 value for processing at 115°C was lower than processing at 121°C.



(A)

Figure 1 Sterilization process of BGJSS in retort pouch at 115°C (A) and at 121°C (B)



(B)

Figure 1 Sterilization process of BGJSS in retort pouch at 115°C (A) and at 121°C (B) (Continue)

3.2 Physicochemical Characteristics of BGJSS During Storage

The pH and total soluble solids content of BGJSS in retort pouches sterilized at 115°C and 121°C during storage for 4 months at ambient temperature (27 - 30°C) are shown in Table 1.

During storage, the pH values of BGJSS processed at 115°C were between 7.75 and 7.91, which is still considered as a low-acid food. The pH value declined slightly with storage time. Sterilized food products are usually safe from microbial spoilage as the main quality deterioration is due to chemical reactions. The changes in pH value may be due to an increase of free fatty acids, which are also found in other pouched food products [5].

The pH values of BGJSS processed at 121°C were between 6.58 to 7.29. The values were lower than those processed at 115°C, but they were still in the range of low-acid foods. However, the pH was relatively constant during storage. The mild alkaline condition of BGJSS was due to sodium carbonate used in extraction of polysaccharides from the black grass leaves. The lower pH value at a higher sterilization temperature may be due to degradation of sugar under alkaline condition into various compounds, including sugar acids, which neutralized alkalinity of product [12].

Total soluble solids (TSS) of BGJSS processed at 115°C was between 26.6 to 31.3°Brix. During preparation, an initial 80 g of BGJ without added sugar was combined with 120 g of 40°Brix sucrose syrup prior to packing in retortable pouches. The initial cut-out Brix of BGJSS processed at 115 and 121°C were 27.7 and 27.3°Brix, respectively. Osmosis of sucrose from the syrup to the BGJ caused a decrease in TSS values of the syrup. The cut-out Brix of BGJSS processed at 115°C decreased slightly during storage, but the values did not change for those processed at 121°C. During storage, the TSS values of BGJSS processed at 121°C were between 25.70 and 27.70°Brix, which were less than those processed at 115°C. This was probably because higher sterilization temperature enhanced mass transfer of osmosis process to an equilibrium condition, while those processed at 115°C reached equilibrium during storage. This is similar to Dario (2019), who reported that the osmosis process is dependent on temperature and that osmosis rate is very strong when the temperature is increased; likewise, the temperature indicates effectiveness of osmosis system [13].

The variations in color of BGJSS in retort pouches during storage are shown in Table 2. The BGJSS was blended before measurement of color. The initial *L** value of BGJSS processed at 115°C was 19.29, which was darker than that processed at 121°C (*L** 16.50). During storage, the *L** of BGJSS processed at 115°C was not significantly changed, but the values of that processed at 121°C increased from 16.50 to 19.32. However, *L** of both sterilization temperatures were not significantly different at the end of storage period.

*a** indicates red color for a positive value and green color for a negative value. *a** of BGJSS processed at 115°C and 121°C tended to decrease slightly during storage, which implies that the jelly became redder. The *b** indicated yellow color for *a** positive value and blue color for a negative value. The initial *b** value of BGJSS processed at 115°C was lower than that processed at 121°C. However, *b** increased during storage for both sterilization temperatures. The decrease of *a** and increase of *b** values suggest that non-enzymatic browning occurred during storage at ambient temperature [12].

The increase of brown color corresponded with a decrease in pH and TSS values observed in the Maillard reaction. The non-enzymatic browning reactions were caused by caramelization and Maillard reaction, with different starting substances. Caramelization of BGJSS occurred in sugar syrup. Sterilization at high temperature caused thermolysis and polymerization of sugar molecules, which formed brown pigments [15]. The sugar was degraded into sugar acids, which could reduce pH and simultaneously form the brown-pigment hydroxymethylfurfural (HMF). Moreover, oxidation of tannins, which were coextracted with polysaccharides, could be another cause of non-enzymatic browning [15]. A similar trend was also found in carrot juice during storage [16].

Table 1 pH and total soluble solids of BGJSS in retort pouch during storage

Shelf life (months)	pH		Total soluble solids (°Brix)	
	115°C	121°C	115°C	121°C^{ns}
0	7.91±0.03^{a}	7.29±0.03^{a}	27.7±0.6^{bc}	27.3±0.6
1	7.80±0.06^{bc}	7.24±0.17^{a}	31.3±1.2^{a}	27.7±0.6
2	7.86±0.06^{ab}	7.19±0.02^{a}	28.8±0.3^{b}	25.7±0.6
3	7.80±0.02^{bc}	6.58±0.26^{b}	29.0±1.0^{b}	26.3±1.5
4	7.75±0.05^{c}	7.07±0.31^{a}	26.6±0.9^{c}	27.3±1.5

The number with the same alphabet (a - c) indicated no significant different ($p > 0.05$) for comparison between different rows of the same columns; ns = non-significant.

Table 2 Color of BGJSS in retort pouch during storage.

Shelf life (month)	Color					
	*L**		*a**		*b**	
	115°C	121°C	115°C	121°C	115°C	121°C
0	19.29±0.27[a]	16.50±0.34[d]	-0.27±0.04[a]	-0.24±0.03[a]	0.62±0.03[d]	0.84±0.06[b]
1	19.08±0.60[b]	17.54±0.54[c]	-0.37±0.01[b]	-0.34±0.04[b]	0.93±0.04[c]	0.86±0.03[b]
2	19.19±0.27[a]	18.29±0.47[b]	-0.38±0.01[b]	-0.39±0.06[b]	0.84±0.06[b]	0.84±0.03[b]
3	19.24±0.18[a]	19.32±0.29[a]	-0.23±0.04[a]	-0.25±0.04[a]	0.93±0.03[c]	0.99±0.05[a]
4	19.22±0.08[a]	19.22±0.06[a]	-0.34±0.01[b]	-0.20±0.01[a]	1.05±0.04[a]	0.99±0.03[a]

The number with the same alphabet (a - d) indicated no significant different (p > 0.05) for comparison between different rows of the same columns.

3.3 Microbiological Analysese

Microbiological analyses of BGJSS during 4-month storage are shown in Table 3. It was found that the total plate count (TPC) and yeast and mold count of BGJSS processed at 115°C and 121°C were less than 10 CFU/ml throughout storage period. In addition, coliforms and *E. coli* were less than 3 MPN/ml for four months. These microorganisms were not able to grow under the anaerobic condition within the retortable pouches. The value obtained also indicated that there was no leakage in the pouches during processing and storage. *Clostridium botulinum* was also absent in BGJSS processed at both temperatures during 4-month storage period.

The microbiological results conformed with standards for food in hermetically sealed containers [17]. The law specifies that food should have total microorganisms of not more than 1,000 CFU/g during storage at 37°C or 55°C, yeast and mold of not more than 100 CFU/g, and coliforms at less than 3 MPN/g. Therefore, the sterilization processes in this study were able to destroy all pathogens and the products were safe for human consumption.

Table 3 Microbiological analyses of BGJSS in retort pouch during storage.

Shelf life (month)	TPC (CFU/ml)		Yeast & mold (CFU/ml)		Coliforms (MPN/ml)		*E. coli* (MPN/ml)	
	115 °C	121 °C	115 °C	121 °C	115 °C	121 °C	115 °C	121 °C
0	<10	<10	<10	<10	<3	<3	<3	<3
1	<10	<10	<10	<10	<3	<3	<3	<3
2	<10	<10	<10	<10	<3	<3	<3	<3
3	<10	<10	<10	<10	<3	<3	<3	<3
4	<10	<10	<10	<10	<3	<3	<3	<3

3.4 Sensory Characteristics

After production, consumer acceptance of the BGJSS processed at 115°C was not significantly different from that of the commercial product (Table 4). However, the value was less than control for BGJSS processed at 121°C. After storage for four months, consumer acceptance of BGJSS processed at 115°C was also not significantly different from that of the commercial product in most criteria, except texture (Table 5). For BGJSS processed at 121°C, the sensory score was lower than other samples in all criteria. The sensory evaluation suggests that processing BGJSS at higher temperatures has a significant effect on its qualities. BGJSS processed at 121°C had a darker syrup and faded-color jelly because the black pigment of BGJ dissolved into the syrup during heat treatment. The mass transfer of sugar also occurred between syrup and BGJ. During sterilization and storage, the sugar in the syrup migrated into the gel, making the jelly sweeter. However, the panelists preferred the less sweet jelly. The taste score of sterilized products was therefore less than that of the pasteurized commercial product. The lower texture score was also observed in sterilized BGJSS due to water leakage by osmotic pressure, which caused the product to become sticky and lack firmness.

Table 4 Sensory evaluation scores of BGJSS after production

Product	Preference scores				
	Color^{ns}	Odor^{ns}	Taste	Texture	Overall acceptability
Control	7.2 ± 1.3	6.5 ± 1.6	6.8 ± 1.3^{a}	7.1 ± 1.4^{a}	6.9 ± 1.4^{a}
BGJSS (115°C)	7.0 ± 1.2	6.2 ± 1.6	6.5 ± 1.7^{ab}	6.7 ± 1.7^{ab}	6.6 ± 1.3^{ab}
BGJSS (121°C)	6.9 ± 1.5	6.0 ± 1.5	6.2 ± 1.4^{b}	6.3 ± 1.6^{b}	6.2 ± 1.3^{b}

The number with the same alphabet (a - c) indicated no significant different ($p > 0.05$) for comparison between different rows of the same columns; ns = non-significant.

Table 5 Sensory evaluation scores of BGJSS after storage for 4 months

Product	Preference scores				
	Color^{ns}	Odor^{ns}	Taste	Texture	Overall acceptability
control	6.5 ± 1.0^{a}	6.4 ± 1.3^{a}	6.7 ± 0.7^{a}	7.2 ± 0.7^{a}	6.9 ± 0.7^{a}
BGJSS (115°C)	6.3 ± 0.8^{a}	6.1 ± 0.9^{ab}	6.4 ± 0.8^{a}	6.3 ± 1.0^{b}	6.7 ± 0.7^{a}
BGJSS (121°C)	5.7 ± 0.9^{b}	5.7 ± 1.1^{b}	5.7 ± 0.8^{b}	5.8 ± 1.0^{c}	6.0 ± 0.8^{b}

The number with the same alphabet (a - c) indicated no significant different ($p > 0.05$) for comparison between different rows of the same columns; ns = non-significant.

4. Conclusions

The sterilization process of BGJSS in retort pouches could be used for commercial production. The F_0 values for sterilization at 115°C for 52.30 min and 121°C for 38.30 min were 5.166 and 6.919 min, respectively. The microorganisms of BGJSS were within the legal limits during 4-month storage period for both sterilization temperatures. However, the BGJSS processed at 115°C had a higher level of consumer acceptance than that processed at 121°C

5. Acknowledgments

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