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อิทธิพลของกรรมวิธีการแปรรูปด้วยความร้อนและบรรจุภัณฑ์ต่อคุณลักษณะทางกายภาพ เคมี และจุลชีววิทยา ของกาแฟสกัดเย็นสายพันธุ์โรบัสต้าผสมน้ำมะพร้าวพร้อมดื่ม

Influence of Thermal Treatments and Packaging on Physicochemical and Microbiological Characteristics of Ready-to-Drink Cold Brew Coffee *cv*. Robusta Blended with Coconut Water

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บทคัดย่อ

ปัจจุบันความนิยมในการบริโภคกาแฟสกัดเย็นมีแนวโน้มเพิ่มสูงขึ้น งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาหาวิธีในการยืดอายุการเก็บรักษาและรักษาคุณภาพของกาแฟสกัดเย็นสายพันธุ์โรบัสต้าผสมน้ำมะพร้าวพร้อมดื่ม โดยศึกษากรรมวิธีการให้ความร้อนสองกรรมวิธีคือ กรรมวิธีที่ 1: การสเตอริไลซ์บรรจุในขวดแก้ว ให้ความร้อนที่ 121ºC, 15 นาที กรรมวิธีที่ 2: การพาสเจอร์ไรซ์บรรจุในถุงลามิเนตเคลือบอลูมิเนียมฟอลย์ให้ความร้อนที่ 65ºC, 30 นาที โดยเมล็ดกาแฟบดหยาบจะถูกนำมาสกัดในน้ำดื่มที่อุณหภูมิห้องเป็นเวลา 6.5 ชั่วโมง ก่อนนำมาผสมกับน้ำมะพร้าว (75:25) บรรจุลงในภาชนะบรรจุแล้วนำไปให้ความร้อน ศึกษาการเปลี่ยนแปลงคุณภาพทางกายภาพ เคมี และจุลชีววิทยาในระหว่างการเก็บรักษาที่อุณภูมิ 25±2ºC สำหรับกรรมวิธีที่ 1 และที่อุณหภูมิ 4±1°C สำหรับกรรมวิธีที่ 2 เป็นเวลา 2 เดือน พบว่า ปริมาณคาเฟอีน (89.42 mg/100mL) ปริมาณสารประกอบฟีนอลลิกทั้งหมด (4.59 mg GAE/mL) และเปอร์เซ็นต์ในการต้านอนุมูลอิสระ DPPH (59.35%) ในกาแฟสกัดเย็นสเตอริไลซ์มีปริมาณลดลงอย่างมีนัยสำคัญทางสถิติ ($p \leq 0.05$) เมื่อเปรียบเทียบกับการ พาสเจอร์ไรซ์ที่มีปริมาณเท่ากับ 92.34 mg/100mL, 6.00 mg GAE/mL and 70.25% ตามลำดับ ค่าสีของกาแฟสกัดเย็น ทั้งสองกรรมวิธีมีค่าความสว่าง (L*) ลดลง และมีค่าความแตกต่างของสี (ΔE) เพิ่มขึ้นเมื่อเปรียบเทียบกับวันเริ่มต้นของการเก็บรักษา และค่าพีเอชของกาแฟสกัดเย็นที่ผ่านกรรมวิธีการสเตอริไลซ์จะมีความเป็นกรดมากกว่ากรรมวิธีพาสเจอร์ไรซ์โดยพีเอชมีค่าลดลงเมื่อระยะเวลาในการเก็บรักษานานขึ้น ผลการทดสอบทางจุลชีววิทยา (Aerobic plate count, *Escherichia coli* และ *Bacillus cereus*) ใช้ยืนยันความปลอดภัยสำหรับกาแฟสกัดเย็นได้ โดยทั้งสองตัวอย่างมีจำนวนไม่เกินเกณฑ์มาตรฐานตลอดระยะเวลาการเก็บรักษา ผลการศึกษาแสดงให้เห็นว่ากรรมวิธีการแปรรูปโดยใช้ความร้อนสามารถรักษาปริมาณสารออกฤทธิ์ทางชีวภาพได้โดยเฉพาะอย่างยิ่งกรรมวิธีพาสเจอร์ไรซ์และกาแฟมีความเป็นกรดน้อยกว่ากรรมวิธีสเตอริไลซ์

คำสำคัญ : กาแฟสกัดเย็น ; เครื่องดื่มพร้อมบริโภค ; กายภาพ-เคมี ; จุลชีววิทยา

Abstract

The popularity of cold brew coffee consumption tends to increase nowadays. This study aimed to investigate the stabilization techniques for prolonging the quality and shelf-life of ready-to-drink cold brew coffee *cv.* Robusta blended with coconut water. The two common thermal techniques, Processing 1: Sterilization, packed in a glass bottle and sterilized at 121ºC for 15 min, and Processing 2: Pasteurization, packed in aluminum foil laminated pouch and heated at 65ºC for 30 min, were evaluated. Coarse grinding coffee beans were extracted with room temperature drinking water for 6.5 hours and then mixed with coconut water (75:25) before being packed and undergoing thermal treatment. Changes in physicochemical and microbiological characteristics of cold brew beverages were observed for 2-month storage at 25±2ºC for processing 1, and 4±1ºC for processing 2. An initial pH of the sample undergoing sterilization (4.78-5.00) showed more acidic than the pasteurization technique (5.41-5.57) and decreased with storage time. Sterilized coffee beverage resulted significantly declined ($p \leq 0.05$) in caffeine content (89.42 mg/100mL), total phenolic acid (4.59 mg GAE/mL), and percentage of DPPH radical scavenging activity (59.35%) compared to pasteurization method, with data being 92.34 mg/100mL, 6.00 mg GAE/mL, and 70.25%, respectively. Lightness (L*) of both treatments decreased during storage and showed a dramatic change in ΔE. The results of the microbial evaluation (Aerobic plate count, *Escherichia coli*, and *Bacillus cereus*) guaranteed food safety risk by the value was within the standard criteria throughout storage periods. The results revealed that all treatments preserved bioactive compounds, especially the pasteurization method which was less acidic than sterilization counterparts.

Keywords : cold brew coffee ; ready-to-drink ; physicochemical ; microbiological

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Introduction

Coffee is one of the world's popular beverages for a reason of unique taste and flavor. In Thailand, the total value of the coffee business was higher than 42 billion baths in 2020, which was divining by 9.7% of fresh-brewed coffee and 90.3% of instant coffee (Food Intelligence Center, 2020). Caffeine in coffee beverages showed desirable effects in improving an individual's cognitive abilities and reducing fatigue and sleepiness. Coffee contains numerous bioactive substances which help lower incidence disorders such as neurodegenerative disease, cardiovascular disease, cancer, type 2 diabetes, liver disease, and metabolic syndrome (Olechno *et al.*, 2021). The most famous coffee varieties among consumers are Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*) which exhibit distinctive taste and flavor. Arabica bean coffee contains half of the caffeine content of the Robusta variety with a sweeter and more pleasant taste (Ghosh, 2014). The Robusta beans are grown on a large scale in the south of Thailand, e.g., Chumphon, Surat Thani, Ranong, Phang-Nga, Krabi, and Nakhon Si Thammarat. Domestic processing of Robusta beans is mainly in the form of a canned coffee drink or instant coffee (Puff & Chamchumroon, 2003).

Because of the popularization of cold brew coffee nowadays, there is an opportunity to develop a cold extraction coffee made from Robusta beans into a ready-to-drink beverage for responding to the greater demand for a versatile drinking product. This beverage has a pleasant-tasting (mellowness or softness), characteristic flavor, and aroma (Olechno *et al.*, 2021). Cold-brew is a brewing method, not a finished product, prepared at low temperature (20 to 25ºC or lower) for a longer immersion time (3 to 24 hours) than a conventional hot brewing method (Rao *et al.*, 2020; Bellumori *et al.*, 2021). Many techniques are used to prepare cold brew coffee, such as drip filtration, cold press, or full immersion (Kwok *et al.*, 2020). However, a long extraction time can cause an enhancement of microbial growth (Kwok *et al.*, 2020; Claassen *et al.*, 2021). Moreover, the pH of cold brew coffee is lower than its hot brew counterpart which seems to inherent food safety risks (Rao & Fuller, 2018).

Cold brew coffee is usually consumed immediately. However, in recent years many commercial traders have gained the ability to invent ready-to-drink cold brew coffee that is packaged for long-term storage (Bellumori *et al.*, 2021). The coffee extract obtained from the cold brewing process showed a reduction in acidity, a brown compound, and total dissolved solids while caffeine content and total caffeoylquinic acid (CQA) were similar in both hot and cold brew coffee (Rao *et al.*, 2020). Moreover, the coffee extracted can be incorporated with other ingredients to create a new recipe, for example, milk (Ikeda *et al.*, 2018), oat milk (ST**Ō**K®), almond milk (ST**Ō**K®), orange juice, and coconut water (Kokomio®) as a ready-to-drink product. Thus, the utilization of subtropical fruit growing locally in the South region, especially coconut in terms of mixed with coffee extract, seems to be of interest. However, there has been evidence of fresh coconut water involving the support of microbial

growth; *Escherichia coli*, and *Klebsiella pneumonia* (Awua *et al*., 2012). Thus, the application of thermal processing technology to preserve quality and extend shelf-life is needed. The integrated thermal treatment with chemical additives is already applied in manufacturing for preserving fresh coconut water before bottling, canning, or hot filling (Prades *et al*., 2012).

Thermal food sterilization and pasteurization are the most common techniques for prolonging food shelf-life. It is inactivated microorganisms and enzyme activity caused food deterioration. The terms of sterilization mean that an application of heat above 100ºC aimed to eliminate bacteria results in a stable shelf-life (Holdsworth & Simpson, 2016). On the other hand, pasteurization has a target to destroy a disease-causing microorganism in which food is exposed to heat below 100ºC (Holdsworth & Simpson, 2016). Therefore, a pasteurized product must be kept under refrigerator temperature throughout the storage period. Bellumori *et al*. (2021) studied five different methods (pasteurization, microfiltration, UV irradiation, high-pressure processing (HPP) and blast chilling) for preserving cold brew coffee, and suggested that pasteurization and HPP have the potential to maintain phytochemicals (caffeine and chlorogenic acids) and microbiological safety during four-month storage. However, there is lacked data for the development of cold brew beverage derived from the Thailand Robusta bean. Thus, the objective of this research was to determine the effect of different thermal treatments and packaging on physicochemical and microbiological characteristics of ready-to-drink cold brew coffee *cv*. Robusta blended with coconut water.

Methods

1. Raw material

Dark roast coffee bean *cv*. Robusta obtained from Community Enterprise in Chumphon province, Thailand. The sample was packed in an aluminum foil bag and transferred by a local postal transport at room temperature to the laboratory for experimentation. Coconut water was prepared from a commercial maturity stage of Sweet Young coconut (*Cocos nucifera* Linn), purchased from a local market, and then kept at $4\pm1^{\circ}$C until used within 2 hours.

2. Cold brew coffee preparation

The preparation of cold brew coffee was followed by the method described by Angeloni *et al*. (2018) with minor modifications. The coarse grinding of the coffee bean was extracted with drinking water in a ratio of 1:10 for 6.5 hours followed by filtered to remove a coffee ground using a cheesecloth. The cold brew extract was mixed with coconut water (75:25) before being packed in two types of packaging, 1) clear glass bottle with metal lid (200 mL) and 2) standup aluminum foil pouch with cap (200 mL). Glass bottle was chosen for sterilization because an inert property and withstand heat and pressure. The sterilized condition was 121ºC for 15 min using a retort before

cooling to reduce the temperature to around 40ºC in tap water (processing 1). The excessed water at the bottle surface was allowed to evaporate by place at room temperature for 2 hours. For the pasteurization method, samples packed in an aluminum foil pouch were heated at 65ºC for 30 min (processing 2) (Bellumori *et al*., 2021). A sample obtained from processing 1 was stored at room temperature (25±2ºC), while the processing 2 was kept in a stainless steel chiller (Sanden Intercool, Thailand) at 4±1ºC for 2 months, respectively. The physical, chemical, and microbiological properties were investigated during storage periods with a 1-month interval.

3. Physical properties

The color of cold brew coffee was measured using a colorimeter (ColorQuest XE, HunterLab, USA) with total transmittance mode (TTRANS), and calibrated with a black card, distilled water, and while standard before measurement. A beverage sample (approximately 30 mL) at room temperature was poured into a transmittance cell for monitoring a color. The color was expressed as L*, a*, and b* values. L* for the lightness which ranged from black (0) to white (100), a* from green (-) to red (+), and b* from blue (-) to yellow (+). For total color change (ΔE) of the cold brew sample (L*, a*, and b*) compared with the color value at initial storage (L_0, a_0, and b_0) was calculated using equation (1).

$$\Delta E=\sqrt{(L^*-L_0)^2(a^*-a_0)^2(b^*-b_0)^2} \qquad (1)$$

4. pH

The changes in coffee brew pH during storage periods were measured using a pH meter (F20; Mettler Toledo, Switzerland). The fifty milliliters of cold brew sample with three replications were used in experiments performed at 25$\pm$1^{o}C.

5. Caffeine content

The caffeine content of coffee brew was examined according to ISO 20481 (2008) as recommended by Gopinandah *et al*. (2014) using high-performance liquid chromatography (HPLC; Shimazdu, Tokyo, Japan) using a reversed-phase C18 column with UV detector at 272 nm. Methanol (24%) was used as a mobile phase under isocratic elution mode. Briefly, the exact weight of the sample in the presented magnesium oxide (5 g) was mixed with 200 mL deionized water and then incubated at 90ºC for 20 min in a water bath, followed by filtered through 0.2-micron nylon filter before injecting to HPLC system. Caffeine content was calculated against the calibration curve and expressed in mg/100mL sample.

6. Total phenolic

Total phenolic content was determined by the Folin Ciocalteu method described by Ngamsuk *et al*. (2019) with slight modification. The 100 µL of cold brew coffee diluted with ethanol (1:10) was mixed with 500 µL folin reagent (tenfold dilution), 400 µL sodium carbonate (0.7 M) in a test tube and then placed in a dark environment for 30 min. After that, the absorbance was measured at 756 nm using a spectrophotometer (UV-1700, Bara Scientific, Thailand). Total phenolic content was calculated using an equation obtained from the calibration curve of gallic acid and expressed in terms of gallic acid equivalents (GAE), in milligram per milliliter sample.

7. Antioxidant activity

DPPH (2, 2 - diphenyl – 1 – picryl –hydrazyl - hydrate) radical scavenging assay in accordance with a recommendation of Choi and Koh (2017) was used to predict the antioxidant activity. One milliliter of sample was mixed with 2 mL of DPPH solution (0.2 mmol/L) and then allowed to stand in the dark at 25ºC. The absorbance of mixed samples was measured at 517 nm (A_{sample}) using a spectrophotometer (UV-1700, Bara Scientific, Thailand) compared to a blank (A_{blank}) which used 1mL of ethanol replacing coffee extract. The percentage of DPPH radical scavenging activity was calculated using equation (2)

$$\text{DPPH radical scavenging activity (\%)} = (A_{blank} - A_{sample}/A_{blank}) \times 100 \qquad (2)$$

8. Microbiological evaluation

The microbial evaluation was evaluated according to the standard of "coffee beverage in a hermetically sealed container" (Ministry of Public Health, 2020) and iced coffee (Thai Community Product Standard, 2011). Aerobic plate count (FDA BAM Online, 2001), *Escherichia coli* (APHA, 2017) and *Bacillus cereus* (FDA BAM Online, 2001) of cold brew coffee mixed with coconut water undergo different processing were investigated during storage periods.

9. Statistical analysis

A Completely Randomized design (CRD) with two treatments (processing 1 and processing 2) was used for this experiment. All experiments were done in triplicate. Data were analyzed using a statistical program (SPSS V.26; IBM, Ontario, Canada) for analysis of variance at $p \leq 0.05$. An independent t-test was used to compare mean values to determine the difference between treatments. The difference in the mean value of each parameter during the storage periods (2-month) was examined using Duncan's multiple range test (DMRT).

Results

1. Color

The production of cold brew coffee mixed with coconut water by different thermal techniques: (1) packed in a glass bottle and sterilized at 121ºC for 15 min (processing 1), (2) packed in aluminum foil laminated pouch and heated at 65ºC for 30 min (processing 2) on color are shown in Table 1. The appearance color of both samples was black, indicated by a lower L* value (7.61 and 5.00) and not significantly different ($p>0.05$) between treatments. During storage, the lightness (L* value) decreased while a* and b* increased, experiencing that both samples had higher intensity of red and yellow shade compared to initial storage. The overall color change was confirmed by a total color difference (ΔE) which was greater distinct from the first month when the storage time increased. Sterilization treatment (processing 1) led to the coffee beverage turning discoloration than pasteurization counterpart (processing 2), however, no statistical difference ($p>0.05$) between treatments. A discoloration is involved with the occurrence of the Maillard reaction induced by thermal treatment.

Table 1 Changes in color values (L*, a*, b* and ΔE) of cold brew coffee mixed with coconut water with different processing.

Month	L*	a*	b*	ΔE
A. Processing 1				
0^{ns}	7.61 ± 0.02^{A}	-4.06 ± 0.24^{C}	-2.71 ± 0.05^{C}	-
1^{ns}	1.64 ± 0.03^{B}	-0.60 ± 0.18^{B}	1.01 ± 0.14^{B}	76.84
2^{ns}	0.64 ± 0.02^{C}	1.60 ± 0.11^{A}	2.18 ± 0.25^{A}	192.91
B. Processing 2				
0^{ns}	5.00 ± 0.05^{A}	-4.25 ± 0.12^{C}	-3.18 ± 0.01^{C}	-
1^{ns}	1.13 ± 0.06^{B}	0.04 ± 0.03^{B}	1.13 ± 0.19^{B}	71.56
2^{ns}	1.05 ± 0.01^{C}	1.54 ± 0.15^{A}	2.16 ± 0.13^{A}	122.13

Data expressed as mean ± SD. $^{A-C}$ Different superscripts in the same column of each processing indicate significant differences ($p\leq0.05$). ^{ns} No significant differences between experimental units (processing method) within the same month.

2. pH

An initial pH value of cold brew coffee mixed with coconut water undergoes sterilizing method (processing 1) resulted in a significantly lower (5.00±0.01) than pasteurizing method (5.57±0.01) (processing 2) as

shown in Table 2, classified as a low acid food. The pH of both treatments showed continuously declined ($p \leq 0.05$) throughout the storage periods. For sterilized beverages, a minimal change (0.6% reduction) was observed after 1-month storage, while the greater depletion (4.4%) was found after 2-month. A reduction of pH values correlated with the development of acidic taste detected by sensory evaluation (data not shown), with the table being 4.78 (processing 1) and 5.41 (processing 2), respectively. The pH dropped around 4.4 and 2.9% for processing 1 and 2, respectively, at the end of the storage. Thus, the experiment for the shelf-life longer than 2-month was not performed for sterilized beverages due to the development of sour taste which is unacceptable for panelists (data not shown).

Table 2 Changes in pH and caffeine content of cold brew coffee mixed with coconut water with different processing.

Month	pH		caffeine content (mg/100mL)	
	Processing 1	Processing 2	Processing 1	Processing 2
0	$5.00 \pm 0.01^{b,A}$	$5.57 \pm 0.01^{a,A}$	$89.42 \pm 2.32^{b,A}$	$92.34 \pm 1.19^{a,A}$
1	$4.97 \pm 0.01^{b,B}$	$5.41 \pm 0.02^{a,B}$	$81.34 \pm 2.87^{ns,B}$	$83.18 \pm 2.65^{ns,B}$
2	4.78 ± 0.01^{C}	-*	80.43 ± 2.50^{B}	-*

Data expressed as mean ± SD. $^{A-C}$ Different uppercase superscripts in the same column of each processing indicate significant differences ($p \leq 0.05$). $^{a-c}$ Different lowercase superscripts indicate significant differences ($p \leq 0.05$) between experimental units (processing method) within the same month. ^{ns} No significant difference between experimental units (processing method). *not analyzed since the expiry of the shelf-life.

3. Caffeine content

The caffeine content of cold brew coffee blended with coconut water is shown in Table 2. Caffeine content was affected by different thermal processing which was significantly reduced ($p \leq 0.05$) after exposure to high-temperature operation (processing 1; 89.42 mg/100mL) compared to low heat treatment counterpart (processing 2; 92.34 mg/100mL). However, it did not exceed 100 mg/100mL, which is the standard for the amount of caffeine in iced coffee (Thai Community Product Standard, 2011). After 1-month storage, the caffeine content in both treatments decreased (9.04% for sterilized beverage, 9.92% for pasteurized beverage) but did not show significantly different ($p>0.05$) between treatments. It was reduced by around 10% at the end of the storage (2 months for sterilized beverage and 1 month for pasteurized beverage).

4. Total phenolic and antioxidant activity

Total phenolic content and percentage of the inhibition on DPPH radical of cold brew coffee blended with coconut water with different processing are shown in Figure 1 (A and B). The result showed that the beverage obtained from processing 2 (pasteurization method) presented a greater amount of phenolic content and antioxidant activity by 6.00 mg GAE/mL sample and 70.25% (Figure 1B), while the processing 1 (sterilization method) sample contained 4.59 mg GAE/mL sample and 59.25%, respectively (Figure 1A). However, phenolic content and antioxidant activity in both beverage samples were declined noticeably during storage periods, especially processing 1 which showed the percent reduction by 16 and 44%, and 15 and 17%, after storage for 1 and 2 months, respectively. The phenolic content and antioxidant activity in beverage obtain from processing 2 showed the percent reduction by 7 and 29%, and 3 and 11%, after storage for 1 and 2 months, respectively. The observation revealed that the level of heat treatment and contact time were affected by the loss of phenolic compounds and their antioxidant efficacy.

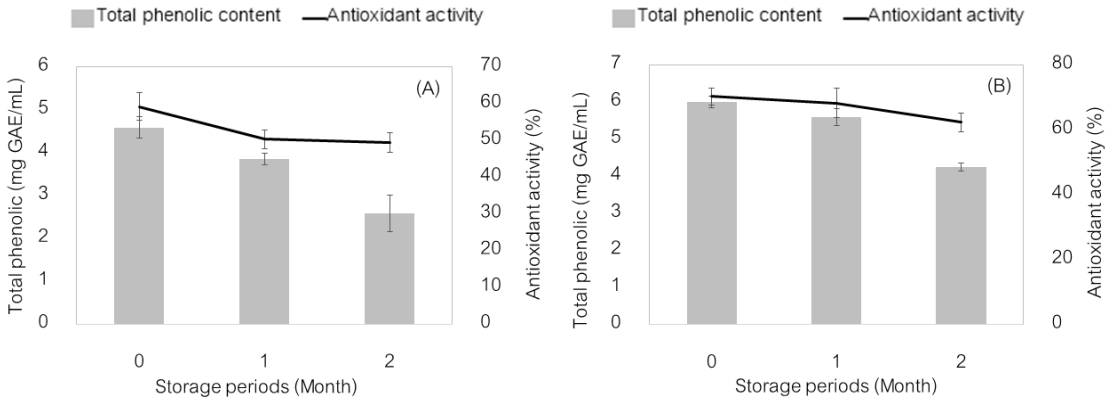

Figure 1 Changes in total phenolic content (mg GAE/mL sample) and DPPH radical scavenging activity (%) of cold brew coffee mixed with coconut water with different processing, (A) packed in glass bottle and sterilized at 121°C for 15 min, (B) packed in aluminum foil laminated pouches and heated at 65°C for 30 min.

5. Microbial evaluation

The microbial population of cold brew coffee blended with coconut water undergoes different two techniques of thermal treatment experienced in the same trend, which was <1 CFU/mL for aerobic plate count and *B. cereus,* and <1.1 MPN/100mL for *E. coli* (Table 4) throughout the storage periods. The number of the microbial count was within the standard criteria of iced coffee (Thai Community Product Standard, 2011) and the coffee

beverage in a hermetically sealed container (Ministry of Public Health, 2020), which was not exceeded 1 x 10^4 CFU/mL for APC, 100 CFU/100mL for *B. cereus,* and not detected *E. coli* in 100 mL of sample.

Table 4 Changes in microbial populations of cold brew coffee mixed with coconut water with different processing.

Month	Aerobic plate count (cfu/mL)	*E. coli* (MPN/100mL)	*B. cereus* (cfu/mL)
A. Processing 1			
1	<1	<1.1	<1
2	<1	<1.1	<1
3	<1	<1.1	<1
B. Processing 2			
1	<1	<1.1	<1
2	<1	<1.1	<1
3	-*	-*	-*

*not analyzed since the expiry of the shelf-life.

Discussion

The color of cold brew coffee was the quality attribute that related to sensory characteristics. The development of melanoidins in coffee beverages indicated an occurrence of Maillard reaction (Torma *et al*., 2019), a non-enzymatic browning reaction between precursors of reducing sugar and amino acids. Quintero *et al*. (2021) reported that under acidic conditions (pH ≤ 5.0) accelerated the change in Maillard reaction products when fructose contents increased. Moreover, thermal processing, especially UHT, resulted in the formation of the advanced glycation end products (AGEs) by Streaker aldehydes degradation pathways in concentrated liquid coffee. There is enough evidence to explain the mechanism of color degradation in coffee beverages undergoing the thermal process, particularly in the sterilization method (processing 1) which showed higher amounts of ΔE after 2-month storage.

The chemical property, mainly pH value, was an important factor correlated to sourness, indicating the quality depletion in coffee beverages. In this study, the pH value of both treatments was significantly decreased ($p \leq 0.05$) during storage periods, evidence of the increment of acidity. The reduction of 5-caffeoylquinic acid (5-CQA) and increase of caffeic and ferulic acid is the cause of undesirable changes (Pérez-Martínes *et al*., 2008; Sopelna *et al*., 2013). Furthermore, the Maillard reaction is might involve lactone hydrolysis which could hydrolyze to acid in aqueous media causing the pH reduction (Manzocco *et al*., 2007; Sopelna *et al*., 2013). It is noticeable that coconut water containing high sugar content and abundant nutrients, a source for microbial metabolism, leads

to changes in organic acid. An initial lower pH value of cold brew coffee conducted by sterilization (processing 1) technique might be correlated with the heat-induced by the polyphenolic compound breaking up into small molecules of phenolic acid.

Cold brewing and thermal processing were affected on phenolic acid content and antioxidant activity. The 5-caffeoylquinic (5-CQA), 4-caffeoylquinic (4-CQA), and 3-caffeoylquinic (3-CQA) are polyphenolic compounds found in cold brew coffee made by the bean from six different locations, which showed a strong correlation between antioxidant activity (Rao and Fuller, 2018). The concentration of antioxidant compounds in cold brew beverages is depended on the solubility at room or cold temperature and soaking time to release the soluble solid into the extract (Rao and Fuller, 2018). However, the hot brewing method had the efficiency to extract the antioxidant compounds better than those of cold brewing counterparts because the high temperature-induced the additional bioactive compound released into the water (Rao and Fuller, 2018). The results of this study revealed that the level of heat treatment and contact time were affected by the loss of phenolic compounds and their antioxidant efficacy, especially in a sterilized beverage. The observation during storage (2 months) showed a higher percentage reduction of total phenolic content (44%) and antioxidant activity (17%) in sterilized beverages than those of pasteurized counterparts (29% and 11%). Interestingly, Bellumoria *et al*. (2021) described that pasteurization is the best technique for maintaining flavor profiles of cold brew coffee over shelf-time compared with high-pressure processing (HPP), microfiltration, or UV irradiation treatment.

Caffeine content (80.43-92.34 mg/100g) of cold brew coffee beverage made by two thermal techniques were in a standard criterion of iced coffee conducted by Thai Community Product Standard (2011), not exceeding 100 mg/100mL. The factors impacting caffeine content depend on many factors, such as species, brewing time, the temperature of the water, roasting the bean, grinding degree, coffee/water ratio, the origin of coffee bean, storage condition of coffee bean, etc. (Olechno *et al*., 2021). From these results, caffeine content significantly reduced ($p \leq 0.05$) during storage which contrasted with work conducted by Bellumoria *et al.* (2021), which remained constant throughout 120 days of evaluation. Generally, higher temperature (100ºC) is an impact factor on caffeine concentration while lower temperature results in a reduction of their contents (Olechno *et al*., 2021). However, it might be depending on the brewing technique reported by Angeloni *et al.* (2018) in a coffee brew prepared using a French press (water temperature of 93ºC, 5 min) had a lower caffeine concentration than cold brew coffee (water temperature of 22ºC). Muzykiewicz-Szymánska *et al*. (2021) noted that caffeine content in cold brew coffee was higher than those with hot extract due to a long-brewing time to infuse this alkaloid.

Cold-brew coffee beverages were susceptible to microbial contamination due to a long immersion time in the water at room temperature (Claassen *et al*., 2021). The pathogenic bacteria, e.g., *Salmonella*, *Listeria*

monocytogenes, and *Escherichia coli* may be viable in cold brew beverages after storage for 7–28 days (Kwok *et al*., 2020). In this study, sterilization and pasteurization were applied to cold brew beverages for preserving shelf-life and concerning microbial hazards. The microbial count of both cold brew beverages was within the standard criteria of "iced coffee" (Thai Community Product Standard, 2011) and the standard of "coffee beverage in a hermetically sealed container" (Ministry of Public Health, 2020). The value was not exceeded 1 x 10^4 CFU/mL for APC, 100 CFU/100mL for *B. cereus,* and not detected *E. coli* in 100 mL of sample. The results guaranteed consumer safety for cold brew coffee blended with coconut water during storage for 2-month in sterilized beverages at room temperature and 4-weeks for pasteurized beverages at 4±1ºC. These agreed with Bellumoria *et al*. (2021) reported that total bacteria count and yeast mold in pasteurized cold brew coffee was lower than 5 CFU/mL after 30 and 60 days storage. Further study should investigate other pathogenic bacteria such as *Staphylococcus aureus* and *Salmonella* spp. *for* concerning the microbial risk of sterilized beverages in a hermetically sealed container recommended by the Ministry of Public Health (2020).

Conclusions

The development of cold brew coffee *cv*. Robusta by blending with coconut water was a new choice and flavor for consumers. Thermal treatments were used to produce shelf-life stable cold brew beverages. Cold-brew coffee packed in an aluminum foil laminated pouch with pasteurization method preserved a phenolic content and antioxidant activity better than those undergoing sterilization, which observed a reduction of approximately 29 and 11% at the end of the storage. The total phenolic content and antioxidant activity of coffee beverages with sterilization decreased around 44 and 17%, respectively, after 2-month storage. The microbial criteria (aerobic plate count, *E. coli*, and *B. cereus*) of both treatments were consistent with the standard throughout storage periods. Further study should be considered for changing aroma compounds after processing and during the storage time.

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