

## Effect of different extraction methods on antioxidant and sensory properties of pasteurized black soymilk

Qamarina Suhaimi<sup>1</sup>, Wan Saidatul Syida Wan Kamarudin<sup>1</sup>, Addion Nizori<sup>2</sup>, Adi Md Sikin<sup>1#</sup>

<sup>1</sup>Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor Darul Ehsan, Malaysia

<sup>2</sup>Agricultural Product Technology Department, Faculty of Agriculture, Jambi University

#Corresponding author: [adisikin@utm.edu.my](mailto:adisikin@utm.edu.my)

**Abstract**— The processing methods of black soymilk (BSM) can significantly affect the retention of its bioactive compounds. The effect of cold water (4 °C, 8 hr) (CWE), hot water (80 °C, 10 min) (HWE) and alkali water extraction (0.25% NaHCO<sub>3</sub>, 54 °C, 10 min) (AWE) of BSM on antioxidant and sensory properties of pasteurized BSM (72 °C /10 min) was evaluated over 10 days of storage at 4 °C. A steep increase in the total phenolic content (TPC) of BSM from HWE was observed over 10 days of storage, although the TPC of that from AWE was steadily the highest. The trend of flavonoids of BSM from HWE was the same as that for TPC, achieving a concentration of 1858.77 mg QAE/100 g after 10 days. The total anthocyanin content was the highest (40.85 mg/100 g) in BSM by HWE at the end of storage. Although the TPC of BSM from all extraction methods increased during storage, the total anthocyanin content showed a decreasing trend. Overall, the DPPH radical scavenging activity of BSM by HWE increased during storage up to 32.19 % on day 10 and a decreasing trend was observed from AWE and CWE. Triangle test showed that there was a significant ( $p < 0.05$ ) sensorial difference among the three extraction methods. The BSM from AWE had the highest score in the 9-hedonic scale for all sensorial parameters after the commercial BSM. Overall, the heat treatment in both HWE and AWE enhanced the antioxidant activity and sensory properties of pasteurized BSM, respectively.

**Keywords**— antioxidants, anthocyanins, black soybean, phenolic

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### I. INTRODUCTION

Legumes such as soybeans (*Glycine max (L.) Merr*) contain bioactive compounds, which can be potentially used as a functional food. The amount of bioactive compound of legumes are usually affected by the weather, soil and geographical place [1]. Soybean is consumed for its protein and lipid all over the world in the form of oil, defatted soybean meal and soy flour. In Asia, the black soybean has been utilized for quite some time, as it is the major source of natural antioxidants due to the rich anthocyanin content found in its seed coat [2]. Anthocyanins are perceived as health-promoting functional food ingredients due to its antioxidant activity and anti-cancer property [3]. Anthocyanins are red, blue, and purple colored pigment. It is important for food quality because of their contribution towards color and appearance. Six main anthocyanins are cyanidin, pelargonidin, petunidin, malvidin, delphinidin and peonidin.

The structures differ by glycosidic substitute at positions 3 and 5 [4].

Black soybean also contains antioxidant components including phenolic, flavonoid compounds and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity. Phenols and flavonoids found in soybean seeds are known as active antioxidants. It is involved with many advantages to human wellbeing. For example, osteoporosis, cancer prevention, cardiovascular diseases, and menopausal symptoms [1]. Despite its antioxidant properties, black soybeans are not as commonly known legumes as yellow soybeans.

Previous studies the antioxidant property of black soybean with the presence of compounds such as anthocyanin, phenolic and flavonoids. However, the stability of these compounds as affected by different extraction methods and storage of black soymilk (BSM) has not been extensively investigated.

Therefore, this study was to determine the effect of cold-water extraction (CWE), hot water extraction (HWE) and alkali water extraction (AWE) method on antioxidant and sensorial properties of pasteurized BSM during storage.

## II. MATERIAL AND METHODS

### A. Material

Black soybean (*Glycine max*) was obtained from organic food area in AEON Mall at Section 13 Shah Alam, Selangor, Malaysia. The chemicals and reagents used were 0.25% sodium bicarbonate solution, 0.1% hydrochloric acid (HCL) (R&M Chemical, Malaysia), methanol (Merck, Germany), Folin-Ciocalteu reagent (Sigma, United States), gallic acid (Sigma, United States), 20% sodium bicarbonate (NaHCO<sub>3</sub>) (Univar, United States), 5% sodium nitrite (NaNO<sub>2</sub>) (Univar, United States), 10% aluminium chloride (AlCl<sub>3</sub>) (Bendosen, Malaysia), 1 M sodium hydroxide (NaOH) (Freinderman Schmidt, Malaysia) and quercetin hydrate (Sigma, United States), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma, United States). All chemicals and reagents used were from analytical standard; purchased from Next Gene Scientific Sdn Bhd., Malaysia.

### B. Methods

#### *Extraction of black soymilk*

Three different extraction methods on black soybeans were conducted following the procedure by Esteves et al. [5]. The black soybean was subjected under running tap water to remove any dirt before subjected to 3 different extraction methods. Firstly, for cold water extraction the 25 g of black soybean was soaked in cold water for 8 hours at 4 °C. Then, it was drained and blended in cold water at 4 °C (1:20, soy: water). Secondly, for hot water extraction the 25 g of black soybean was heated in water for 10 minutes at 80°C. Then, it was drained and blended in warm water at 43°C (1:20, soy: water). The third extraction method was alkali extraction. The 25 g of black soybean was soaked in cold water for 8 hours at 4 °C. The cold water was drained, and black soybean was heated in alkali water (0.25% sodium blended carbonate solution) before being drained and blended in hot water at 54 °C (1:20, soy: water). Then, all extracted samples were centrifuged for 10 minutes to separate the liquids and solid of black soymilk BSM and pasteurize for 10 minutes at 72 °C. The BSM samples were stored in refrigerator at 4 °C for storage analysis. The analysis was carried out at 2 days interval for a duration of 10 days.

#### *Determination of Total Phenolic Content (TPC)*

The total phenolic content was measured following the procedure described by Josipovic et al. [1]. Absorbance was measured at 765 nm by using Ultraviolet-Visible Spectrophotometer (Thermo Scientific). Total phenols were calculated from a gallic acid standard curve and reported as mg gallic acid equivalents (GAE)/100 g of dried weight.

#### *Determination of Total Flavonoids Content (TFC)*

The flavonoids content was measured following the procedure by Josipovic et al. [1]. Absorbance was measured at 415 nm by using Ultraviolet-Visible Spectrophotometer (Thermo Scientific). Total flavonoids are calculated from quercetin hydrate standard curve and report as mg quercetin hydrate (QAE/100 g) of dried weight.

#### *Determination of Total Anthocyanin Content (TAC)*

The total anthocyanin content was measured according to procedure described by Ivanoic et al. [6]. The absorbance of the solution was measured at 528 nm by Ultraviolet-Visible Spectrophotometer (Thermo Scientific), using a 0.1% (v/v) solution of hydrochloric acid in methanol as the compensation liquid. The percentage content of anthocyanins in black soybean extracts, expressed as cyanidin-3-glucoside, calculated:

$$\text{TAC (g/100 g)} = \frac{A_{528}}{718.m} \times 4.5000$$

A as an absorbance at 528 nm; 718 as the specific absorbance of cyanidin-3-glucoside at 528 nm; m as the mass of the substance in grams.

#### *Determination of DPPH radical scavenging capacity*

The DPPH radical scavenging capacity was measured following the procedure by Azalina and Mohammad [7]. The absorbance was measured against the blank (distilled water) at 517 nm by using Ultraviolet-visible Spectrophotometer (Thermo Scientific). Inhibition of DPPH radical scavenging was calculated in percentage (%) using equation below:

$$\text{DPPH scavenging effect \%} = 1 - \left( \frac{\text{Absorbance of sample at 517nm}}{\text{Absorbance of control at 517nm}} \right) \times 100$$

#### *Determination of colour*

The colour of the BSM was determined using chroma meter (CR-400, Konica Minolta Business Technologies, Tokyo, Japan) in the reflection mode and calibrated with a standard white plate (Y=94.00, x=0.3158, y=0.3322). The net colour difference was evaluated with the following equation [8], using the parameters L\* (lightness), a\* (green chromaticity) and b\* (yellow chromaticity) coordinates, and comparing the colour of BSM at day 2, 4, 6, 8 and 10 with day 0.

#### *Sensory evaluation*

A triangle test was conducted following UNI ISO 4120 Norm (Standard-ISO, 2004) to evaluate whether the panelists could discriminate BSM from three different extraction methods. Also, a 9-point hedonic scale was used to evaluate the acceptability of BSM extracted from different extraction methods against commercial BSM as control. Samples were presented in random order and panelists were asked to rate their liking for appearance, colour, texture, sweetness, taste and overall acceptability on a 1-9 hedonic scale (Meilgaard, Civille, & Carr, 1999): 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely. 60 panelists were randomly selected from the Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Shah Alam, Malaysia. They were students aged 20 years and above, capable of understanding the study procedure and providing an informed consent to participate in the analysis. All of them had prior experience (from modest to good) in sensory evaluation tests.

#### Statistical analysis

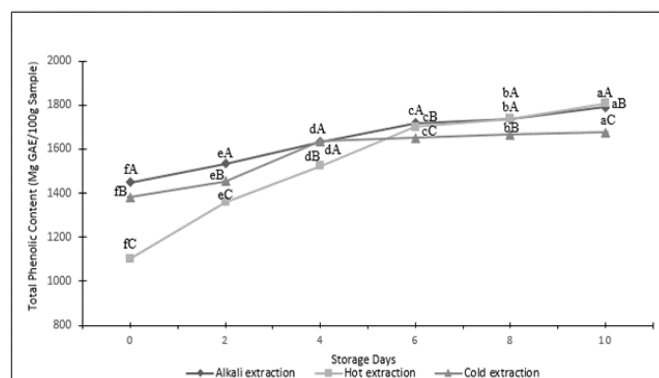
All the analysis was done in triplicate and expressed as mean  $\pm$  standard deviation. Data was analysed using ANOVA using SPSS 15. Duncan's multiple range test was used to assess the difference in means. A significant difference was considered at the level of  $p < 0.05$ . All measurements were done in triplicate.

### III. RESULT AND DISCUSSION

#### Total phenolic content (TPC)

Total phenolic content (TPC) can be used as an important indicator of antioxidant capacity in a preliminary screen for any functional food [9]. Figure 1 shows the effect of storage on TPC of black soymilk (BSM) from cold (CWE), hot (HWE) and alkali water extraction (AWE). It was shown that the TPC of all BSM significantly increased ( $p < 0.05$ ) during storage. During the analysis of TPC, some compounds such as hydroxycinnamic acid and rutin being formed during storage and reacted with the Folin-Ciocalteu's reagent, resulting in an enhanced TPC during storage [10]. Of all the extraction methods, AWE shows the highest TPC which was 1448.29 g GAE/100 g at 0 day. It could be hypothesized that  $\text{NaHCO}_3$  provided stress to enhance the activity of phenylalanine ammonialyase (PAL), an enzyme responsible for phenolic accumulation. This result agreed with Qin et al. [11] who suggested the role of  $\text{NaHCO}_3$  (0.05-0.2%) that enhanced the PAL activity during buckwheat sprouts germination. After 10 days of storage, the TPC of BSM from HWE was the highest 1807.33 g GAE/100g followed by AWE and CWE, suggesting that heat treatment during HWE and AWE enhanced the TPC of BSM. Zhou et al. [12] reported the increase of antioxidant activity as a result of thermal processing. Similarly, Park et al.

[13] claimed that changes of temperature affect phenolic content and antioxidant activity. These suggest that heat treatment during HWE and AWE increased the TPC of BSM. Fig 1. The effect of storage on total phenolic content (mg



GAE/100g sample) in pasteurized black soybean milk (BSM) (72 °C/10 min) as a results of different extraction methods<sup>2</sup>.

<sup>1</sup>All BSM samples were stored in refrigerator at 4 °C in a bottle wrapped with aluminum foil.

<sup>2</sup>AWE is alkaline water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C and then heated in alkaline (0.25% sodium bicarbonate) water; HWE is Hot water extraction in which black soybeans were soaked in hot water for 10 minutes at 80 °C; CWE is cold water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C.

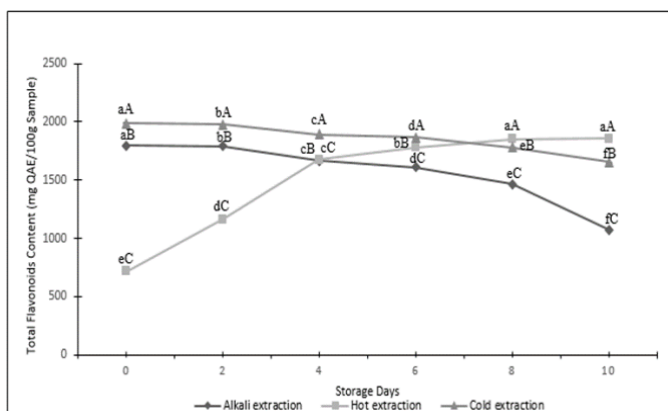
#### Total flavonoid content (TFC)

Figure 2 shows the effect of storage on total flavonoid content in pasteurised SBM as a result of different extraction methods. The results showed that BSM from HWE had the lowest flavonoid content, followed by that from AWE and CWE at day 0. This indicated that the flavonoid content decreased with increasing extraction temperatures of SBM. This agreed with Sharma et al. [14] who reported that the involvement of heat during processing caused lower of total flavonoids. However, the flavonoid content of BSM from HWE gradually increased, while the flavonoid content from that of AWE and CWE decreased over 10 days of storage.

This results in some of the flavonoids declining. As shown in Figure 2, alkali and cold extraction methods were decreasing when store for 10 days. Alkali use high temperature during processing cause the total flavonoids content in black soymilk decrease. This might cause the degradation of flavonoids. Furthermore, the amount of flavonoids content depends on the structure of flavonoids [14]. Hot extraction method shows an increase in flavonoid content. Since the hot extraction method has higher antioxidant activity, it poses higher flavonoid

content [15]. In addition, thermal processing caused antioxidant activity to increase [12].

Fig. 2. The effect of storage1 on total flavonoids content (mg QAE/100 g sample) in pasteurized black soybean milk (BSM)



(72 °C /10 min) as a results of different extraction methods<sup>2</sup>.

<sup>1</sup>All BSM samples were stored in refrigerator at 4 °C in a bottle wrapped with aluminum foil.

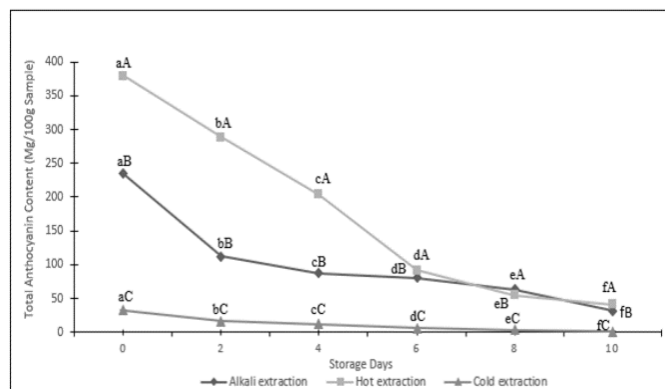
<sup>2</sup>AWE is alkaline water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C and then heated in alkaline (0.25% sodium bicarbonate) water; HWE is hot water extraction in which black soybeans were soaked in hot water for 10 minutes at 80 °C; CWE is cold water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C.

#### Total anthocyanin content (TAC)

Figure 3 shows the effect of storage on total anthocyanin content in pasteurised SBM as a result of different extraction methods. The results showed that SBM from HWE had the highest amount of anthocyanin content followed by that from AWE and CWE throughout the storage. It was shown that CWE was not effective to extract anthocyanin and maintained the lowest amount of anthocyanin throughout the storage days. These results agreed with Esteves et al. [5] who reported that black soymilk extracted by HWE method has the highest amount of cyanidin-3-glucoside compared to alkali and cold extraction methods. In this regard, Sarkis et al. [16] highlighted that the involvement of heating process gives strong influence in anthocyanin stability. According to Patras et al. [17], it was not possible to predict the exact effect of thermal treatment on anthocyanin retention. Furthermore, the inclusion of heating to approximately 50 °C has a positive effect on anthocyanin retention because polyphenol oxidase, which degrades anthocyanin can be inactivated at mild heat treatment. Moreover, the conditions selected for thermal processing may have changed monomeric anthocyanin content during storage. Another reason could be due to condensation reactions of anthocyanins with other phenolic compounds, including flavan-3-ols [17]. However, there was a decline in anthocyanin content over storage for all extraction methods as shown in Figure 3.

The high temperature applied on black soybean during the extraction method caused higher loss of anthocyanins during storage [18]. It was also possible that the general loss of anthocyanins in the BSM during storage could be enhanced by the pasteurization process [5].

Fig. 3. The effect of storage1 on total anthocyanin content (mg/100g sample) of black soymilk (BSM) (72 °C /10 min)



from different extraction methods<sup>2</sup>.

<sup>1</sup>All BSM samples were stored in refrigerator at 4 °C in a bottle wrapped with aluminum foil.

<sup>2</sup>AWE is alkaline water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C and then heated in alkaline (0.25% sodium bicarbonate) water; HWE is hot water extraction in which black soybeans were soaked in hot water for 10 minutes at 80 °C; CWE is cold water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C.

#### DPPH radical scavenging capacity

The storage of BSM by HWE shows an increasing trend of DPPH radical scavenging capacity reaching the value of 32.19% at the end of storage. According to Castro-Lopez et al. [19], stated that the antioxidant activity rises may be because of strong possibility of polyphenols to encounter polymerization reactions. Therefore, results in oligomers have bigger areas accessible for charge delocalization, which cause high in antioxidant activity. The standard (ascorbic acid) which had DPPH free radical scavenging activity was about 98.22%. The increment corresponded with an increase in TPC of BSM from HWE. However, BSM from both CWE and AWE decreased slightly, reaching an almost similar capacity of approximately

27%. According to Carmen et al. [20] the decrease of antioxidant activity was due to the light and high temperature.

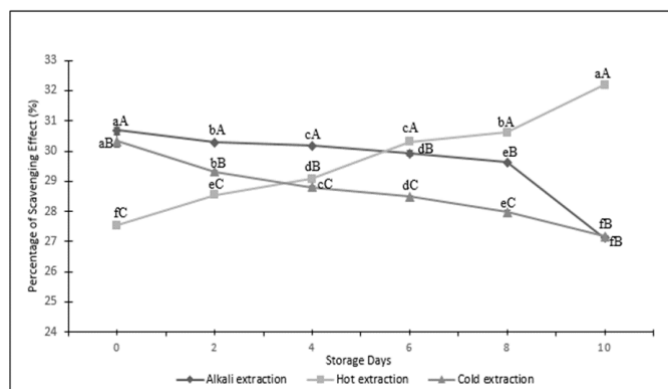


Fig. 4. The effect of storage on DPPH scavenging activity in pasteurized black soybean milk (BSM) (72 °C /10 min) as a results of different extraction methods<sup>2</sup>.

<sup>1</sup>All BSM samples were stored in refrigerator at 4 °C in a bottle wrapped with aluminum foil.

<sup>2</sup>AWE is alkaline water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C and then heated in alkaline (0.25% sodium bicarbonate) water; HWE is hot water extraction in which black soybeans were soaked in hot water for 10 minutes at 80 °C; CWE is cold water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C.

#### Effect of storage on colour

The effect of water extraction on colour of pasteurized BSM was measured on the day of extraction (0 day) and after 2, 4, 6, 8 and 10 days of storage using CIE L\*(lightness) a\*(green/red) b\*(blue/yellow) coordinates as shown in Table 1a), 1b) and 1c), respectively. As shown in Table 1a), the lightness (L\* value) of pasteurized BSM was significantly decreased ( $p < 0.05$ ) in the order of CWE (61.47), HWE (53.17) and AWE (52.47). Conversely, the redness (a\* value) was significantly increased in the order of CWE (-0.6), HWE (-0.34) and AWE (3.77) at 0 day of storage (Table 1b). This could be attributed to the release of anthocyanin assisted by heat treatment during the extraction process as mentioned previously. During the hot extraction method, the black soybean was heated, the anthocyanin pigment dissolved in water caused the colour of water during heating become reddish-purple. Furthermore, the increased lightness (L\*), and the reduced redness (a\*) were observed during storage for all three extraction methods, which could be correlated with the decrease in anthocyanin content as shown in Figure 3. This agreed with Kasim et al. [21], who reported that the L\* value higher with the storage time results in lower anthocyanin. Table 1 c) shows that AWE method resulted in the highest b\* (yellowness) value, followed by CWE and HWE in most cases during storage. Also, the b\* values were generally reduced over storage time for all extraction methods. Likewise, the lower value of yellowness for hot extraction method indicated higher anthocyanin content, and this was evidenced

by Kasim et al. [21], who reported that the b\* value increased with storage time as a results of decreased anthocyanin content. Table 1d) showed that the HWE method generally had the highest degree of colour change ( $\Delta E$ ) throughout storage, followed by AWE and CWE. The colour change was noticeable starting from day 2, 4 and 6 for HWE, AWE and CWE, respectively. According to Chugh et al. (2014), the  $\Delta E$  could be classified as “not noticeable” for the score of 0 to 0.5, “slightly noticeable” for the score of 0.5 to 1.5, and “noticeable” for the score of more than 1.5.

TABLE 1 (A)

Day of storage	L*		
	AWE	HWE	CWE
0	52.49±0.01 <sup>fc</sup>	53.17±0.01 <sup>fb</sup>	61.47±0.01 <sup>fa</sup>
2	53.61±0.01 <sup>eb</sup>	53.50±0.01 <sup>ec</sup>	61.58±0.01 <sup>ea</sup>
4	54.04±0.01 <sup>dc</sup>	59.01±0.00 <sup>db</sup>	61.71±0.01 <sup>da</sup>
6	58.69±0.01 <sup>cc</sup>	61.58±0.02 <sup>cb</sup>	64.41±0.02 <sup>ca</sup>
8	59.46±0.02 <sup>bc</sup>	62.46±0.00 <sup>bb</sup>	65.37±0.01 <sup>ba</sup>
10	59.59±0.01 <sup>ac</sup>	62.61±0.00 <sup>ab</sup>	66.01±0.00 <sup>aa</sup>

TABLE 1 (B)

Day of storage	a*		
	AWE	HWE	CWE
0	3.77±0.02 <sup>aA</sup>	-0.34±0.01 <sup>cdB</sup>	-0.60±0.01 <sup>ac</sup>
2	3.56±0.02 <sup>bA</sup>	-0.26±0.02 <sup>bB</sup>	-0.83±0.01 <sup>ec</sup>
4	3.58±0.00 <sup>bA</sup>	-0.31±0.01 <sup>cb</sup>	-0.78±0.01 <sup>dc</sup>
6	2.82±0.02 <sup>aA</sup>	-0.40±0.10 <sup>fb</sup>	-1.15±0.03 <sup>fc</sup>
8	2.91±0.02 <sup>aA</sup>	-0.12±0.01 <sup>ab</sup>	-0.72±0.00 <sup>bc</sup>
10	2.86±0.03 <sup>dA</sup>	-0.38±0.01 <sup>efB</sup>	-0.75±0.02 <sup>cc</sup>

TABLE 1 (C)

Day of storage	b*		
	AWE	HWE	CWE
0	8.68±0.01 <sup>eA</sup>	3.03±0.02 <sup>fc</sup>	5.00±0.01 <sup>eb</sup>
2	8.16±0.02 <sup>fA</sup>	4.80±0.01 <sup>ab</sup>	4.52±0.00 <sup>fc</sup>
4	8.73±0.00 <sup>dA</sup>	4.34±0.01 <sup>bc</sup>	4.72±0.01 <sup>eb</sup>
6	10.40±0.01 <sup>bA</sup>	4.09±0.01 <sup>cc</sup>	4.93±0.00 <sup>db</sup>
8	10.28±0.01 <sup>cA</sup>	3.90±0.02 <sup>dc</sup>	5.15±0.01 <sup>bb</sup>
10	10.59±0.01 <sup>aA</sup>	3.64±0.02 <sup>ec</sup>	6.19±0.00 <sup>ab</sup>

TABLE 1 (D)

Day of storage	Total color difference ( $\Delta E$ ) <sup>3</sup>		
	AWE	HWE	CWE
0	-	-	-
2	1.25	1.80	0.54
4	1.56	5.99	0.41
6	6.50	8.48	2.99
8	7.04	9.33	3.90
10	7.41	9.46	4.70

Values are expressed as mean ± standard deviation. Means within column (a, b, c) and within a row (A, B, C) marked with different letters were significantly different at the level of  $p < 0.05$ . Lower case letters indicate the effect of different storage days. Capital letters indicate different extraction methods.

<sup>1</sup>All extracted samples were stored in refrigerator at 4 °C in a bottle wrapped with aluminum foil.

<sup>2</sup>AWE is alkaline water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C and then heated in alkaline (0.25% sodium bicarbonate) water; HWE is hot water extraction in which black soybeans were soaked in hot water for 10 minutes at 80 °C; CWE is cold water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C.

<sup>3</sup> $\Delta E$  calculated as  $\sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$ ; where,  $\Delta L = L(\text{Day } 0) - L(\text{Different days})$ ,  $\Delta a = a(\text{Day } 0) - a(\text{Different day})$ ,  $\Delta b = b(\text{Day } 0) - b(\text{Different day})$ . According to Chugh *et al.* (2014), stated that  $\Delta E$  were classified as: 0 to 0.5 were “not noticeable”, 0.5 to 1.5 were “slightly noticeable” and more than 1.5 were “noticeable”.

### Sensorial quality of pasteurised BSM

A triangle sensory test was conducted to determine the noticeable differences between extraction methods. The number of correct responses for 60 panelists should be at least 28 to indicate a noticeable sensorial difference as a result of different extraction methods (UNI ISO 4120). As shown in Table 2, 53, 57 and 33 of the 60 panelists were able to discriminate overall sensory difference between the CWE vs AWE, HWE vs AWE and HWE vs CWE, respectively. This suggests that there is detectable sensory difference between all the samples as of different extraction methods.

The 9-hedonic test was also conducted to compare the effect of extraction method on appearance, colour, texture, sweetness, taste, and overall acceptability of pasteurized BSM. As shown in Table 3, the BSM from AWE had the highest average score for sweetness, taste, and overall acceptability, while the BSM from CWE had the highest average score for appearance, colour and texture. There is no significant difference ( $p < 0.05$ ) in terms of sweetness between all the samples. The BSM from HWE had the lowest number of attributes with an average score higher than 5, a score considered limit of acceptability [22]. This result gives a negative connotation that the release and retention of anthocyanin in BSM from HWE, known for its enhanced antioxidant activity may not necessarily favour the consumer’s acceptability due to its darker colour and appearance. In this regard, Mian *et al.* [23] claimed that the preference of BSM appears to be related to its appearance.

TABLE 2

The effect of extraction methods on the number of correct response for 60 panellists by triangle test for pasteurized BSM (72 °C /10 min) at day 0.

Extraction methods <sup>1</sup>	No. of correct responses
CWE vs AWE	53
HWE vs AWE	57
HWE vs CWE	33

<sup>1</sup>AWE is alkaline water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C and then heated in alkaline (0.25% sodium bicarbonate) water; HWE is hot water extraction in which black soybeans were soaked in hot water for 10 minutes at 80 °C; CWE is cold water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C.

TABLE 3

Sensory acceptance analysis of pasteurized BSM (72 °C /10 min) from different extraction methods<sup>1</sup> using a 9-hedonic scale<sup>2</sup> at day 0.

Parameter	AWE	HWE	CWE
Appearance	4.97±1.99 <sup>b</sup>	4.08±1.76 <sup>c</sup>	5.70±1.65 <sup>a</sup>
Colour	4.73±1.94 <sup>b</sup>	3.88±1.57 <sup>c</sup>	5.48±1.76 <sup>a</sup>
Texture	5.55±1.62 <sup>a</sup>	4.87±1.53 <sup>b</sup>	5.60±1.50 <sup>a</sup>
Sweetness	6.42±1.63 <sup>a</sup>	5.37±1.66 <sup>a</sup>	5.17±1.88 <sup>a</sup>
Taste	6.33±1.64 <sup>a</sup>	4.67±1.72 <sup>b</sup>	4.82±2.05 <sup>b</sup>
Overall acceptability	6.10±1.64 <sup>a</sup>	4.77±1.71 <sup>b</sup>	5.13±1.87 <sup>b</sup>

Values are expressed as mean ± standard deviation. Means within each row marked with different letter are significantly different at  $p < 0.05$ . The lower case indicates the different extraction methods on black soymilk.

<sup>1</sup>AWE is alkaline water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C and then heated in alkaline (0.25% sodium bicarbonate) water; HWE is hot water extraction in which black soybeans were soaked in hot water for 10 minutes at 80 °C; CWE is cold water extraction in which black soybeans were soaked in cold water for 8 hours at 4 °C.

<sup>2</sup>The 9-hedonic scale includes 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely.

## IV. CONCLUSION

The extraction temperature before pasteurization process affects the antioxidant activity and sensorial properties of BSM. Overall, HWE enhanced the antioxidant activity of BSM, except for the total anthocyanin content. However, the BSM from HWE resulted in the lowest score in 9-hedonic scale for most sensorial attributes as compared to that from AWE and CWE. It is recommended that the pasteurized BSM prepared by HWE should be formulated to improve its sensory quality, while maximizing the benefits of antioxidant capacity.

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