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Identification and Reduction of Bitter Taste Determinant Compounds in Chocolate Spread Formulated with Candlenut

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Abstract

Candlenuts can be used as a substitute in producing chocolate spread due to their high-fat content. However, the limitation of using candlenut in the spread formulation is the existence of a bitter taste. This study aimed to investigate the effect of sodium bisulfite and sodium bicarbonate soaking treatment (100, 300, and 500 ppm) on reducing the bitter taste of candlenut seed. In order to reach the goal, bitter compounds of candlenut and chocolate spread were identified using Liquid chromatography-mass spectrometry (LC-MS). The sensory characteristics of chocolate spread were profiled using the Rate All That Apply (RATA) descriptive method. It was shown that the panelists identified 14 sensory attributes in the samples, one of which was bitter taste. Treatment with sodium bisulfite, as well as sodium bicarbonate at the level of 300 ppm, reduced the intensity of the bitter taste. The treatment reduced flavonoid and tannin levels by 85.21% and 82.08% in candlenut seeds. It also reduced flavonoid and tannin levels by 88.47% and 72.71% in chocolate spread, respectively. LC-MS identified 13 compounds that cause the bitter taste, namely quercetin, oleuropein, kaemferol, resveratrol dimer, luteolin, epicatechin, theobromine, caffeine, iso-humolones, colaflavone, chlorogenic acid, feruloyl-caffeoylquinic acid, and coumaroylquinic acid. This study creates a new technique for producing chocolate spread formulated with candlenuts with lower bitterness levels.

Keywords: bicarbonate; bisulfite; chocolate spread; flavonoid; RATA; tannin

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INTRODUCTION

The sustainable agro-food industry is an emerging issue at present. Continuous improvement conducted in the industry is projected to result from sustainable production (De Ron, 1998). In the chocolate industry, for instance, continuous efforts have been made to improve the quality of the chocolate (Praseptiangga et al., 2020). Chocolate formulated with plant-based functional ingredients and probiotics is an example of innovative chocolate (Muhammad et al., 2021b; 2021c; 2022). Continuous innovation has been done in the chocolate bar, chocolate drinks, and spread products (Acan et al., 2021a; Faiqoh et al., 2021; Muhammad et al., 2021a).

Chocolate spread is a food product made from the market's cocoa powder, vegetable oil, sugar, milk powder, and nuts. In biscuits, cakes, and breads, chocolate spread can be used as a filling (Acan et al., 2021b; Tolve et al., 2021). Adding

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nuts, particularly those high in fat and protein, like candlenuts, can improve the nutritional value of chocolate spread. Candlenut (*Aleurites moluccana*) is a cultivated plant that can be used in all its parts. Candlenut contains 19 g protein, 8 g carbohydrates, 63 g fat, and 639 calories, flavonoids, saponin, diterpenoids, phenolic components, and tannin compound (Yusnita et al., 2001; Krisnawati et al., 2011; Perdani et al., 2017; de Castilho et al., 2021; Yu et al., 2021). However, candlenuts have not been utilized optimally in the food sector, except as food seasoning (Krisnawati et al., 2011). Substituting hazelnuts with candlenut seeds can reduce production costs because they are cheaper than hazelnuts and local nuts. In addition, it can provide a nutty and creamy flavor like macadamia nuts, almonds, or roasted walnuts, giving a soft texture and taste and a flavor similar to hazelnuts, walnuts, and macadamias (Köksal et al., 2006; Raghavan, 2007; Pramesthi et al., 2020).

However, adding candlenut to the chocolate spread can give the product a bitter taste. The bitter taste of bitternut is suspected to come from determinant compounds (flavonoids, tannins, and diterpenoids). Based on research studies, secondary metabolites such as tannins, saponins, glycosides, and alkaloids have been shown to cause bitter taste in vernonia and *sukun* (breadfruit) (Maris and Djiwanti, 2019; Widowati et al., 2019). Moreover, determinant compounds that cause bitter taste in food are phenols, flavonoids (isoflavones, flavone, flavonols, naringenin), glycosides (terpenes, limonins, and saponins), glucosinolates, alkaloids (quina, theobromine, caffeine), and pyrazines (Fennema, 1996; Valls et al., 2009; Khairy et al., 2018).

Usually, the bitter taste in some products is disliked by consumers, so a treatment is needed to reduce it. The bitter taste of the product can be minimized or eliminated by pre-treatment of the raw material by various means, such as soaking it in sodium. Soaking treatment with sodium bisulfite (Na-bisulfite) for one hour can reduce the tannin content of *sukun*, and soaking with sodium bicarbonate (Na-bicarbonate) has been shown to reduce the bitter taste of moringa seeds (Widowati et al., 2019; Gunawan et al., 2020). Decreasing the determinant compound can be reduced by soaking the ingredients with sodium bisulfite or bicarbonate. The soaking process can cause damage to the α-1,4 glycosidic bonds due to the alkaline conditions produced. Damage to glycosidic bonds can reduce the concentration of determinant compounds and bitter taste levels. In addition, soaking treatment with sodium bicarbonate can facilitate the process of removing non-volatile compounds from the moringa seed matrix into the water due to the alkaline conditions. Soaking produces carbon dioxide (CO_2) by reacting sodium bicarbonate with water. This gas will enter and form cavities in the seeds, causing damage or softening of the tissue, which can cause oxidation of non-volatile compounds in the material (Gunawan et al., 2020).

Meanwhile, soaking using sodium metabisulfite forms alkaline conditions and sulfur dioxide gas (SO_2). The mechanism is the same as CO_2 gas, but the role in this treatment is SO_2 gas. If too much gas is produced, it can cause a dominant bitter taste in the product (Sulaswatty and Roestamsjah, 1991). This mechanism is the basis for the effect of soaking in sodium bisulfite.

To our knowledge, studies on candlenut pre-treatment and its application in chocolate have not been carried out. There is no specific information on secondary metabolites that contribute to the bitter taste of candlenut seeds. So, the treatment of soaking candlenut seeds with sodium bisulfite and bicarbonate was used in the study. The purpose of this study, therefore, was to identify and reduce the determinant compounds of bitter taste and sensory attributes of chocolate spread formulated with candlenut, which was soaked in sodium bisulfite and sodium bicarbonate solution (100, 300, and 500 ppm, respectively).

MATERIALS AND METHOD

Materials

Fresh peeled candlenut seeds in Songgo, East Java, Indonesia, low-fat cocoa powder (Burgundy Tulip, FreyAbadi Indotama Inc., Bandung, Indonesia), palm oil, skim milk (CV Sari Indo Prima, Indonesia), refined sugar (Rosebrand, Adi Karya Gemilang Inc., Bandar Lampung, Indonesia), soy lecithin (Lansida), vanilla (Gunacipta multirasa Inc., Tangerang, Indonesia), cracker biscuits (Monde, Jadi Abadi Corak Biscuit Factory Indonesia Inc., Surabaya, Indonesia), white bread, mineral water (Bayuadji Nusantara Inc., Semarang, Indonesia), sodium bicarbonate, and sodium bisulfite. Reagents used for chemicals analysis are methanol, aquadest, Folin-Ciocelteu reagent, Na_2CO_3 20% (Merck KgaA, Darmstadt, Germany), aluminum chloride ($AlCl_3$) 0.1 M (E. Merck, Darmstadt, Germany),

tannic acid and quercetin standard (Sigma-Aldrich Company Ltd., Gillingham, Inggris).

Chocolate spread preparation

The process of making chocolate spread was conducted by first soaking the candlenuts in sodium bicarbonate or sodium bisulfite solutions with concentrations of 100, 300 and 500 ppm for one hour (Widowati et al., 2019; Gunawan et al., 2020). Afterwards, the ingredient was washed and then dried in the oven (Memmert, Germany) for 20 minutes at a low temperature (< 100 °C). Then, candlenut was ground with a blender (Phillips turbo 0899, CKM, China) and sieved to produce powder with a particle size of 80 mesh. The candlenut powder was then mixed with other ingredients (7.6 g cocoa powder, 0.6 g lecithin, 7.4 g skim milk, 0.3 g vanilla, 50.1 g sugar, and 21 g palm oil) using a blender (Phillips turbo 0899, CKM, China) for 10 minutes until homogeneous. The formulation was set based on the in-house formula developed by the trial. After that, the chocolate spread was put in a clean plastic jar and stored in a dark room at room temperature (David et al., 2021).

Identification of bitter determinant compound with Liquid chromatography-mass spectrometry (LC-MS)

The chocolate spread sample was macerated in methanol. The extracted sample was extracted using a 0.22-micrometer millex sieve, then the sample extract was injected into a 5 microliter LC-MS column equipped with a UPLC BEH C18 column (1.7 µm; 2.1 x 50 mm), with source temperature 500 °C, capillarity voltage is 30 kV, positive polarity mode, and 50 to 1200 mass range. The mobile phase A was formic acid water (0.1%) and the mobile phase B was formic acid (0.1%). The results of the LC-MS test are in the form of a chromatogram equipped with the m/z value and retention time of each detected compound. The compound's name is identified by determining the fundamental peak, which has an intensity of 100%, as measured by m/z. Then, compare other m/z fragmentation patterns with fragmentation patterns from literature data and PubChem applications (https://pubchem.ncbi.nlm.nih.gov/, accessed on December 2022).

Flavonoid analysis

Flavonoid content determinantion was referred to Udayaprakash et al. (2015). Briefly, 0.2 ml of sample was added to 5 ml of 0.1 M $AlCl_3$ in a test tube and shaken with a vortex. The absorbance value was measured using a spectrophotometer at 415 nm after being incubated for 40 minutes at room temperature. The standard curve was prepared using standard quercetin at a concentration range of 0 to 0.15 mg ml^{-1} formulated similarly (Muhammad et al., 2017). The absorbance value obtained was then entered as y in the linear equation of the standard curve so that the value of x (concentration) was obtained. Linear equations for standard quercetin were used to evaluate the flavonoid results: $Y = 0.0218x - 0.1468$, and the error value (R^2) was 0.9923. The total value of flavonoids was expressed in mg quercetin equivalent per gram sample.

Tannin analysis

Tannin content determination was referred by Fitriani et al. (2021). A 1.25 g sample (candlenut powder and chocolate spread sample) was extracted with 250 ml of aquadest and heated over boiling water for two hours. The extract was left to cool and filtrated with Whatman paper No.1, 1 ml of extract was added to 0.5 ml of Folin-ciocalteu reagent and 2 ml of Na_2CO_3, then mixed and incubated for 30 minutes at room temperature. The solution was subjected to UV-VIS spectrophotometry at 748 nm. The standard curve was prepared using a standard tannic acid at a concentration of 0.00, 0.02, 0.04, 0.06, 0.08, and 0.1 mg ml^{-1} prepared in the same manner. All the samples were examined thrice. Linear equations for standard tannic acid used to evaluate the results of tannins were $Y = 24.513x + 0.0667$ and the value of R^2 was 0.9907.

Sensory evaluation of chocolate spread

The sensory test was a descriptive test using the Rate All That Apply (RATA) method by Meyners et al. (2016). Sensory testing was performed on 30 to 75 untrained panelists. The RATA method was used to identify the sensory profile of the sample and intensity based on the sensory attributes of the food product. The test was carried out by placing a checklist on the RATA form which contained sensory attributes that were considered to be able to describe the sample. For the selected attribute, panelists were asked to provide an intensity scale according to the panelist's perception using five scales (low/weak, slightly low, moderate, slightly high, and high). Data was analyzed using PCA analysis to identify sensory attributes and Friedman's test analysis to compare differences in each sensory attribute (Adawiyah et al., 2020). If the data from Friedman's test

shows a difference in effect on the product (p-value < 0.05, rejected H_0), followed by the Wilcoxon test with a significance of 0.05.

Sensory attributes were determined by a focus group discussion (FGD) consisting of 8 to 12 participants. Each participant would describe the sensory attributes of taste, texture, and odor in the sample provided. Then, they discussed together and selected the right sensory attributes for the next sensory test (Meilgaard et al., 2020). From the FGD process, 14 attributes were selected to describe the words considered appropriate to describe the product's attributes in sensory testing with the RATA method. The sensory test followed recommendations based on the principles of ethical clearance: KE-FK-1478-EC-2022.

Statistical analysis

The chemical data were presented as the mean ± standard deviation (SD) from one independent experiment. The data were analyzed using one-way ANOVA followed by Duncan Multiple Range Test (DMRT). Statistical significance is determined by $p < 0.05$. Friedman's test was used.

RESULTS AND DISCUSSION

The first sensory analysis by Friedman's test is shown in Table 1. There are five significant sensory attributes ($p < 0.05$): earthy odor, bitter taste, thick texture, melt texture, and spreadability. Attributes that are not significantly different occur due to the panelists' response, which can be said to be the same for each treatment, or the perception of the panelists is not different for each treatment. Significantly, attributes occur due to the panelists' response, which can be said to be the same for each treatment, or the perception of the panelists is not different for each treatment. That is, panelists have multisensory perceptions that are not much different or can be said to be the same in each sample for each attribute.

The Wilcoxon test follows the five sensory attributes with a significant value < 0.05. Based on the results of Table 2, samples K3 and S3 have a different intensity of bitter taste than sample R. Samples S5 and K1 have different attributes of melt texture and spreadability than R. The differences in the samples are influenced by the concentration of sodium used, which causes damage to seed tissue or candlenut cells. Damaged cells will easily release water during roasting, so the candlenut seeds are drier and optimally release their oil (Darmawan et al., 2019). However, there is no significant difference in the earthy odor because most of the earthy aroma in a chocolate spread is obtained from the same aromatic compound (LC-MS data). Likewise, the thick texture attribute was used in all treatment samples.

Table 1. Friedman's test of the sensory attributes of chocolate spread

Sensory attribute	p-value	Sample						
		R	S1	S3	S5	K1	K3	K5
Odor								
Roasted	0.501	3.82	3.78	4.17	3.84	3.98	4.30	4.10
Nut	0.981	3.94	3.92	4.03	4.15	3.88	4.00	4.07
Earthy	0.008	3.95	3.66	4.08	4.43	3.76	3.65	4.46
Taste								
Sweet	0.603	4.05	4.11	3.84	3.70	4.25	4.07	3.98
Chocolate	0.606	4.05	3.91	4.10	4.18	4.16	3.91	3.67
Nut	0.532	4.34	3.93	3.96	4.12	3.92	3.70	4.03
Bitter	0.045	4.45	3.88	3.68	4.33	4.17	3.62	3.87
Bitter aftertaste	0.931	4.02	4.14	3.83	4.06	3.87	3.95	4.13
Nut aftertaste	0.115	4.27	3.82	3.84	4.38	3.93	3.59	4.18
Texture								
Sandy	0.233	3.54	4.06	4.14	4.29	4.11	3.84	4.03
Oily/greasy	0.453	3.85	4.33	3.99	3.76	3.84	4.03	4.20
Thick	0.000	3.24	4.22	3.80	4.63	4.34	4.02	3.76
Melt	0.007	4.22	3.93	4.43	3.61	3.43	4.13	4.25
Spreadability	0.001	4.41	4.16	4.04	3.39	3.45	4.21	4.33

Note: *P*-value in bold indicates significance at alpha < 0.05. R = control sample; S1 = samples treated with 100 ppm Na-bisulfite; S3 = samples treated with 300 ppm Na-bisulfite; S5 = samples treated with 500 ppm Na-bisulfite; K1 = sample treated with 100 ppm Na-bicarbonate; K3 = samples treated with 300 ppm Na-bicarbonate; K5 = samples treated with 500 ppm Na-bicarbonate

Table 2. The significance test of the Wilcoxon

Sensory attribute	Sample treatment with R					
	S1 - R	S3 - R	S5 - R	K1 - R	K3 - R	K5 - R
Earthy odour	0.252	0.709	0.245	0.257	0.087	0.183
Bitter taste	0.132	0.019	0.954	0.304	0.051	0.082
Thick texture	0.061	0.337	0.763	0.555	0.427	0.470
Melt texture	0.146	0.553	0.010	0.009	0.483	0.917
Spreadability	0.429	0.273	0.003	0.022	0.423	0.826

Note: *P*-value in bold indicates significance at alpha < 0.05 (different from R on some attributes). R = control sample; S1 = samples treated with 100 ppm Na-bisulfite; S3 = samples treated with 300 ppm Na-bisulfite; S5 = samples treated with 500 ppm Na-bisulfite; K1 = sample treated with 100 ppm Na-bicarbonate; K3 = samples treated with 300 ppm Na-bicarbonate; K5 = samples treated with 500 ppm Na-bicarbonate

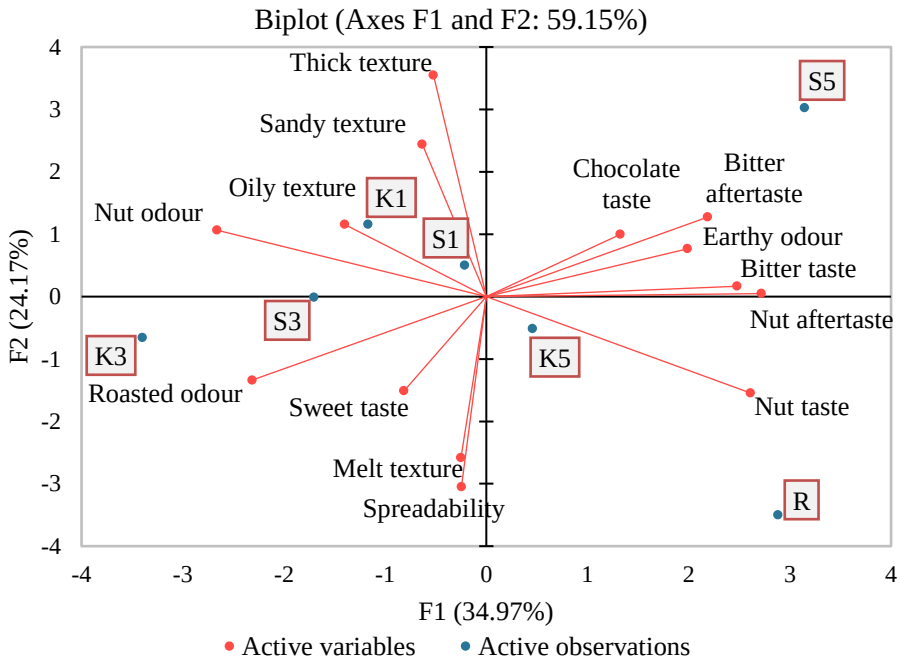

Figure 1. PCA plots of sensory attributes using the RATA method. R = control sample; K1 = sample treated with 100 ppm Na-bicarbonate; K3 = samples treated with 300 ppm Na-bicarbonate; K5 = samples treated with 500 ppm Na-bicarbonate; S1 = samples treated with 100 ppm Na-bisulfite; S3 = samples treated with 300 ppm Na-bisulfite; S5 = samples treated with 500 ppm Na-bisulfite

PCA plots identify the distribution of sensory attributes that differentiate between products. Figure 1 shows that the horizontal axis is associated with taste (chocolate, bitter, aftertaste of bitter and nutty, sweet) and smell (earth, peanut, roasted). The vertical axis is associated with texture, e.g., gritty, viscous, melted and oily. The correlations between the original data and each principal component are computed using the correlation procedure to interpret each component. The interpretation of the principal components relies on identifying the variables that exhibit the strongest correlations with each component. This involves examining which correlation coefficients are large in magnitude, indicating a substantial association with the component, regardless of whether they are positive or negative. In this results, samples K1, K3, and S3 have the same sensory attributes, namely the dominant taste of peanuts, roasted aroma, sweet taste, and oily texture. Samples K5 and R have the same sensory attribute characteristics in the quadrant. At the same time, sample S5 has different characteristics from other samples, such as a bitter taste, bitter aftertaste, chocolate taste, peanut taste, peanut aftertaste and earthy smell.

So, it can be concluded from the sensory test the substitution of candlenut in the chocolate spread detected a bitter taste in the product.

Table 3. Flavonoid and tannin content in candlenut and chocolate spread

Compound	Treatment	Candlenut	Chocolate spread
Flavonoid (mg QE g^{-1})	R	1.433 ± 0.061^{b}	1.796 ± 0.058^{b}
	S1	1.411 ± 0.067^{b}	1.780 ± 0.068^{b}
	S3	1.378 ± 0.050^{b}	1.743 ± 0.048^{b}
	S5	1.341 ± 0.047^{ab}	1.711 ± 0.049^{ab}
	K1	1.346 ± 0.070^{ab}	1.713 ± 0.070^{ab}
	K3	1.221 ± 0.115^{a}	1.589 ± 0.0118^{a}
	K5	1.353 ± 0.036^{b}	1.721 ± 0.036^{b}
Tannins (mg g^{-1})	R	0.173 ± 0.006^{d}	0.480 ± 0.025^{d}
	S1	0.157 ± 0.006^{bc}	0.411 ± 0.089^{c}
	S3	0.151 ± 0.006^{ab}	0.383 ± 0.011^{b}
	S5	0.143 ± 0.002^{a}	0.326 ± 0.023^{a}
	K1	0.163 ± 0.005^{c}	0.410 ± 0.006^{bc}
	K3	0.142 ± 0.007^{a}	0.349 ± 0.011^{a}
	K5	0.146 ± 0.008^{a}	0.384 ± 0.049^{b}

Note: R = control sample; S1 = samples treated with 100 ppm Na-bisulfite; S3 = samples treated with 300 ppm Na-bisulfite; S5 = samples treated with 500 ppm Na-bisulfite; K1 = sample treated with 100 ppm Na-bicarbonate; K3 = samples treated with 300 ppm Na-bicarbonate; K5 = samples treated with 500 ppm Na-bicarbonate

The K3 and S3 treatments could reduce the bitter taste in the product compared to sample R. This is by Gunawan et al. (2020), soaking treatment with sodium bicarbonate can facilitate the process of removing non-volatile compounds from the moringa seed matrix into water due to the alkaline conditions produced. Sodium ions can eliminate the effect of isoflavone, which gives soybeans an undesirable taste. However, using a higher concentration of Na-bisulfite can also give a more dominant aroma and bitter taste effect, as in sample S5.

Table 3 shows that soaking treatment with Na-bicarbonate 300 ppm (K3) can reduce flavonoid levels by 85.21% in candlenut seeds and 88.47% in chocolate spread. Soaking treatment with Na-bicarbonate 300 ppm (K3) and Na-bisulfite 500 ppm (S5) can reduce tannin content in candlenut seeds and chocolate spread. The K3 treatment can reduce tannin levels in candlenut seed and chocolate spread by 82.08% and 72.71%, respectively. Then, the S5 treatment can reduce tannin levels by 82.65% and 67.91% in chocolate spread. The Na-bicarbonate can cause oxidation of flavonoid compounds during soaking. According to Gunawan et al. (2020), soaking treatment with sodium bicarbonate can facilitate removing non-volatile compounds from the moringa seed matrix into water because of the alkaline conditions produced. The soaking process produces CO_2 gas due to the reaction of sodium bicarbonate with water. This gas will enter and form cavities in the seeds, resulting in tissue damage or softening, which can cause oxidation of non-volatile compounds in the material. Then, sodium ions depromote the activity of isoflavones in soya beans (Bollegala and Rajapakse, 2015). Likewise with the effect of soaking using sodium bisulfite. Sodium bisulfite reacts with water to produce alkaline conditions and SO_2 gas. It has the same working system as CO_2 gas, but too much SO_2 gas is produced, which can cause a bitter taste (Sulaswatty and Roestamsjah, 1991). Therefore, using S5 treatment on samples produces a sensory profile that is different from the others.

LC-MS testing was used to identify specific compounds contained in five samples. Based on the LC-MS data tentative in Table 4. In peaks of the chromatogram in Figure 2, 27 compounds were obtained in all samples. Most of the identified compounds belonged to the flavonoid group; a small portion were tannin and alkaloid. These flavonoid compounds are thought to cause a bitter taste in the sample. It is in line with Drewnowski and Gomez-Carneros (2000); Valls et al. (2009) that these compounds also cause a bitter taste in breadfruit (Widowati et al., 2019), moringa leaves (Gunawan et al., 2020) and other nuts (Kimani et al., 2019). However, the research hypothesis that flavonoid and tannin compounds cause a bitter taste in candlenut seed and chocolate spread with candlenut has been proven to be true.

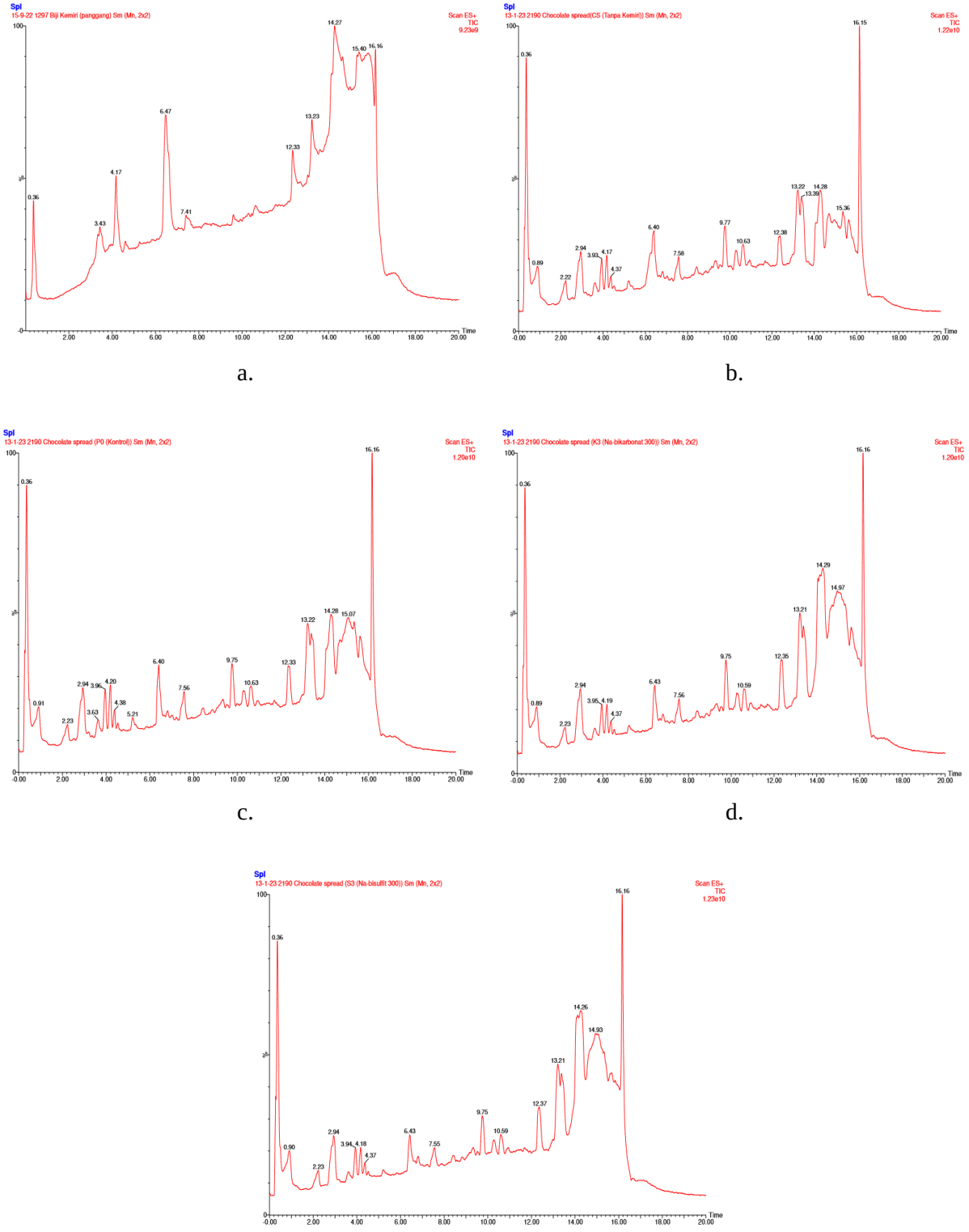

Figure 2. Peak of chromatogram. a) candlenut sample; b) chocolate spread; c) chocolate spread with candlenut; d) samples treated with 300 ppm Na-bicarbonate; e) samples treated with 300 ppm Na-bisulfite

Table 4. Identification of determinant compounds in a sample

Base peak (m/z)	MW (g mol^{-1})	Molecular formula	Tentative identification	Fragmentasi m/z	Sample					References
					Candlenut seed	CS without candlenut	CS (R)	CS (K3)	CS (S3)	
Alkaloid										
181.4	180.16	$C_7H_8N_4O_2$	Theobromine	182; 181	-	√	√	√	√	Marcucci et al.
195.4	194.18	$C_8H_{10}N_4O_2$	Caffeine	196	-	√	√	√	√	(2021); Pubchem
83.39	356.2	$C_{21}H_{26}NO_4$	Tetrahyrophal matine (THP)	356; 337; 279	-	-	√	√	-	Singh et al. (2015)
Flavonols and flavone										
60.2	302.33	$C_{15}H_{10}O_7$	Quercetin	302; 288	√	-	-	-	-	Barbosa-Pereira et al. (2021)
60.2	758.6	$C_{32}H_{38}O_{21}$	Quercetin 3-O-alpha	338; 523; 551; 758	√	-	-	-	-	PubChem
83.32	927.6	$C_{30}H_{42}N_7O_{19}$	Quercetin 3-glucoside dimer	453; 927	-	-	-	-	√	PubChem
60.2	662.6	$C_{34}H_{30}O_{14}$	Caemferol 3	226; 295; 576; 663	√	-	-	-	-	Hussain et al. (2018)
83.32	814.96	$C_{37}H_{34}O_{21}$	Luteolin	816; 815	-	√	√	√	√	Marczak et al. (2016)
Tannin										
6.47	289	$C_{15}H_{14}O_6$	(E) catechin	230;274;275;290	√	-	-	-	-	Barbosa-Pereira et al. (2021)
143.15	578.14	$C_{30}H_{26}O_{12}$	Procyanidin B2 / (E) catechin-(E) catechin		-	√	√	-	-	Massbank; Calderón et al. (2009)
152.33	782.5	$C_{34}H_{22}O_{22}$	Punicalin/ellagitannin		-	-	-	√	√	Man et al. (2022) Pubchem

Base peak (m/z)	MW (g mol^{-1})	Molecular formula	Tentative identification	Fragmentasi m/z	Sample					References
					Candlenut seed	CS without candlenut	CS (R)	CS (K3)	CS (S3)	
Anthocyanins										
60.2	476.4	-	Petunidin 3-galactose	453; 475; 491;273;	√	-	-	-	-	PubChem
83.39	697.6	$C_{31}H_{37}O_{18}$	Malvidin 3-5-glucoside	696.96; 534.81	-	-	√	-	-	Sharma et al. (2015)
152.33	818.89	$C_{55}H_{61}O_{29}$	Cyanidin 3-5-glucoside	818.89; 665.9; 162.3	-	-	√	-	-	Sharma et al. (2015)
Hydroxycinnamic acid										
60.1	338.31	$C_{16}H_{18}O_8$	3-O-coumaroylquinic acid	337	√	-	-	-	-	Ana Plazonić et al. (2009)
60.2	296.23	$C_{13}H_{12}O_8$	Caffeoylmalic acid	251; 219	√	-	-	-	-	Świątek et al. (2021)
381	705	-	3.9-di-caffeoyl-2.7-anhydro-3-deoxy	705.59; 543.5; 381.4; 365.4	-	√	√	√	√	Zhang et al. (2007)
83.32	365.34	$C_{17}H_{10}O_9$	Feruloyl-caffeoylquinic acid	365.3; 246.58	-	-	√	-	-	Simirgiotis et al. (2015)
83.32	353.5	$C_{16}H_{18}O_9$	Chlorogenic acid	353.54	-	-	√	√	√	Sánchez-Rabaneda et al. (2003)
Other flavonoid										
83.23	701		Oleuropein glucoside		√	√	√	√	√	Silva et al. (2006); Ruslin et al. (2022); Pubchem
83.39	453.87	-	Resveratrol dimer		-	-	√	-	-	Ruslin et al. (2022)
143.4	783	-	di-Galloyl hexahydroxid		-	-	-	√	√	Gordon et al. (2011)
83.25	362.4	$C_{21}H_{30}O_5$	Iso humulones	230; 274; 363	-	√	√	-	-	Ruslin et al. (2022)
83.39	588.5	$C_{29}H_{30}O_{13}$	Kolaflavone		-	√	-	-	-	Reed (2009)
83.32	520.7	$C_{30}H_{18}O_{10}$	4’dehydroxyamentoflavone	542;521;478	-	-	√	√	√	Yao et al. (2017)
274	362.6	$C_{20}H_{26}O_6$	secorsolariciresinol	274; 318; 362	-	-	-	√	√	Cádiz-Gurrea et al. (2017)

Base peak (m/z)	MW (g mol^{-1})	Molecular formula	Tentative identification	Fragmentasi m/z	Sample					References
					Candlenut seed	CS without candlenut	CS (R)	CS (K3)	CS (S3)	
Unidentified										
	83.32	-	-	512; 337; 279; 263; 153	-	-	-	-	√	
	83.39	-	-	247; 266; 339; 357; 554	-	√	-	-	-	
	14.25	-	-	239; 265; 325; 406	-	√	-	-	-	
	15.35	-	-	226; 413; 455; 928; 944	-	√	-	-	-	
782.94	803	-	-		-	-	-	√	√	

Note: MW = molecular weight. R = control sample; K3 = samples treated with 300 ppm Na-bicarbonate; S3 = samples treated with 300 ppm Na-bisulfite

Further research was carried out using the website reference by Bagler (2017) or https://cosylab.iiitd.edu.in/flavordb/search to find out the specifications of which compounds cause the bitter taste. A group of flavonoids detected to cause a bitter taste were quercetin, oleuropein, kaemferol, resveratrol dimer, and luteolin. Meanwhile, tannin compounds were detected that cause a bitter taste, namely epicatechin. A part from that, there are compounds theobromine, caffeine, iso humolones, colaflavone, and chlorogenic acid which also cause a bitter taste in the sample.

Chromatography using LC-MS may also be useful in quantifying each type of flavonoid compound detected in this study. However, as mentioned, this study focuses on reducing the bitter taste of the chocolate spread formulated with candlenuts. As discussed, flavonoid is the key compound of the bitterness in the product. In fact, there is no study differing the bitterness level of different flavonoid compounds, meaning that all types of flavonoids are believed to contribute in similar levels of bitterness. Therefore, it is unnecessary to quantify each type of flavonoid by using LC-MS. Thus, "total flavonoid content" analysis by spectrophotometry is sufficient to examine the effect of pre-treatment on the reduction of bitterness level (i.e., flavonoid content).

CONCLUSIONS

Based on sensory analysis, 14 sensory attributes were identified as the sensory profile of candlenut spread chocolate, one of which was bitter taste. The application of K3 and S3 treatment had a significant effect in reducing the bitter taste of the samples. Meanwhile, in chemical analysis, the application of K3 treatment reduced the levels of flavonoids and tannins. The LC-MS test results identified 27 compounds, 13 of which were determinant compounds that cause bitter taste. This research is useful for the food industry dealing with bitter raw materials and the rheological properties of innovative chocolate products.

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