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Physicochemical properties of caramel toffee products supplemented with whey protein and rice bran hydrolysate

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Abstract A caramel toffee product (CTP) supplemented with whey protein concentrate (WPC) and rice bran hydrolysate (RBH) produced in pilot scale process was investigated. Three recipes of CTPs were prepared using different WPC levels (0.0, 2.0, and 5.0%). The sensory attributes were then evaluated using a 9-point hedonic scale. The results showed that the addition of WPC at 2.0% had no negative effect on all sensory attributes, with an overall acceptability score of 7.73 (Like moderately to like very much), when compared with the CTP without the addition of WPC (control). Therefore, 2.0% WPC was selected for further production of CTPs supplemented with different RBH concentrations (0.0-2.0%). α-Amino contents and antioxidant activities of CTPs were determined. The CTP supplemented with 2%RBH plus 2.0% WPC (CTP_2% RBH_WPC) exhibited the highest α-amino content (0.47 mg Leucine Eq./g sample), ABTS radical scavenging activity (0.88 mg Trolox Eq./g sample) and ferric reducing antioxidant power (0.52 mg Trolox Eq./g sample). However, the CTP_2% RBH_WPC had lower acceptability by panelists than that of control (0% RBH). Therefore, the CTP_2% RBH_WPC was selected for further development by adding vanilla and caramel flavors. The a_w, color, chemical compositions, sensory evaluation (n=311), and microbial load of flavored CTP were then determined. The a_w, moisture content, and L*, a*, and b* values were 0.45, 3.90%, 57.90, 12.47, and 21.62, respectively. Protein, fat, ash, and carbohydrate of the flavored CTP were 2.60, 15.10, 1.12, and 77.33%, respectively. The overall acceptability score of the flavored CTP was 7.57 and the microbial load were within the Thai community product standard of Toffee. These results suggested that WPC and RBH have potential as nutritive and antioxidative substance supplementation in the caramel toffee product.

Keywords: Caramel toffee products, Whey protein, Rice bran hydrolysate, Antioxidant activity, Consumer test

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Introduction

Toffees are chewable confectionery items containing sugar, milk solids, and butter or vegetable fat as major ingredients (Bhokre *et al.*, 2010). Toffees are made by caramelizing sugar or molasses (creating inverted sugar) along with butter, and milk solids. The unique thing about toffees is "Maillard Reaction" which is manifested by interaction between reducing sugar (glucose and/or lactose) and milk protein content (Xiang *et al.*, 2021). Maillard reaction plays an important role in the formation of flavors and colors of some confectionery products. In addition, Maillard reaction products (MRPs) also exhibit an antioxidant activity (Nooshkam *et al.*, 2019; Wang *et al.*, 2019). An antioxidant is a molecule capable of inhibiting the oxidation of other molecules and prevents free radical-induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition (Lobo *et al.*, 2010). However, toffee products cannot be attributed to healthy foods because they have a low nutritional value and high sugar content. Thus, many researchers have attempted to develop toffees by supplementing them with functional ingredients to improve their nutritive value. For example, Mazur *et al.* (2018) reported that the candy caramel with plant extracts exhibited an antioxidant activity due to the presence of total phenolic compounds. Gehlot *et al.* (2018) developed the taste and nutritive value of fruit toffee using a mixture of guava and mango blends. Kohinkar *et al.* (2014) developed a mixed fruit toffee from Fig and Guava fruits. Khapre (2010) developed a guava – soybean toffee as a protein enriched product. Therefore, incorporating toffee with nutritive ingredients and antioxidant substances should continue to be sought.

Milk whey proteins are one of the most valued constituents due to their nutritional and techno-functional attributes which are rich in bioactive peptides such as antioxidant, antihypertensive, and antimicrobial activities (Minj and Anand, 2020). Whey protein is separated from the casein in milk or created as a byproduct from cheese production (Sangwan and Seth, 2021). Milk whey contains high levels of amino acids that are readily available and easy to digest, making it effective to incorporate into body cells (Smithers, 2008). Whey powder is used to make whey toffees which provide protein for Maillard browning reaction to caramel color and flavor, in particular the whey proteins (Mohos, 2010). In addition, it also helps in emulsification. Therefore, incorporating toffee with whey protein could increase the nutritive value and the formation of MRPs with antioxidant activity.

Besides, it has been reported that some food protein hydrolysates, derived from various food-derived plants, animals, and marine protein sources, have been reported to exhibit antioxidant activity such as Atlantic salmon (Auwal *et* *al.*, 2017), sesame protein (Lu *et al.*, 2019), rice bran protein (Wattanasiritham *et al.*, 2016), perilla seed protein (Yang *et al.*, 2018), soybean (Chen *et al.*, 2018). Protein hydrolysates which mainly compose of antioxidant peptides have gained growing interest as potential natural antioxidants due to their little or no negative side effects, lower molecular weight, easy absorption, high activity, and low cost (Sarmadi and Ismail, 2010; Liao *et al.*, 2020). The use of protein sources from byproducts to produce antioxidant peptides could increase the value of byproducts. Our previous study revealed that a rice bran hydrolysate (RBH) prepared by Alcalase exhibited an antioxidant activity and could be applied in broken rice beverage and milk tablets with antioxidant properties (Khongla *et al.*, 2022a,b). Thus, in this work, the use of RBH incorporation in toffees could increase their antioxidant properties.

Therefore, the objective was to develop caramel toffee products (CTP) supplemented with whey protein and rice bran hydrolysate. In addition, physicochemical properties including a_w, color values, sensory attributes, chemical compositions, α-amino contents, and antioxidant activities of the CTP were determined. Furthermore, consumer acceptability, purchase test, and microbiological qualities of the developed CTP were investigated.

Materials and methods

Materials

The rice bran hydrolysate powder (RBH) was prepared using Alcalase in a pilot scale process (moisture of 9.57%, ash of 11.00%, fat of 9.64%, protein of 16.14% and carbohydrate of 53.65%, total phenolic compounds of 0.70 mg gallic acid Eq./g RBH, α-amino contents of 51.56 mg Leucine Eq./g RBH, ABTS radical scavenging activity of 20.17 mg Trolox Eq./g RBH, and FRAP value of 5.77 mg Trolox Eq./g RBH) which was obtained from our previous work (Khongla *et al.*, 2022b). 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2, 4, 6-tripyridyl-s-triazine (TPTZ) were purchased from Biochemika (Buchs, Switzerland). Trinitrobenzenesulfonic acid (TNBS) and 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals and reagents used were of analytical grade.

Development of caramel toffee products (CTPs) supplemented with whey protein concentrate (WPC)

Preparation of CTPs

Ingredients of 500 g of caramel toffees supplemented with various WPC levels (0.0, 2.0, and 5.0% w/w) were prepared as described in Table 1. Salted butter was melted in brass pan by a gas flame. Solid sugar was then added and mixed until a homogeneous mixture was obtained. Sweetened condensed milk was added in the mixture, and whey protein concentrates (WPC) at 0, 2.0, and 5.0% were added and continued to mix. Glucose syrup was added. The CTP was poured into a plastic mold and stored at 4 ℃ for 20 min, cut into small pieces and wrapped in plastic bag for determination of a_w, moisture, color values, and sensory evaluation.

Table 1. Ingredients of caramel toffee products supplemented with various whey protein levels (0, 2.0, and 5.0% w/w)

Ingredients	**Content (g)**		
	0% WPC	**2.0% WPC**	**5.0% WPC**
Glucose syrup	132	132	127
Sugar	130	120	120
Salted butter	92	92	87
Sweetened condensed milk	146	146	141
Whey protein concentrate	0	10	25
Total	**500**	**500**	**500**

a_w, moisture and color values

The a_w of CTPs supplemented with WPC at concentration of 0, 2.0, and 5.0% was measured at 25±2 ℃ by using a water activity meter (AQUA LAB 4TE dew point, Pullman, WA, USA). Samples were cut into small pieces and placed in the sample holder of the water activity meter. All measurements were performed in triplicate. The moisture content of CTPs was measured using hot air oven according to the method of AOAC (2000). The color values of CTPs were recorded in terms of CIE L*, a*, b* values using a color measurement device (Hunter Lab, Color flex 4510, USA), where L*, a*, and b* was lightness, redness, and yellowness, respectively.

Sensory evaluation

Sensory attributes (color, odor, taste, texture, and overall acceptability) of CTPs supplemented with WPC at concentrations of 0, 2.0, and 5.0% were evaluated by 30 untrained panelists using a preference test with a 9-point hedonic scale (1= dislike extremely, 9= like extremely). The untrained panel of assessors selected comprised of 15 female and 15 male panelists aged between 17 to 25. All panelists declared no food allergies.

Development of CTPs supplemented with various levels of RBH (0.0-2.0%) plus 2.0% WPC

Ingredients of caramel toffees supplemented with various RBH levels (0.0-2.0 %w/w) plus 2.0% WPC were prepared as described in Table 2. Salted butter was heated in brass pan by a gas flame until melted. Solid sugar was then added and mixed until a homogeneous mixture was obtained. Sweetened condensed milk was added in the mixture and 2.0% WPC and various concentrations of RBHs (0.0-2.0%) were added and continued to mix. Glucose syrup was added to the mixture and mixed until a homogeneous mixture was obtained. The CTP was poured into a plastic tray and stored at 4 ℃ for 20 min. The CTP was cut into small pieces, wrapped in plastic bag, and used for determining α-Amino content and antioxidant activities as described below, and a_w, moisture, color values, and sensory evaluation as mentioned before.

Table 2. Ingredients of CTPs supplemented with various RBH levels (0, 0.5, 1.0, 1.5, and 2.0 %w/w) plus 2.0% WPC

Ingredients	Content (g)				
	0%RBH	**0.5%RBH**	**1.0%RBH**	**1.5%RBH**	**2.0%RBH**
Glucose syrup	132	132	132	132	132
Sugar	120.0	117.5	115.0	112.5	110.0
Salted butter	92	92	92	92	92
Sweetened condensed milk	146	146	146	146	146
Whey protein concentrate	10	10	10	10	10
RBH	0	2.5	5	7.5	10
Total	500	500	500	500	500

α-Amino content and antioxidant activities assays

Five grams of CTPs was mixed with 25 ml of 75% Ethanol. The mixture was homogenized at 8,000 rpm for 1 min and then filtered by using Whatman no.1. The filtrate was collected, and the α-amino content determined using trinitrobenzenesulfonic acid (TNBS) with L-leucine as a standard (Adler-Nissen, 1979). The α-amino content was expressed as mg Leucine equivalents (Eq.) /g sample.

ABTS radical scavenging activity of the filtrate was determined according to the method of Wiriyaphan *et al.* (2012). In brief, 20 µl of the filtrate was mixed with ABTS working solution (Absorbance of 0.700±0.02) and incubated in the dark for 5 min. The absorbance was measured at 734 nm. The result was expressed as mg Trolox equivalents (Eq.)/g sample.

The ferric reducing antioxidant power (FRAP) of the filtrate was measured according to the method of Benzie and Strain (1996). In brief, 100 µl of supernatants were mixed with FRAP working solution. The mixtures were incubated at 37 ℃ for 15 min in water bath. The absorbance was measured at 593 nm. The results were expressed as mg Trolox Eq./ g sample.

Flavor development of CTP by the addition of vanilla and caramel flavors

The CTP supplemented with 2.0% RBH exhibited the highest antioxidant activity and thereby was selected for further development by adding 30 ml of caramel and 15 ml of vanilla flavors into the main composition to improve the acceptability of products. The product was referred to as flavored CTP. Physicochemical properties including, a_w, color, chemical compositions (moisture, crude protein, crude fat, and crude ash), microbiological quality test, and consumer and purchase tests of flavored CTP were determined as described below.

Physicochemical properties

The a_w and color values of flavored CTP were determined as mentioned before. Chemical compositions including moisture, crude protein, crude fat, and crude ash of flavored CTP were determined according to AOAC (2000).

Microbiological quality test

Microbiological qualities (Total plate count, *Staphylococcus aureus*, *Salmonella* spp., and *E. coli*) of flavored CTP were determined following the United States Food and Drug Administration (U.S. FDA) Biological Analytical Manual (BAM).

Consumer and purchase tests

A consumer panel of 311 volunteers was recruited from the Rajamangala University of Technology Isan. The untrained panel of selected assessors comprised of 161 female and 150 male panelists. Of the panelists, 78% aged between 17 to 25, 11% aged between 26 to 35, and 8% aged below 17. The untrained panel evaluated the acceptability of color, odor, taste, texture, and overall acceptability of the flavored CTP using a preference test with a 9-point hedonic scale (1= dislike extremely, 9= like extremely). All panelists declared no food allergies. In addition, purchase intention test was done by using the question "Would you buy it?" accompanied by a structured scale of 4 points ("No", "Yes", "Maybe buy", and "Maybe not buy").

Statistical analyses

Statistical analyses were carried out using a SPSS package (SPSS 17.0 for Windows, SPSS Inc., Chicago, IL, USA). The results were analyzed using a one-way analysis of variance (ANOVA) at $p < 0.05$. Duncan's multiple-range test (DMRT) was used to determine significant differences of means at the 95% confidence level. Sensory evaluation was analyzed using a randomized complete block design (RCBD). The a_w, moisture content (%), color values, α-Amino content, and antioxidant activity were performed using completely randomized design (CRD).

Results

Development of caramel toffees supplemented with various WPC levels

a_w, moisture, and color values

The a_w of caramel toffee product (CTP) with 5.0% WPC was 0.5083 which was significantly lower than those with 0% WPC (0.5478) and 2.0% WPC (0.5496) ($p<0.05$, Table 3). The moisture content of CTP with 2.0% WPC (4.25%) and 5.0% WPC (3.82%) was not significantly different when compared to 0% WPC (4.01%) ($p\geq0.05$). The CTP with 5.0% WPC presented the lowest L* value and the highest a* and b* values (Table 3).

Table 3. a_w, moisture content (%), and color values of caramel toffees supplemented with 0.0, 2.0, and 5.0% (w/w) of WPC

Treatments	a_w	Moisture content (%)	Color values		
			L*	a*	b*
0.0% WPC	0.5478 ± 0.0030^{a}	4.01 ± 0.08^{ab}	62.94 ± 0.18^{b}	8.52 ± 0.05^{b}	19.36 ± 0.13^{c}
2.0% WPC	0.5496 ± 0.0035^{a}	4.25 ± 0.26^{a}	63.28 ± 0.16^{a}	8.31 ± 0.03^{c}	20.38 ± 0.06^{b}
5.0% WPC	0.5083 ± 0.0078^{b}	3.82 ± 0.15^{b}	59.92 ± 0.04^{c}	10.19 ± 0.04^{a}	21.54 ± 0.05^{a}

Different superscripts within a column indicate significant differences ($p<0.05$); 0.0% WPC was caramel toffee without the addition of whey protein concentrate; 2.0% WPC was caramel toffee with the addition of 2.0% whey protein concentrate; 5.0% WPC was caramel toffee with the addition of 5.0% whey protein concentrate.

Sensory evaluation

All sensory attributes scores of CTP with 2.0% WPC were not significantly different with the CTP without the addition of WPC ($p\geq0.05$), with an overall acceptability score of 7.73 (like moderately to like very much) (Table 4). However, the addition of WPC at the concentration of 5.0%

decreased texture and overall acceptability of the CTP, with scores of 6.07 and 7.10, respectively (Table 4). Therefore, 2.0% WPC was selected to incorporate in CTPs supplemented with different rice bran hydrolysate (RBH) levels.

Table 4. Sensory evaluation of CTPs supplemented with 0.0, 2.0, and 5.0% (w/w) of WPC

Treatments	Color [ns]	Odor [ns]	Taste [ns]	Texture	Overall acceptability [ns]
0.0% WPC	7.57 ±0.97	7.43±1.07	7.87±1.11	7.07±1.58[a]	7.73±1.11[a]
2.0% WPC	7.77±0.94	7.53±1.07	7.43±1.25	7.4±1.59[a]	7.73±1.11[a]
5.0% WPC	7.53±0.90	7.23±1.16	7.33±1.27	6.07±2.35[b]	7.10±1.27[b]

N = 30

Different superscripts within a column indicate significant differences ($p<0.05$); ns means not significantly different in the same column ($p\geq0.05$); 0% WPC was caramel toffee without the addition of whey protein concentrate; 2.0%WPC was caramel toffee with the addition of 2% whey protein concentrate; 5.0% WPC was caramel toffee with the addition of 5.0% whey protein concentrate.

Development of caramel toffee products supplemented with various RBH levels (0.0-2.0%) plus 2.0% WPC

a_w, moisture content, and color values

The a_w and moisture content of CTPs tended to decrease with an increase in RBH levels where the a_w ranged from 0.5313-0.5496 (Table 5). The addition of 2.0% RBH resulted to a CTP with the lowest a_w. The moisture content of CTPs ranged from 2.51-4.25% which was within the Thai Community Product Standard (TCPS 265/2559; Toffee) (moisture content lower than 12%). L* (lightness) of CTPs tended to decrease with an increase in RBH levels. The CTP incorporated with 2.0% RBH had the lowest L* value and the highest a* (redness). The b* value (yellowness) of CTPs ranged from 20.38-21.90 (Table 5).

Table 5. a_w, moisture content (%), and color values of CTPs supplemented with RBH at levels plus 2.0% WPC

Treatments	a_w	Moisture content (%)	Color values		
			L*	a*	b*
0.0% RBH	0.5496±0.0035[a]	4.25±0.26[a]	63.28±0.16[a]	8.31±0.03[c]	20.38±0.06[c]
0.5% RBH	0.5466±0.0032[ab]	3.56±0.27[b]	60.94±0.06[b]	7.81±0.18[d]	21.90±0.14[a]
1.0% RBH	0.5434±0.0013[ab]	3.31±0.12[b]	58.61±0.04[c]	9.43±0.05[b]	21.14±0.18[b]
1.5% RBH	0.5450±0.0031[b]	2.60±0.05[c]	57.29±0.13[d]	9.46±0.02[b]	20.55±0.14[c]
2.0% RBH	0.5313±0.0034[c]	2.51±0.44[c]	55.40±0.02[e]	10.18±0.04[a]	20.38±0.06[c]

Different superscripts within a column indicate significant differences ($p<0.05$).

Sensory attributes

The color attribute score of CTPs supplemented with 0.5-2.0% RBH was significantly lower than the CTP without the addition of RBH ($p<0.05$, Table 6). Texture attribute score of CTPs supplemented with 1.5-2.0% RBH was significantly lower than the 0% RBH. The addition of 1.0-2.0% into CTPs decreased the odor, taste, and overall acceptability attributes score of products. The CTPs without RBH and with 0.5% RBH exhibited an overall acceptability attribute score of 7.73 (like moderately to like very much), while the CTPs with 1.0-2.0%RBH exhibited an overall acceptability attribute score of 6.63-6.80 which was described as like a little bit to like moderately (Table 6).

Table 6. Sensory attributes score of CTPs supplemented with RBH at levels of 0.0-2.0% plus 2.0% WPC

Treatments	Color	Odor	Taste	Texture	Overall acceptability
0.0% RBH	7.77 ±0.94[a]	7.53 ±1.07[a]	7.43 ±1.25[a]	7.40 ±1.59[a]	7.73 ±1.11[a]
0.5% RBH	7.00 ±1.36[b]	7.40 ±1.33[a]	7.63 ±1.07[a]	7.27 ±1.14[a]	7.73 ±0.87[a]
1.0% RBH	6.53 ±1.83[b]	6.00 ±1.95[b]	6.70 ±1.60[b]	6.67 ±1.69[ab]	6.80 ±1.67[b]
1.5% RBH	6.67 ±1.69[b]	5.93 ±2.05[b]	6.60 ±1.57[b]	6.37 ±1.73[b]	6.73 ±1.64[b]
2.0% RBH	6.43 ±1.63[b]	6.10 ±1.83[b]	6.23 ±1.72[b]	6.10 ±2.09[b]	6.63 ±1.59[b]

N = 30

Different superscripts within a column indicate significant differences ($p<0.05$).

α-Amino content and antioxidant activity

α-Amino content of CTPs supplemented with 0.5 and 1.0% was not significantly different from 0.0% RBH ($p\geq0.05$). However, the α-amino content increased after the addition of 1.5% RBH and reached postulate when the concentration of RBH increased up to 2.0% (Figure 1a). α-Amino content of the CTP incorporated with 2.0% RBH was 0.47 mg Leucine Eq./g sample (Figure 1a). ABTS radical scavenging activity and FRAP values of the CTPs tended to increase with an increase in RBH levels (Figure 1b and c). The addition of 2.0% RBH exhibited the highest ABTS radical scavenging activity and FRAP values with values of 0.88 and 0.52 mg Trolox Eq./g sample, respectively (Figure 1b and c).

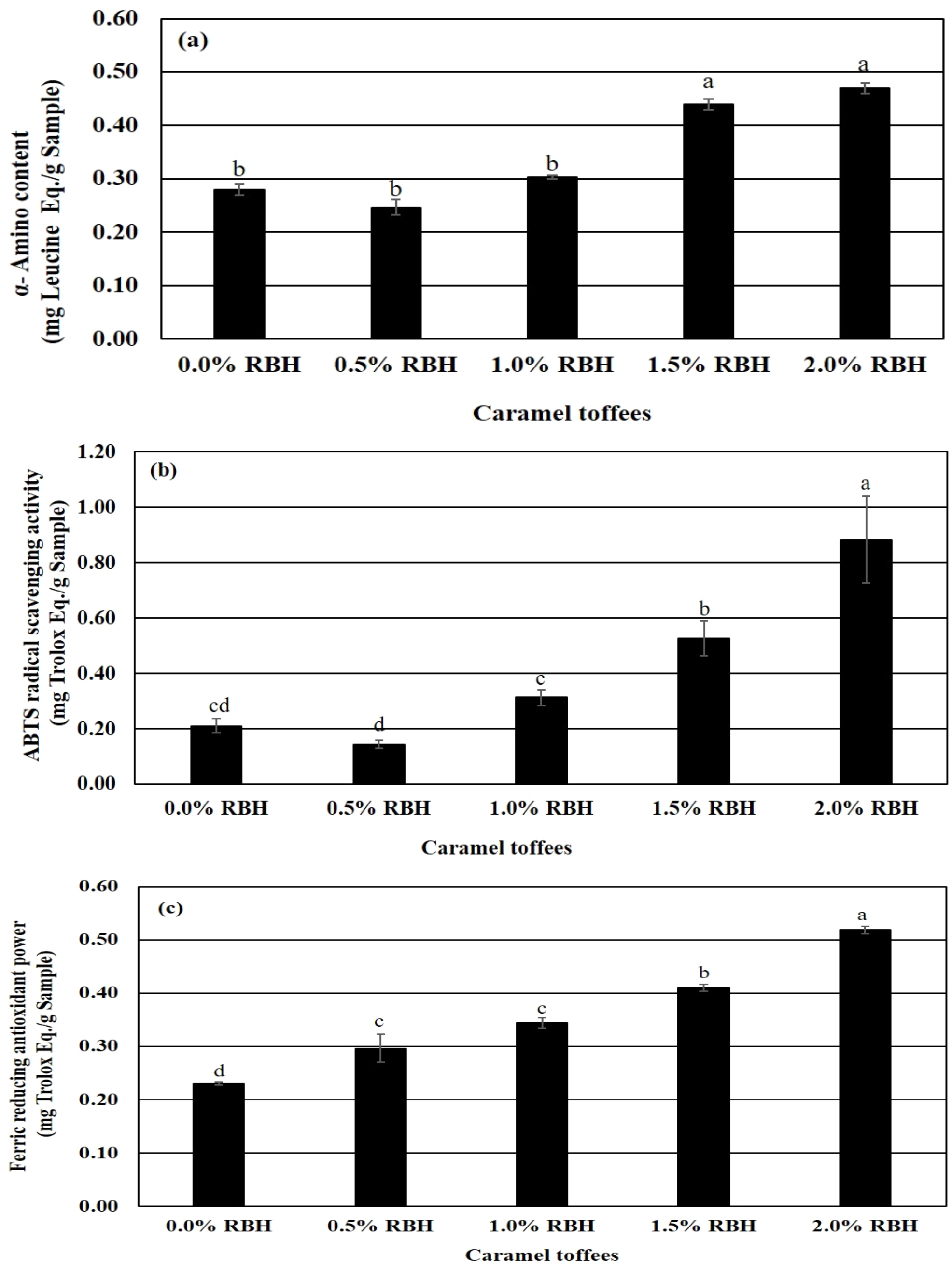

Figure 1 α-Amino content (a), ABTS radical scavenging activity (b), and ferric reducing antioxidant power (c) of caramel toffees supplemented with 2% WPC plus RBH at levels of 0.0, 0.5, 1.0, 1.5, and 2.0%

Bars with different letters indicate means with significant differences ($p<0.05$).

Development of the CTP supplemented with 2.0% RBH plus 2.0% WPC by adding Vanilla and caramel flavors

Due to higher antioxidant activity and lower sensory attributes score of the CTP supplemented with 2.0% RBH plus 2.0% WPC, it was further developed by adding vanilla and caramel flavors. The product was referred to as flavored CTP. The moisture content and a_w of flavored CTP were 3.9% and 0.45, respectively, which was within the standard limits of toffee (no more than 12%) (Table 7). L*, a*, and b* of the flavored CTP were 57.90, 12.47, and 21.62, respectively. Ash, crude fat, crude protein, and total carbohydrate of flavored caramel were 1.12, 15.10, 2.60, and 77.33, respectively (Table 7).

Table 7. Physicochemical properties of the flavored CTP

Physicochemical properties	Content
Moisture (%w.b.)	3.90 ±0.09
Ash (%w.b.)	1.12 ±0.29
Crude fat (%w.b.)	15.10 ±0.35
Crude protein (%w.b.)	2.60 ±0.08
Total carbohydrate (%w.b.)	77.33 ±0.00
a_w	0.4497 ±0.0022
Color values	
L*	57.90 ±0.17
a*	12.47 ±0.27
b*	21.62 ±0.14

Microbiological qualities

Total plate count of flavored CTP was lower than 2×10^3 CFU/g. Yeasts and Molds, *E. coli*, *S. aureus*, and *Salmonella* spp. were not detected in 1 and 25 g of flavored CTP, respectively (Table 8).

Table 8. Microbiological qualities of flavored caramel toffee supplemented with 2% RBH

Microbiological Test	Microbiological qualities	
	Flavored caramel toffee	Standard
Total Plate Count	< 2 $\times 10^3$ CFU/g	< 1 $\times 10^6$ CFU/g
Yeasts and Molds	Not detected in 1 g	< 100 CFU/g
E. coli	Not detected in 1 g	< 3 MPN /g
S. aureus	Not detected in 1 g	< 10 CFU/g
Salmonella spp.	Not detected in 25 g	Not detected in 25 g

Consumer and purchase tests

Sensory attributes score including color, odor, and texture of flavored CTP ranged from 7.21-7.45, which was described as like moderately. The taste and overall acceptability of flavored CTP were 7.70 and 7.57, respectively, which was described as like moderately to like very much (Table 9). Purchase test of flavored CTP supplemented with 2% RBH is shown in Table 10. Of the panelists, 48.87% answered “I would buy it”; 46.94% of panelists answered “maybe buy it”; only 4.18% of panelists answered “maybe not buy it”; nobody answered "I would not buy it" to the poll.

Table 9. Consumer test of flavored caramel toffee supplemented with 2% RBH

Sensory attributes	Score (Means ± SD)	Description
Color	7.42 ± 0.89	Like Moderately
Odor	7.45 ±1.00	Like Moderately
Taste	7.70 ± 0.92	Like Moderately to Like very much
Texture	7.21 ± 1.23	Like Moderately
Overall acceptability	7.57 ± 1.00	Like Moderately to Like very much

N = 311

Table 10. Purchase test of flavored CTP supplemented with 2% RBH

Purchase test	Number of People	Percentage
“I would buy it”	152	48.87
“maybe buy it”	146	46.94
“maybe not buy it”	13	4.18
"I would not buy it"	0	0.00

N = 311

Discussion

Development of caramel toffees supplemented with various WPC levels

Water is one of the most important components of confections, and of most foods, and is called moisture content in the food industry. The moisture content affects the physical or textural characteristics of the food product (Ergun *et al.,* 2010) while the amount of free water could be described as water activity (a_w). Both are the critical factors affecting food quality and safety as well as the food’s shelf stability (Ergun *et al.*, 2010). The a_w of the CTP with the addition of 5.0% WPC was the lowest due to lower glucose syrup, salted butter, sweetened condensed milk, and higher WPC levels than the others. The moisture content of the CTP with the addition of 2.0 and 5.0% WPC was not significantly different when compared to 0.0% CTP ($p \geq 0.05$). This might be due to lower solid sugar content of 2.0% and 5.0% WPC than 0.0% CTP (Table 1), therefore, the addition of WPC might have contributed to the retention of moisture content in 2.0% and 5.0% WPC, resulting in no difference in moisture content between 2.0% and 5.0% WPC and 0.0% CTP. Thai Community

Product Standard suggested that moisture content in toffee do not contain more than 12% (TCPS 265/2559; Toffee). In our experiments, the moisture content of CTPs ranged from 3.8-4.0%, which was within the Thai Community Product Standard. In addition, CTPs were classified as dried foods that generally do not contain more than 25% moisture content and have an a_w between 0.00 and 0.60 (Jay *et al.*, 2005).

Color plays an essential role in food appearance and is one of the most important factors in determining consumer acceptance of food (Corradini, 2019). The addition of 5.0% WPC in CTP decreased L* value, and increased a* and b* values of CTPs which indicated a darker, redder, and more yellow color of 5.0% WPC than others, respectively. The decrease in L* and increase in a* values could have been probably due to the Maillard and caramelization reactions occuring during the heating process of caramel toffee. Mohos (2010) reported that milk proteins were also essential to caramel color and flavor, in particular the whey proteins, via the Maillard browning reaction. On the other hand, the increase in b* of CTP with 5.0% WPC could have been due to the yellow color of whey protein concentrate powder, making the caramel toffees more yellow.

The addition of WPC at concentration of 5.0% had a negative effect on the texture and overall acceptability of the CTP), with scores of 6.07 and 7.10, respectively. Mendenhall and Hartel (2016) reported that higher protein caramels (4% and 7%) rapidly formed much larger aggregates than lower protein samples resulting in producing harder and tougher caramels. These might have contributed to lower texture and overall acceptability attributes score of the CTP with 5.0% WPC.

Development of caramel toffee products supplemented with various RBH levels (0.0-2.0%) plus 2.0% WPC

Based on the sensory evaluation, 2.0% WPC was selected to be incorporated in CTPs supplemented with different rice bran hydrolysate (RBH) levels. The results showed that the addition of 2.0% RBH had the lowest a_w due to the presence of higher total solid content than others. In addition, the moisture content of CTPs tended to decrease with an increase in RBH levels which ranged from 2.51-4.25%, which were within the Thai Community Product Standard (TCPS 265/2559; Toffee) (moisture content lower than 12%).

The addition of RBH with increased RBH levels tended to decrease L* and increase a* values. It could be probably due to the brown color of RBH making the caramel toffees darker. In addition, the Maillard and caramelization reactions occuring during the heating process of caramel toffee might have contributed to the darker color of toffee. The Maillard reaction involves a condensation reaction between carbonyl groups of various reducing sugars and amino groups of amino acids/peptides/proteins which is the starting point for the formation of the mixture of intermediate stage products such as Hydroxymethylfurfural (HMF), and insoluble dark-brown polymeric pigments called melanoidins (Stojanovska *et al.*, 2017; Xiang *et al.*, 2021). Therefore, at higher concentrations of RBH, it contained higher level of amino groups in peptides sequences and could interact with reducing sugars resulting in more Maillard reaction and leading to a daker color of toffee. However, the addition of RBH had a slight effect on b* value (yellowness) of CTP.

For sensory evaluation, the color attribute score of CTPs supplemented with 0.5-2.0% RBH was significantly lower than the CTP without the addition of RBH due to the darker color of products. These results were correlated well with the L* value of CTPs. The addition of 1.0-2.0% into CTPs decreased the texture, odor, taste, and overall acceptability attributes score of CTPs. These results indicated that the addition of 1.0-2.0% RBH had a negative effect on all sensory attributes score of the products due to unacceptable flavor and dark color of rice bran. Therefore, the CTP with the addition of high RBH levels should be further flavor developed to improve the acceptability of the consumer.

For α-amino content and antioxidant activity, the increase of α-amino content in CTPs with an increased RBH levels represented an increase in peptides content derived from RBH. The lack of correlation of α-amino content and RBH levels might be due to the Maillard reaction occurring during the CTP process. The α-amino in peptide sequences of RBH might be interacted with reducing sugars (glucose in glucose syrup, and lactose in WPC and sweet concentrated milk), and generated the MRPs resulting in no significant difference between α-amino content of 1.5 and 2.0% RBH.

ABTS radical scavenging activity and FRAP values of the CTPs tended to increase with an increase in RBH levels. These results indicated that the addition of the RBH could improve antioxidant activity of the CTP. The increase in antioxidant activity of the CTP could be due to the presence of antioxidative amino acids in peptides sequences of RBH which donated hydrogen/electron to free radicals. The antioxidant activity of rice bran hydrolysate has been widely reported in literature (Chanput *et al.*, 2009; Thamnarathip *et al.*, 2016; Wattanasiritham *et al.*, 2016; Cho, 2020). An aromatic amino acid such as His, Phe, Try, and Trp with a phenol group in hydrolyzed peptides might be contributed to ABTS radical scavenging activity (Zaky *et al.*, 2019). In addition, phenolic compounds in RBH contributed the to antioxidant activity of the CTP supplemented with RBH. The patterns of ABTS radical scavenging activity and FRAP values of the CTP did not correlate well with α-amino content. These results suggested that not only did the α-amino content affect the antioxidant activity, but also other factors which contributed to the antioxidant activity of the CTP. During preparation of caramel toffee, many reactions occurred including caramelization and Maillard reaction. It has been reported that the intermediates or the final brown polymer in caramel products and Xylose–bovine casein hydrolysate MRPs derived from Maillard reaction product acted as antioxidant activity (Jafary *et al.*, 2018; Chen *et al.*, 2019). Jafary *et al.* (2018) reported that dextrose and D-glucose were a good source of natural antioxidant involving caramelization. Authors suggested that either intermediates or the final brown polymer in caramel products could function as hydrogen donors and can be beneficial to scavenge free radical scavenging capacity. The hydroxyl and pyrrole groups of MRPs are important ways to contribute to the reducing activity (Jiang *et al.*, 2013; Vhangani and Van Wyk, 2013). Therefore, amino acid in peptide sequences, phenolic content, and the intermediates or the final brown polymer from caramelization and Maillard reaction could contribute to the antioxidant activity of CPTs.

Development of the CTP supplemented with 2.0% RBH plus 2.0% WPC by adding Vanilla and caramel flavors

The CTP supplemented with 2.0% RBH plus 2.0% WPC was further developed by adding vanilla and caramel flavors to improve acceptability. Chemical compositions and physicochemical properties of final product were recorded. Mendenhall *et al.* (2016) reported that caramel formulations contained protein content ranging from 1% to 4% with typical commercial caramels between 2% to 2.5% protein. It was reported that total sugars content of fig and guava toffees was between 73.6 to 75.8% and 75.1 to 77.2%, respectively (Kohinkar *et al.*, 2014). The developed nipa palm fruit toffee contained 3.50% moisture, 85.72% carbohydrate, 7.43% crude protein, 2.86% total fat content, and 0.49% ash contents (Supaking, 2019). Gunathilake *et al.* (2010) reported that dikiri coconut toffee comprised 4.95% moisture, 0.81% minerals, 16.59% crude fat, 4.59% crude protein, 0.62% crude fiber and 72.44% carbohydrate. The difference of chemical compositions and physicochemical properties of toffees in our work and previous works maybe due to different recipes.

The microbiological qualities including total plate count, yeasts and molds, *E. coli*, *S. aureus*, and *Salmonella* spp. of flavored CTP were within the standard limits of toffee. All attributes score of flavored CTP (with the addition of caramel and vanilla flavors) with 2% RBH were higher than that of the CPT incorporated with 2% RBH without the addition of caramel and vanilla flavors. These results indicated that the addition of caramel and vanilla flavors could improve the acceptability of CTP incorporated with 2%RBH. The purchase test exhibited that 48.87% of panelists answered, “I would buy it”; 46.94% of panelists answered, “maybe buy it”. Our results suggested that the flavored CTP were accepted by consumer.

In conclusions, the caramel toffee supplemented with 2.0% WPC plus 2.0% RBH exhibited the highest antioxidant activity. The addition of caramel and vanilla flavors could improve the negative effect of all sensory attributes score of CTP supplemented with 2.0% RBH where the overall acceptability was 7.57 (Like Moderately to like very much). The moisture content and microbiological qualities of flavored caramel toffee were within the standard limits of toffee. Our findings suggested that the addition of 2.0% WPC plus 2.0% RBH could improve nutritive value of toffee. In addition, RBH has a potential use as antioxidant substance supplementation in caramel toffee products which were accepted by consumer.

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