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Research Article
Functional Properties and *in vitro* Bio-Accessibility Attributes of Light Ice Cream Incorporated with Purple Rice Bran

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Abstract
Background and Objective: The consumption of purple rice has become popular where the bran contains a high amount of fibers, vitamins, minerals and bioactive components such as phenolic compounds, flavonoids and antioxidants. This research aims to produce light ice cream using Purple Rice Bran (PRB) as a fat replacer and source of bioactive components. **Materials and Methods:** The ice cream formulations were assessed for the physicochemical properties, the content of bioactive components (antioxidants, phenolic compounds and flavonoids) and sensory characteristics. Also, *in vitro* bioaccessibility of the bioactive components was evaluated during the oral and gastrointestinal digestion. **Results:** The ice cream formulations supplemented with PRB contained a high content of total solids, protein, ash and fiber. Furthermore, the addition of PRB had a slight effect on the physical properties of ice cream. Ice cream supplemented with PRB contained a significant increase in the total phenolic compounds, flavonoids and antioxidant activity (DPPH and FRAP assay) compared to the plain ice cream (without PRB). The addition of PRB in the ice cream formulation didn't have a significant impact on sensory characteristics. In the stimulation digestion, the release of phenolic, flavonoids and antioxidants compounds from the ice cream samples supplemented with PRB was in a higher amount compared to the control sample. **Conclusion:** The overall results indicated that PRB can display a favorable natural factor to produce novel light ice cream with health benefits.

Key words: Purple rice bran, light ice cream, physiochemical properties, phenolic compounds, flavonoids, antioxidants, *in vitro* bioaccessibility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

With the increased food awareness and the spread of different social media, the consumer has become sufficiently educated in the food culture[1], which has led to his constant search for healthy, low-calorie, especially low-fat food products[2-4]. This is has made a continuous struggle between finding new food products that meet the needs of consumers in terms of delicious and attractive taste as well as nutritional and health values[5,6]. Dairy manufacturers, producers and researchers are constantly trying to produce products for a different structure that is low in fat or free fat and at the same time high in health and nutritional value[3,7,8]. Ice cream is a refreshing, nutritious and also popular dairy product that may be eaten a lot by different age groups, especially children and they are the most beloved category of these products[9]. For these reasons, there have been many attempts through studies to produce ice cream for a low-fat, low-calorie and high nutritional and healthy. The traditional ice cream formulations had 10-16% fat which can be substituted by different types of fat replacers[10]. Many studies have confirmed that foods contain low fat and fiber can reducing and protecting the injury of colon cancer, obesity, cardiovascular diseases and other disturbances[8]. Many fat alternatives have been used in the manufacture of ice cream such as inulin[11]; soy protein hydrolysate/xanthan gum[12]; maltodextrin[13], maltodextrin combined with inulin[14]; polydextrose[15], milk proteins[16], dietary fibers[17], a combination between starch with β-glucan[18] and starches[19]; etc. Dietetic fibers have been used in different dairy products as fat substitutes[20]. Carbohydrates based on fat substitutes can hold water; raise the viscosity and improving the texture[3]. Purple rice (*Oryza saitva* L. *var. glutinosa*) is a domestic Thai agricultural product distinguished by the purple pigments in the bran. The Purple Rice Bran (PRB) contains a high amount of valuable phytochemicals and micronutrients. Several types of research pointed to the purple rice contain a high level of phytochemicals such as phenolic acids, flavonoids, tocopherols free fatty acids[21,22] also, these compounds had an important role in the inhibition of growth-cancer cell[23]. Food rich in phenolic compounds is associated with reducing the risk of many diseases, such as diabetes (type-2) and atherosclerosis[24]. The bioavailability of phenolic compounds is the main issue where show the beneficial effects. However, the bioavailability varies completely from one phenolic compound to another which depends on the source of these compounds. The bioavailability of any compound relies on its digestive stability, liberation from the food system and its efficiency passing through an epithelium[25].

With an increased need for more natural and healthy products, this research was carried out to elucidate the possibility of purple rice bran as a fat replacer and a source of bioactive compounds to produce functional ice cream with acceptable sensory attributes.

MATERIALS AND METHODS

Study area: The present study was conducted during July-September, 2020 at the Department of Dairy, Food Industries and Nutrition Division, National Research Centre, Egypt.

Materials: Purple rice was purchased from the local supermarket in Chiang Mai, Thailand. A polyethylene bag was used to store the separated bran at 28±2°C. Fresh skim buffalo's milk and cream (fat 33% fat, protein 1.9%, carbohydrate 3%) were obtained from Animal Production Research Institute, Egypt. Low heat skim milk powder (moisture 4%, fat 1.5%, protein 32%, lactose 50% and ash 8%) was obtained from a local market. Commercial cane sugar was obtained from the local market. All chemicals and solvents were obtained from MERCK, USA.

Methods

HPLC analysis of phenolic compounds in the purple rice bran (PRB): The phenolic compounds of PRB were determined by reversed-phase HPLC with gradient elution. The separation was carried out using a Kromasil C18 column (4.6×250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 1 mL min^{-1}. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0-5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (85% A) and 15-16 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 µL for each of the sample solutions. The column temperature was maintained at 35°C[26].

Determination of chemical composition and minerals of the PRB: The data in Table 1 is shown the gross composition (moisture, protein, fat, ash and crude fiber) and the minerals content (iron, manganese, zinc, sodium, calcium and potassium) of PRB[27]. Total carbohydrate was determined based on the differential analysis of moisture, ash, protein and fat.

Preparation of light ice cream with PRB: The basic formulation of ice cream was prepared according to

Table 1: Proximate composition and minerals of purple rice bran

Constituents (%)		Minerals (mg kg^{-1})	
Total solids	88.50	K	2600
Moisture	11.5	Ca	228
Protein	12.5	Na	200
Carbohydrate	17.34	Zn	21
Fat	19.6	Mn	33
Crude fiber	29.5	Fe	24
Ash	9.56		

Schmidt[28]. The composition of this formula was milk solids non-fat (MSNF) 12%, fat 10%, sucrose 15%, stabilizer (CMC) 0.2% and emulsifier 0.1%. The ice cream formulations were prepared as follows:

Control: contain milk-fat 10%, T_1: contain PRB 2% (w/w) and 7% milk-fat, T_2: contain PRB 4% (w/w) and milk-fat 7%, T_3: Contain PRB 2% (w/w) and milk-fat 4%, T_4: Contain PRB 4% (w/w) and milk-fat 4%. All formulations were heated at 80±1°C for 30 sec then the temperature was reduced to 42°C. Every formulation was kept at 4°C for 2 hrs. To obtain ice cream, previous formulations were whipped for 30 min using an ice cream machine (Model: BL1380) then put in plastic containers (1 L) then frozen at -5.5°C for 2 hrs. The ice cream samples were packed into small cups and then stored at -20±2°C for 24 hrs before analysis.

Physicochemical analysis: All ice cream formulations were evaluated for the total solids, protein, fat, ash and crude fiber as stated by Verma *et al.*[27]. pH values were measured using a Jenway 3510 pH meter. The apparent viscosity was measured using Brookfield digital viscometer (Middleboro, MA 02346, USA). According to Marshall[9], overrun percent was calculated as described in the following Eq:

$$\text{Overrun} = \frac{\text{Vol. of ice milk-Vol. of mix used}}{\text{Vol. of mix used}} \times 100$$

The specific gravity was determined according to Arbuckle[29]. Melting properties were determined according to Arndt and Wehling[30].

Determination of total phenolic compounds, total flavonoids and antioxidant activity: The Total Phenolic Compounds (TPC) were determined according to Naczk and Shahidi[31] and the results were expressed as mg of (+)-catechin equivalent per gram. The method illustrated by Leong and Shui[32] was used to determine DPPH radical-scavenging activity and the results were expressed as µmol Trolox equivalent per gram. Total Flavonoids (TF) were measured according to Djeridane *et al.*[33] and the results were expressed as mg Rutin equivalent per gram. Ferric Reducing Antioxidant Power (FRAP) was evaluated as stated by Benzie and Strain[34] and the results were expressed as µmol Trolox equivalent per gram.

Sensory evaluation: Sensory attributes were characterized according to the method of Arbuckle[29]. All ice cream samples were evaluated based on flavor (50 points), body and texture (40 points), melting property (5) and appearance (5) by the staff member of the Department of Dairy in the National Research Centre, Egypt.

***In vitro* gastrointestinal digestion:** According to the method illustrated by Oliveira and Pintado[35], all ice cream samples were subjected to simulating digestion (oral, gastric and intestinal stage). The oral stage, two gram of ice cream was added to 3200 U mL^{-1} of porcine α-amylase in carbonate buffer (0.2 M, pH 7). Twenty seconds after the start of the oral stage, the gastric stage initiated using porcine pepsin (3200 U mL^{-1}) in HCl (0.02 M, pH 2) at 37°C for 1 hr. The final stage is ice cream samples were subjected to intestinal stimulated fluids. Pancreatin (2 mg mL^{-1}) in 0.2 M sodium acetate buffer pH 6 and bile salts (12 mg mL^{-1}) was added into the digesta and incubated at 37°C. Fluids from the oral, gastric and intestinal stages were assessed in the total phenolic compounds, total flavonoids and antioxidant activity.

Statistical analysis: Statistical analysis for the results was made by SPSS program[36] using Duncan's test where $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Identification and quantitative phenolic and flavonoid compounds in the purple rice bran (PRB): The data in Table 2 and Fig. 1 showed the phenolic and flavonoid compounds in the PRB using HPLC. The results showed that the PRB contained eight phenolic acids and four flavonoids. The highest Retention Time (RT) peak was Gallic acid (3.328 min) then followed by Chlorogenic acid (4.115 min), Caffeic acid (5.681 min), Syringic acid (6.522 min), Pyro catechol (7.069 min), Rutin (7.421 min), Ellagic acid (7.961 min), Coumaric acid (8.809 min), Vanillin (9.842 min), Ferulic acid (10.099 min), Naringenin (10.273 min) and Taxifolin (12.393 min), respectively. The Gallic acid recorded the highest

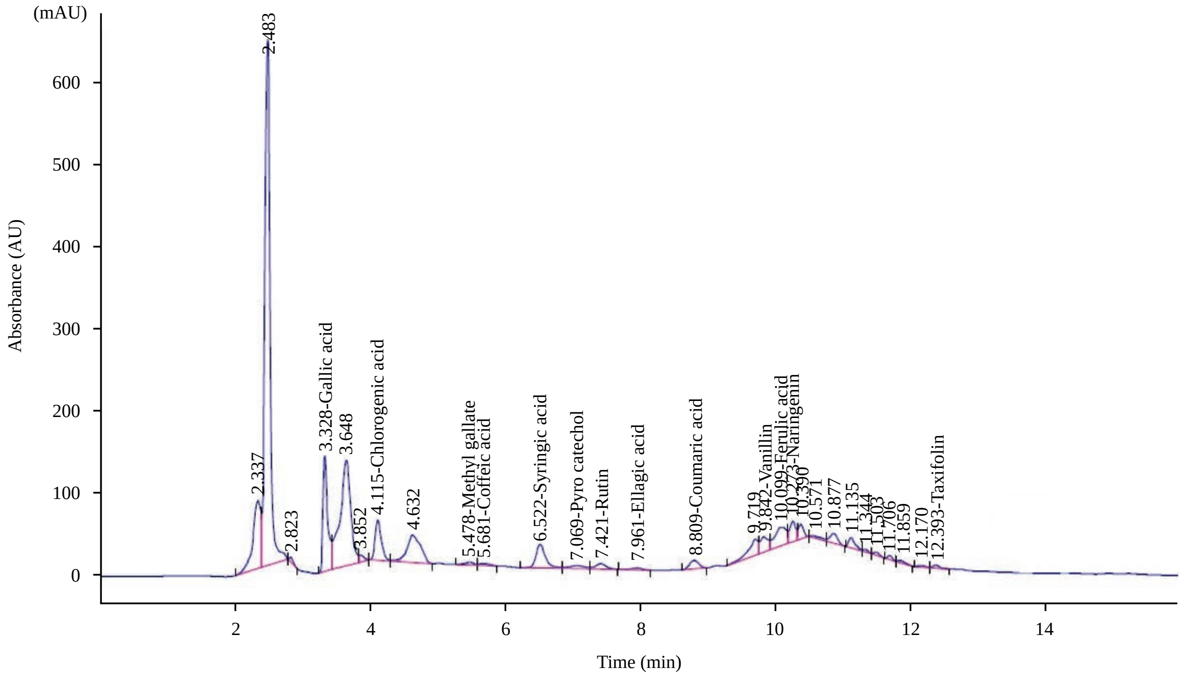

Fig. 1: HPLC chromatograph of phenolic and flavonoid compounds in the PRB

Table 2: Phenolic and flavonoid compounds of the PRB

Phenolic compounds	Concentration (µg g^{-1} rice bran)
Phenolic acid	
Gallic acid	2668.06
Chlorogenic acid	1225.84
Caffeic acid	52.85
Syringic acid	497.72
Ellagic acid	74.75
p-Coumaric acid	116.09
Vanillic acid	188.40
Ferulic acid	524.67
Total phenolic acids	5348.38
Flavonoids	
Pyro catechol	292.52
Rutin	472.60
Naringenin	464.99
Taxifolin	135.52
Total flavonoids	1365.63
Methyl gallate	37.67
Total phenolic compounds	6751.68

concentration (2668.06 µg g^{-1} PRB) then Chlorogenic acid (1225.84 µg g^{-1} PRB), Ferulic acid (524.67 µg g^{-1} PRB), Syringic acid (497.72 µg g^{-1} PRB), Vanillic acid (188.40 µg g^{-1} PRB), p-Coumaric acid (116.09 µg g^{-1} PRB) and Ellagic acid (74.75 µg g^{-1} PRB) respectively whereas Caffeic acid was recorded the lowest concentration (52.85 µg g^{-1} PRB). The highest concentration of flavonoids in the PRB sample was Rutin (472.60 µg g^{-1} PRB) then Naringenin (464.99 µg g^{-1} PRB), Pyro catechol (292.52 µg g^{-1} PRB) and Taxifolin (135.52 µg g^{-1} PRB), respectively. The total amount of flavonoids identified and quantified in the PRB was 1365.63 (µg g^{-1}), corresponding to the 5348.38 (µg g^{-1}) of total phenolic compounds. These results are in agreement with the finding of Tian *et al.*[26].

Physicochemical characteristics: The chemical composition and physical properties of ice cream samples with or without PRB were shown in Table 3. It is evident from the results that, there is a significant difference ($p<0.05$) between the supplemented ice cream samples and control sample (without PRB) in total solids where the total solids values for the control, T_1, T_2, T_3 and T_4 were 39.27, 36.14, 40.26, 35.68 and 40.14%, respectively and still T_2 was the highest in its total solids content (40.26%). The high total solids in the supplemented samples is due to the high content of PRB, where the total solids content in it was 88.5% (Table 1). The same trend was observed when estimating protein, ash and fiber in ice cream samples. The supplemented samples with PRB had high content in protein, ash and fibers than the control sample and T_2 still the highest content in protein (4.44%), ash (1.64%) and fiber (1.81%). This is due to the high protein, ash and fiber content of PRB which was 12.50, 9.56 and 29.50% respectively (Table 1). These results are in agreement with Temiz and YeşilSu[37] and Cody *et al.*[38]. It can be seen from Table 3, there wasn't a significant difference ($p<0.05$) between control and the supplemented samples in pH values where the pH values were 6.91, 6.77, 6.85, 6.80 and 6.84 for control, T_1, T_2, T_3 and T_4

Table 3: Physicochemical properties of ice cream incorporating the PRB

	Experiments				
Characteristics	Control	T_1	T_2	T_3	T_4
Moisture (%)	60.73±0.03^a	63.87±0.02^a	59.74±0.01^a	64.33±0.02^a	59.87±0.02^a
Total solids (%)	39.27±0.03^c	36.14±0.02^d	40.26±0.01^a	35.68±0.02^e	40.14±0.02^b
Protein (%)	1.33±0.01^d	1.54±0.01^c	4.44±0.02^a	1.51±0.01^c	4.37±0.02^b
Fat (%)	10.25±0.07^a	7.30±0.14^b	7.30±0.14^b	4.25±0.07^c	4.05±0.07^c
pH	6.91±0.01^a	6.77±0.12^a	6.85±0.01^a	6.80±0.01^a	6.84±0.01^a
Ash (%)	1.13±0.01^d	1.24±0.02^b	1.64±0.01^a	1.19±0.01^c	1.64±0.02^a
Fiber (%)	-	0.57±0.02^b	1.81±0.01^a	0.52±0.01^c	1.80±0.01^a
Overrun (%)	70.91±0.64^c	75.45±1.47^b	84.30±1.48^a	70.89±1.51^c	75.45±1.28^b
Specific gravity	0.84±0.01^b	0.86±0.02^b	0.94±0.03^a	0.75±0.01^c	0.76±0.02^c
Melting resistance (%)					
5 min	0.96±0.01^d	7.40±0.07^a	7.03±0.04^b	0.70±0.01^e	4.38±0.04^c
10 min	13.50±0.39^d	25.91±0.03^a	19.73±0.15^c	12.26±0.05^e	22.50±0.14^b
15 min	30.64±0.51^b	42.63±0.11^a	33.65±0.04^c	24.69±0.05^d	38.25±0.21^b
20 min	0.96±0.05^d	7.40±0.28^a	7.03±0.26^b	0.70±0.04^e	48.26±0.37^c
25 min	60.43±0.43^b	62.03±0.08^a	48.08±0.11^d	50.29±0.05^c	50.07±0.06^c
30 min	0.96±0.05^d	7.40±0.01^a	7.03±0.07^b	0.70±0.1^e	4.38±0.02^c

Results are expressed as the mean (n = 3). Means with different letters in a row are significantly different ($p<0.05$). Control: Ice cream without PRB, T_1: Ice cream contains PRB 2% (w/w) and 7% milk-fat, T_2: Ice cream contains PRB 4% (w/w) and milk-fat 7%, T_3: Ice cream contains PRB 2% (w/w) and milk-fat 4%, T_4: Ice cream contains PRB 4% (w/w) and milk-fat 4%

respectively. This means that the addition of PRB in the ice cream formulation hadn't affected the pH values and these results are consistent with the finding of Salama *et al.*[39,40].

Also, the results showed that the specific gravity values were increased with an increase in the PRB level in the ice cream samples compared to the control sample where the specific gravity values were 0.84, 0.86, 0.94, 0.75 and 0.76 for control, T_1, T_2, T_3 and T_4, respectively and T_2 had the highest the specific gravity value (0.94) than all ice cream samples. These results are in agreement with El-Kholy[41]. He studied the effect of substituting milk fat with pomegranate peel in frozen yoghurt and determined the quality of a final product. He stated that the air has a massive effect on the ice cream density, therefore, blending air in the ice cream mixture before the freezing process reduces the specific gravity of the produce ice cream and so, the specific gravity of the ice cream mixture was higher than the resultant ice cream. These results are consented with finding Abd El-Rashid and Hassan[42].

Overrun is an important economic estimate in the frozen desserts. However, the overrun values were increased significantly ($p<0.05$) in the supplemented samples with PRB compared to the control sample where the overrun values were 70.91,75.45, 84.30, 70.89 and 75.45% for control, T_1, T_2, T_3 and T_4, respectively and T_2 has gained the highest overrun value (84.30%) than other samples. This may due to the increasing level of PRB in the ice cream mixture which led to increase in the total solids of the ice cream formula which was followed by an increase in the overrun.

The melting rate of ice cream is one of the most important factors that influence the sensory evaluation of the final product[43]. However, many factors affect the melting rate, including the composition of the ice cream, the shape and size of the ice crystals and the fat particles during the freezing process, the additives and the overrun[44]. It is noticed from the results, the addition of PRB to ice cream formulation increased significantly ($p<0.05$) the melting resistance of ice cream. It was found that the supplemented samples with PRB (T_1, T_2, T_3 and T_4) were resistant to melt and took a long time to melt and followed by a control sample. This may be due to the high solids content of the supplemented samples, which include some starch from the PRB. These results in agreement with the finding of El-Samahy *et al.*[45], they found that the ice cream samples supplemented with pomegranate peel melted more slowly compared to the control sample. Usually, decreasing the melting rate indicates the quality of ice cream and processed amla[46].

Viscosity: One of the most important features that have a role in evaluating the quality of ice cream is the viscosity which has a responsibility for the desired body and texture of ice cream. Therefore, the measurement of viscosity is important to know the effect of addition PRB on the ice cream attributes. It could be seen in the present study the addition of PRB significantly ($p<0.05$) affected the viscosity behavior of the ice cream samples (Fig. 2). The viscosity of the supplemented samples was higher than the control sample ($T_2>T_4>T_1>T_3>$control) and T_2 was the highest value. These results could be attributed to the high fiber content of PRB, which holds extra water thus increasing the viscosity of the final product. These results are in agreement with the finding of Erkaya *et al.*[47], Yangılar[48].

Table 4: Total Phenolic Compounds (TPC), Total Flavonoids Content (TFC) and antioxidant activity (DPPH and FRAP) of ice cream incorporating PRB

Experiments	TPC (mg catechin g^{-1} ice cream)	TFC (mg rutin g^{-1} ice cream)	DPPH (µmol trolox g^{-1} ice cream)	FRAP (µmol trolox g^{-1} ice cream)
Control	0.221 ± 0.14^{e}	ND*	0.0236 ± 0.26^{e}	0.057 ± 0.45^{e}
T_1	0.471 ± 0.18^{d}	0.253 ± 0.18^{c}	0.327 ± 0.33^{d}	0.452 ± 0.11^{d}
T_2	0.794 ± 0.45^{b}	0.453 ± 0.13^{b}	0.603 ± 0.59^{b}	0.465 ± 0.23^{b}
T_3	0.497 ± 0.25^{c}	0.246 ± 0.63^{d}	0.354 ± 0.46^{c}	0.460 ± 0.37^{c}
T_4	0.812 ± 0.23^{a}	0.463 ± 0.17^{a}	0.630 ± 0.26^{a}	0.795 ± 0.40^{a}

Results are expressed as Mean±STD (n = 3). Means with different letters are significantly different (p<0.05). *ND: Not detected, Control: Ice cream without PRB, T_1: Ice cream contains PRB 2% (w/w) and 7% milk-fat, T_2: Ice cream contains PRB 4% (w/w) and milk-fat 7%, T_3: Ice cream contains PRB 2% (w/w) and milk-fat4%, T_4: Ice cream contains PRB 4% (w/w) and milk-fat 4%

Table 5: Sensory attributes of ice cream incorporating PRB

Experiments	Flavor (50)	Body and texture (40)	Melting properties (5)	Appearance (5)	Total score (100)
Control	38.90 ± 0.63^{d}	32.49 ± 0.54^{b}	2.75 ± 0.07^{d}	3.35 ± 0.21^{c}	77.49 ± 0.05^{c}
T_1	44.76 ± 0.31^{b}	35.59 ± 0.27^{a}	3.60 ± 0.14^{bc}	4.20 ± 0.14^{b}	88.15 ± 0.86^{b}
T_2	46.35 ± 0.47^{a}	36.50 ± 0.54^{a}	4.30 ± 0.28^{a}	4.75 ± 0.21^{a}	91.90 ± 0.57^{a}
T_3	41.73 ± 0.37^{c}	33.62 ± 0.52^{b}	3.40 ± 0.28^{c}	3.50 ± 0.14^{c}	82.25 ± 1.32^{c}
T_4	$45.54\pm0.58a^{b}$	35.50 ± 0.68^{a}	3.95 ± 0.07^{ab}	4.60 ± 0.28^{ab}	89.59 ± 0.91^{b}

Results are expressed as Mean±STD (n = 3). Different superscript lowercase letters in the same column indicate significant differences (p<0.05). Control: Ice cream without PRB, T_1: Ice cream with fat 7% and PRB 2% (w/w), T_2: Ice cream with fat 7% and PRB 4% (w/w), T_3: Ice cream with fat 4% and PRB 2% (w/w), T_4: Ice cream with fat 4% and PRB 4% (w/w)

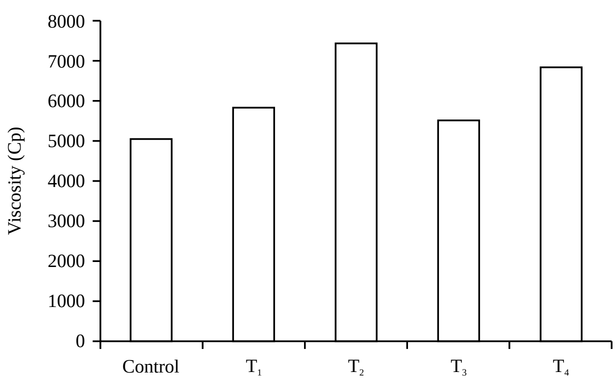

Fig. 2: The viscosity of ice cream

Results are expressed as the mean (n = 3). Control: Ice cream without PRB, T_1: Ice cream contains PRB 2% (w/w) and 7% milkfat, T_2: Ice cream contains PRB 4% (w/w) and milk fat 7%, T_3: Ice cream contains PRB 2% (w/w) and milk fat4%, T_4: Ice cream contains PRB 4% (w/w) and milk fat 4%

Total phenolic compounds (TPC), total flavonoids content (TFC) and antioxidant activity (AA): The total phenolic, flavonoid compounds and antioxidant activity using DDPH and FRAP methods of ice cream samples were shown in Table 4. From the results, it was found that the control sample had antioxidant activity and phenolic content. This may be due to the presence of antioxidants in milk which are derived from protein (tyrosine, tryptophan and phosphoserine residues), minerals (such as Zinc and Phosphorus), fat (α-tocopherol, Vitamin-E, carotenoids), enzymes (SOD, Catalase, GSH reductase)[49]. Also, phenolic compounds can be formed in milk during the pasteurization process[50,51]. The total phenolic compounds for the supplemented samples were 0.471, 0.794, 0.496 and 0.812 mg catechin g^{-1} for T_1, T_2, T_3 and T_4 respectively compared to the control sample (0.221 mg catechin g^{-1}) while the total flavonoids for the supplemented samples were 0.252, 0.453, 0.246 and 0.463 mg Rutin g^{-1} for T_1, T_2, T_3 and T_4 respectively compared to the control sample. The total phenolic compounds and the total flavonoids in the supplemented samples with PRB were higher compared to the control sample (without PRB), around 2.1-4.6 times more than those found in the control sample. This may due to the presence of PRB which is a rich source of phenolic and flavonoid compounds (Table 2 and Fig. 1), thus the addition of PRB to the ice cream formulation increased the ice cream's content phenolic compounds and flavonoids in an important amount. The antioxidant activity using DPPH was 0.0236, 0.327, 0.602, 0.354 and 0.630 for control, T_1, T_2, T_3 and T_4 respectively while antioxidant activity using FRAP was 0.057, 0.452, 0.465, 0.460 and 0.795 for control, T_1, T_2, T_3 and T_4 respectively. It was noticed from these results, the DPPH and FRAP values of the ice cream samples increased with an increase in the addition of PRB. Many previous studies indicated that the addition of phenolic sources such as dark cocoa, hazelnut, green tea extracts, sugarcane and grape juice residue could increase the antioxidant activity of ice cream[52,53]. This may due to the phenolics and flavonoids which presence in PRB is responsible for the powerful antioxidant activity. Also, it is known The DPPH radical scavenging activity is related to the nature of phenolic compounds which are considered a donor of electrons[54,55].

Sensory evaluation: Sensory attributes of the ice cream samples supplemented with or without PRB was illustrated in Table 5. The results revealed that the addition of PRB in ice

Table 6: Release of phenolic compounds, flavonoids and antioxidants of ice cream incorporating PRB during *in vitro* gastrointestinal digestion

	In vitro gastrointestinal		
Experiments	Mouth	Gastric	Intestinal
TPC* (mg Catechin g^{-1})			
Control	0. 277±0.29^{d}	0.342±0.91^{d}	0.340±1.09^{c}
T_1	0.587±0.25^{b}	0.671±0.36^{c}	0.835±0.38^{b}
T_2	0.907±0.14^{a}	1.044±0.38^{a}	1.192±0.68^{a}
T_3	0.575±0.54^{c}	0.654±0.25^{c}	0.843±0.20^{b}
T_4	0.915±0.45^{a}	1.038±0.23^{b}	1.181±2.26^{a}
TF* (mg Rutin g^{-1})			
Control	ND*	ND*	ND*
T_1	0.334±0.31^{d}	0.404±0.41^{c}	0.606±0.79^{d}
T_2	0.519±0.14^{b}	0.695±0.64^{a}	0.806±0.52^{b}
T_3	0.344±0.14^{c}	0.400±0.47^{c}	0.623±0.29^{c}
T_4	0.530±0.71^{a}	0.648±0.01^{b}	0.835±0.45^{a}
DPPH* (µmol Trolox g^{-1})			
Control	0.772±0.52^{d}	0.172±0.33^{c}	0.251±0.51^{d}
T_1	0.418±0.52^{c}	0.783±0.66^{b}	0.865±0.17^{c}
T_2	0.835±0.33^{a}	1.003±1.07^{a}	1.143±0.61^{a}
T_3	0.438±0.72^{b}	0.796±0.41^{b}	0.875±0.20^{c}
T_4	0.838±0.07^{a}	1.016±0.58^{a}	1.126±0.71^{b}
FRAP* (µmol Trolox g^{-1})			
Control	0.125±0.23^{d}	0.242±0.01^{e}	0.315±0.29^{c}
T_1	0.559±0.23^{c}	0.845±0.21^{c}	0.944±0.30^{b}
T_2	0.944±0.38^{b}	1.054±0.41^{a}	1.155±5.37^{a}
T_3	0.566±0.29^{c}	0.823±0.30^{d}	0.926±0.16^{b}
T_4	0.966±0.61^{a}	1.035±0.54^{b}	1.176±0.45^{a}

The results are expressed as Mean±STD (n = 3). Different superscript lowercase letters in the same column indicate significant differences (p<0.05). Control: Ice cream without PRB, T_1: Ice cream with fat 7% and PRB 2% (w/w), T_2: Ice cream with fat 7% and PRB 4% (w/w), T_3: Ice cream with fat 4% and PRB 2% (w/w), T_4: Ice cream with fat 4% and PRB 4% (w/w)

cream formulation had a significant effect on flavor, body and texture, melting properties and appearance. However, ice cream with PRB 2% (T_2) had the highest scores in flavor (46.35), body and texture (36.50), melting properties (4.30) and appearance (4.75), as well as total scores (91.90) compared to the control sample (flavor 38.90, body and texture 32.49, melting properties 2.75, appearance 3.35 and total score 77.49). The high acceptability of T_2 may be attributed to the PRB, unlike other fibers, which has sweet and delicious savor. However, Anuyahong *et al.*[56] reported yogurt enrich with rice berry extract was improved their content of the phytochemicals without a significant impact on the sensory characteristics.

In vitro bioaccessibility

Phenolic and flavonoid compounds: The Total Phenolic Compounds (TPC) of ice cream with or without PRB during *in vitro* gastrointestinal digestion are illustrated in Table 6. It is observed that TPC values of all ice cream samples were increased significantly (p<0.05) after the oral phase which was 0.277,0.587,0.907,0.575 and 0.915 mg Catechin g^{-1} for control, T_1, T_2, T_3 and T_4. This may due to the content of the samples from the phenolic compounds before digestion. This increase in TPC may due to an increase in the solubility of phenolic compounds or stirring or the enzyme activity during the gastrointestinal conditions stimulated in vitro, all of these could contribute to the breakdown of phenolic compounds of high molecular weight to compounds low in molecular weight which increase TPC[57]. Likewise to the oral phase, after the gastric digestion step, the TPC of all ice cream samples increased significantly (p<0.05) which were 0.342, 0.671, 1.044, 0.654 and 1.038 mg Catechin g^{-1} for control, T_1, T_2, T_3 and T_4. Saura-Calixto[58] indicated that during the digestion step, there are many factors like enzyme activity, pH and others that can facilitate the hydrolysis of phenolic compounds which bounded with protein or carbohydrate in the food system. These results indicated that the digestive stage could facilitate the release of phenolic compounds in the ice cream system and these results are in agreement with Rodríguez-Roque *et al.*[59] and Cutrim and Cortez[60]. However, the TPC of all ice cream samples increased significantly (p<0.05) after pancreatic digestion where were 0.340, 0.835, 1.192, 0.843 and 1.181 mg Catechin g^{-1} for control, T_1, T_2, T_3 and T_4. This conduct may be due to the ph of the system (5-5.50) or the enzyme activity or both which promote the liberation of phenolic compounds and increasing their level in the intestinal fluids. The progressive increase of TPC in ice cream samples during in vitro digestion is in the agreement of de Carvalho *et al.*[61], Granato *et al.*[4] and Tagliazucchi *et al.*[62]. Also, it was noticed from the results the Total Flavonoids (TF) of the supplemented ice cream samples with PRB were increased after oral digestion where were 0.334, 0.519, 0.344 and 0.530 mg Rutin/g for T_1, T_2, T_3 and T_4 (control sample didn't contain flavonoids). The TF were 0.404, 0.695, 0.400 and 0.648 mg Rutin g^{-1} for T_1, T_2, T_3 and T_4 after gastric digestion while were 0.606, 0.806, 0.623 and 0.835 mg Rutin g^{-1} for T_1, T_2, T_3 and T_4 after pancreatic digestion. The high flavonoids content of the samples is due to high flavonoids in PRB which used as a supplement in the ice cream formulation (Table 2 and Fig. 1).

Antioxidant activity: The results of the antioxidant activity estimated during *in vitro* digestion are shown in Table 6. The ice creams with PRB had higher antioxidant activity compared to the control sample in both DPPH and FRAP evaluation. After pancreatic digestion, the DPPH values of all the samples increased significantly (p<0.05) (0.251, 0.865, 1.143, 0.875 and 1.126 µmol Trolox g^{-1} for control, T_1, T_2, T_3 and T_4) compared to the oral and gastric phases where the DPPH values were 0.772, 0.418, 0.835, 0.438 and 0838 µmol Trolox g^{-1} for control, T_1, T_2,

T_3 and T_4 after oral digestion. This conduct may be related to liberating antioxidant compounds from milk[25]. These results are in agreement with the findings of Helal[63]. They reported at the end of the intestinal digestion step, the fortified yogurt with cinnamon had the highest antioxidant activity. As well, Lafarga *et al.*[64] noticed phenolic compounds had a positive corresponding with FRAP value. Thus, increasing FRAP values in all ice cream samples after gastric (0.242, 0.845, 1.054, 0.823 and 0.242 µmol Trolox g^{-1} for control, T_1, T_2, T_3 and T_4) and intestinal digestion (0.315, 0.944, 1.155, 0.926 and 0.315 µmol Trolox g^{-1} for control, T_1, T_2, T_3 and T_4) were related to liberating of polyphenolic compounds from samples. Furthermore, Bouayed *et al.*[65] proposed that increase the antioxidant activity of phenolic compounds during in vitro gastric and intestinal digestion may be due to change from acidic to an alkaline condition which let to remove proton of the hydroxyl radicals exists in the benzene rings.

Indeed, *in vitro* digestion of the PRB supplemented ice cream resulted in the release of phenolic compounds, flavonoids and antioxidants at the end of the digestion. The amount of these compounds in the supplemented ice cream samples was higher than that found in the control sample (without PRB) ($p<0.05$). These results showed that the ice cream matrix improves the bioaccessibility of PRB phenolics, flavonoids and antioxidants. The PRB supplemented ice cream can be considered an important source of dietary bioaccessible bioactive components.

CONCLUSION

Purple rice bran was successfully utilized to produce functional ice cream. The chemical and physical properties of ice cream were not significantly affected by the addition of PRB. All ice cream formulas had a high amount of phenolics. The ice cream samples supplemented with PRB exhibit a high content of phenolic compounds and antioxidant activity than the control sample during gastrointestinal digestion stimulated in vitro. Finally, the supplemented ice cream had considerable potential as a functional dairy product. The results of this research proposed that ice cream can be a good matrix for the delivery of bioactive compounds in PRB. Ice cream is a popular dairy product and had high acceptability in the entire world and it is easy to the addition of PRB as a novel fat substitute and source of bioactive compounds at an industrial scale.

SIGNIFICANCE STATEMENT

This study discovered that the addition of purple rice bran to ice cream formulation can be beneficial for the production of light ice cream rich in phenolic and flavonoids content and antioxidants activity. It was also discovered that the release of phenolics and flavonoids compounds increased at the last stage (intestinal) of *in vitro* gastrointestinal stimulation. This study will help the researchers to uncover the critical areas of assessment of purple rice bran as a functional ingredient in ice cream, which many researchers were not able to explore.

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