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Original Article

Optimization of pumpkin and feed moisture content to produce healthy pumpkin-germinated brown rice extruded snacks

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ABSTRACT

The effects of three levels of pumpkin flour (PF; 10, 20 and 30%) and three levels of feed moisture content (FM; 13, 16 and 19%) on the physical properties, antioxidant activity and sensory properties of pumpkin-germinated brown rice extrudates were investigated. The increase in the PF increased the bulk density, hardness, total phenolic content (TPC) and antioxidant activity, but decreased the expansion ratio of the extruded snack. Decreasing the FM caused an increase in the TPC and antioxidant activity of the snack product. The predicted optimum formulation of extrudates using response surface methodology was 10–13% PF and 13–14% FM under extrusion conditions at 140 °C (zone 6) and 350 rpm screw speed. These conditions produced extruded snacks with TPC values of 20–28 mg GAE/100 g sample on a dry basis and appearance and hardness liking scores of more than 6.5 (on a 9-point hedonic scale). Therefore, the results of this study supported the utilization of PF and germinated brown rice flour to develop healthy snack products.

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Introduction

Snack foods are popular products that are easy to eat, have an attractive taste and an enjoyable texture (Anton et al., 2009; Brennan et al., 2013). Nevertheless, snack products tend to be energy dense and have poor nutritional value because their ingredients mostly comprise starch flour, fat and sugar, and especially high sodium content. (Dehghan-Shoar et al., 2010; Brennan et al., 2013). Consuming a large amount of snacks can lead to health problems such as cardiovascular disease, obesity and malnutrition (Ebbeling et al., 2002; Lobstein et al., 2004; Kerver et al., 2006). Therefore, available foods should aim to provide nutritive value and be a healthy product. There have been some unsuccessful trials attempting to develop healthy snacks to improve the nutritional content as well as the sensory qualities of taste, smell and texture that are attractive to consumers. (Potter et al., 2013). Thus, consumers continue to consume popular and unhealthy snacks.

Extrusion-cooking technology has been widely applied in healthy snack production. This process involves a continuous mixing, cooking and forming process carried out at high temperature over a short time. The advantage of the extrusion process for snack food manufacture is its very efficient technology, with high production volumes and continuous production (Matz, 1984; Moscicki, 2011). In addition, the extrusion process provides ease of operation and the ability to produce a variety of desirable sizes, shapes and textures of snack product (Brennan et al., 2013). The most popular snack raw materials are starch-based products derived from wheat, maize, potato and rice. Other ingredients obtained from fruit and vegetables are used in small amounts, mostly in order to give nutritional enrichment to the product. (Brennan et al., 2013; Potter et al., 2013). There have been several attempts to research and investigate fortifying the functional ingredients to improve the nutritional value of extruded snacks such as with bean flour, green banana flour (Sarawong et al., 2014), chestnut flour (Obiang-Obounou and Ryu, 2013), peanut flour (Suknark et al., 1997), fruit powders (apple, banana, strawberry and tangerine) (Potter et al., 2013) and tomato paste and tomato skin (Dehghan-Shoar et al., 2010). The optimization of the functional ingredient proportions for the extruded snack seems to vary according to the nature of each raw material. However, functional ingredients must be incorporated in carefully controlled quantities because of their variable effects on quality such as increasing the hardness of the

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extruded product and decreasing expansion after fortification with pea grits (Singh et al., 2007).

Germinated brown rice flour (GBRF) and pumpkin flour (PF) have become interesting as ingredients of snack product as they are good sources of nutrition (Chalermchaiwat et al., 2015; Promsakha na Sakon Nakhon et al., 2017). Rice (*Oryza sativa* L.) is the major source of carbohydrates. Brown rice (unpolished rice), especially germinated processed brown rice flour with greater nutritional value has been paid more attention for use as a raw material (Songtip et al., 2012). GBRF is a well-known excellent source of nutrients (Patil and Khan, 2011), such as total phenolic content, antioxidant activity, ferulic acid (Tian et al., 2004), γ-oryzanol, especially γ-aminobutyric acid (GABA). In addition, pumpkin (*Cucurbita moschata* L.) is a nutritious source of phenolics, flavonoids, β-carotene and carbohydrates (Promsakha na Sakon Nakhon et al., 2017). Thus, flours from both these raw materials have potential for use as ingredients in expanded or puffed, healthy, extruded snack products. Therefore, the objective of this study was to optimize the formulation of a healthy snack from PF and GBRF using response surface methodology (RSM).

Materials and methods

Materials

The paddy rice Khao Dawk Mali 105 (*Oryza sativa* L. cv. KDML-105) was provided by the Sakon Nakhon Rice Research Center, Sakon Nakhon province, Thailand. Pumpkin (*Cucurbita moschata* L.) was sourced from a local market, Bangkok, Thailand. Corn grit (moisture content 6.0%) was manufactured by Thai Maize Products Co. Ltd, Bangkok, Thailand. Soy protein isolate (5.4% moisture content) was purchased from Eurofins Gene Scan Incorporated, LA, USA. Calcium carbonate ($CaCO_3$; 0.2% moisture content) was supplied by Thai Food and Chemical Co. Ltd, Bangkok, Thailand.

Preparation of germinated brown rice flour (GBRF) and pumpkin flour (PF)

The GBRF was prepared from brown rice Khoa Dawk Mali 105 according to the method of Songtip et al. (2012). The germinated brown rice was dried in a hot-air dryer (BWS-model; Frecon, Bangkok, Thailand) at 55 °C for 5.5 h to obtain a moisture content of 9–12%. The PF was produced from pumpkins washed, peeled, deseeded and cut into 0.5 cm thickness slices using a food processor (Combimax 600, Braun, USA). The sliced pumpkins were dried in a hot-air dryer at 50 °C for 24 h to obtain a moisture content of 9–12%. Both the GBRF and PF samples were ground in a blender (NC.4695, NESCO, Spain) and passed through a 60-mesh sieve, sealed in plastic bags and stored at 25 °C before use.

Extrudates preparation and extrusion process conditions

The flour mixtures for extruded snack preparation were comprised 90% of GBRF and PF mixtures. PF was substituted for GBRF at three levels (10, 20 and 30%). The remaining 10% of ingredients consisted of 5% corn grit, 4.5% soy proteins isolate and 0.5% $CaCO_3$. The composite flour was mixed, packed in LLDPE bags and stored at 25 °C for 24 h to equilibrate the moisture before extrusion. Extruded ring-shaped snack samples were produced using a co-rotating twin-screw extruder (ZE 25x33D; Hermann, Berstorff Laboratory, Hanover, Germany) comprised of a barrel with seven parts ending in a 24.5 mm thick die plate. The ring-shaped die configuration consisted of a 9 mm external diameter and a 5 mm internal diameter. The barrel temperature profile was set at 30 °C (zone 1), 40 °C (zone 2), 55 °C (zone 3), 120 °C (zone 4), 130 °C (zone 5), 140 °C (zone 6), and 130 °C (zone 7 at the die plate). The feed rate was kept constant at 4.0 kg/h and the screw speed was maintained at 400 rpm. The feed moisture content was pumped into the first barrel section of extruder and varied to experimental requirements. After extrusion, the extrudates were dried in a tray dryer at 80 °C for 10 min, sealed in laminated aluminum foil and stored at 25 °C until further analysis.

Experimental design

A 3 × 3 factorial arrangement in a completely randomized design (CRD) was studied to investigate the three levels of PF (10, 20 and 30%) and the three levels of feed moisture content (13, 16 and 19%) used. The extruded snacks were analyzed for physical properties (color, expansion ratio, bulk density and hardness), total phenolic content, antioxidant activity and sensory acceptability. Formula optimization of the extruded snack was carried out using RSM. The non-extruded flour mixture served as the control.

Measurement of physical properties

Color

The color of the extruded snacks was performed using a spectrophotometer (Minolta CM-3500d; Konica Minolta Holdings Inc., Tokyo, Japan) with a D65 illuminant at 10° observation and reported as the CIELAB parameters (L*, a* and b* values) (Koksel and Tugrul Masatcioglu, 2018). The whole pieces of extrudates were randomly arranged in groups weighing 5 g each and placed in upright position to fill in a glass measurement container. Color readings were taken from 4 separate points on the surface of each replication. The results were reported as the mean of color measurements with three replications.

Expansion ratio and bulk density

The expansion ratio was measured as the mean of 10 samples using digital vernier calipers (Model 500-150-30, Mitutoyo, Japan). The expansion ratio of extruded, ring-shaped, snack samples was calculated according to Equation (1):

$$\text{Expansion ratio} = \frac{(\text{External diameter of extrudate} - \text{Internal diameter of extrudate})}{(\text{External diameter of die} - \text{Internal diameter of die})} \quad (1)$$

The bulk density was measured using a seed displacement method. The 200 mL of sesame seeds was placed in a 250 mL measuring cylinder and tapped 20 times. The volume and weight of 10 extrudates were recorded to calculate the bulk density by unit mass per unit volume (g/cm^3) (Zhuang et al., 2010).

Hardness

The textural property of extruded, ring-shaped snack was analyzed using a TA-XT plus Texture Analyzer (Stable Micro System,

Texture Technologies Corp., NY, USA). A Kramer, shear cell, five-blade probe was used to determine the hardness in newtons (N) by measuring the maximum peak force to break the extruded sample and the result was expressed as the average of 10 measurements. A 50 kg load cell was used. The test speed was 2 mm/s and the distance between the two supports was 48 mm. Each extruded sample was weighed prior to hardness determination.

Measurement of chemical properties

Preparation of extracts from extrudates

Ground extrudate (2.0 g) were extracted with 25 mL methanol (80% v/v) by agitation using a magnetic stirrer at room temperature (28 ± 2 °C) for 30 min. The mixtures were centrifuged at 2500 *g* for 10 min and then the supernatants were collected. The residues were re-extracted twice under the same conditions, resulting finally in 50 mL extract in 80% methanol (Chalermchaiwat et al., 2015). All extracts were protected from light and kept in a refrigerator before determining the total phenolic content and antioxidant activity.

Total phenolic content (TPC) and antioxidant activity

The TPC was determined using the Folin-Ciocalteau method according to Adom and Liu (2005) and Boateng et al. (2008). Gallic acid (0.01–0.06 mg/mL) was used as a standard. The TPC was expressed as mg of gallic acid equivalents (GAE) per 100 g extrudate (dry basis, db). For antioxidant activity, the ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay was measured according to the method of Re et al. (1999) and Bravo et al. (2013) with slight modifications. Testing for gallic acid was performed at a standard curve range of 0.5–3.0 μg/mL. The DPPH (2,2-Diphenyl-1-picprylhydrazyl) assay was analyzed using a slightly modified method of Ozgen et al. (2006). Testing for gallic acid was performed using a standard curve range of 0.5–2.5 μg/mL. The FRAP (ferric reducing antioxidant power) of the sample extract was determined using a slightly modified method of Benzie and Strain (1996). The standard curve was performed with ferrous sulfate ($FeSO_4.7H_2O$) in the range 0.1–1.0 mM. The absorbance values from TPC, ABTS, DPPH and FRAP assay were measured using a UV-visible spectrophotometer (UV-160A, Shimadzu Co., Japan). All determinations were performed in triplicate. The ABTS and DPPH results were reported as mg of GAE per 100 g extrudate, db and the FRAP results were expressed as mg of $FeSO_4$ equivalent per 100 g extrudate, db.

Sensory acceptability

Untrained panelists (n = 60) from Kasetsart University, Bangkok, Thailand were asked to evaluate the extruded snack samples using a liking score for appearance, odor, taste, hardness and overall liking based on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely). Each panelist was presented with two sets of extruded samples, with the first set containing four samples and the second set containing five samples (5 g/serving). Between testing samples, panelists were instructed to rinse their mouths with water and to have a 10 min break between sample sets.

Statistical analysis

Experimental data were analyzed using analysis of variance (ANOVA) in the SPSS© statistical package, version 12.0 (SPSS (Thailand) Co., Ltd., Bangkok, Thailand). Duncan's Multiple Range Test was used to determine multiple comparisons of mean values with statistically significant difference established at $p \leq 0.05$. For optimum formulation, RSM was used to investigate relationships between independent and dependent variable using a regression model (second-order polynomial model; $Y_i = \beta 0+\beta_1X_1+\beta_2X_2+\beta_3X_1X_2+\beta_4X_1^2+\beta_5X_2^2$). Contour plots were analyzed using a trial version of the STATISTICA 10 software package (StatSoft, Inc., Tulsa, OK, USA). To evaluate the model, the correlation coefficient of determination (R^2) and the lack-of-fit term were used to judge the adequacy of the model fit. Model verification was evaluated using the root mean-square error (RMSE) values.

Results and discussion

Physical properties of extrudates

Color

The color of the extrudates made from the GBRF and PF was yellowish-brown with the presence of high b* values. The color was significantly affected by both the amount of PF and the feed moisture content (Table 1). Increasing the PF content decreased L* (lightness) but increased a* (redness) and b* (yellowness). This color effect was probably due to the high carotenoid pigment from the PF and Maillard browning reaction of ingredients in the flour mixture occurring during extrusion process. Moreover, the higher PF level (30%) produced extrudates with lower puffing and greater density resulting in a deep brown color and less lightness. Increasing the feed moisture content from 13 to 19% changed the product color from yellow to brown. The correlation between expansion and the lightness of extrudates was revealed by Altan et al. (2008). The decrease in feed moisture content tended to increase lightness. It was described that the puffed products gave more lightness in extrudates because of large air cells. A similar observation was found in extrudates of dry brewer's spent grain and dry red cabbage reported by Stojceska et al. (2009).

Table 1
Physical properties of extruded snacks as affected by pumpkin flour (PF) content and feed moisture content.

PF (%)	Feed moisture content (%)	Physical properties					
		Color			Bulk density (g/cm^3)	Expansion ratio	Hardness (N)
		L*	a*	b*			
10	13	68.03^{a} ±0.37	10.91^{f} ± 0.25	39.02^{g} ± 0.51	0.14^{e} ±0.01	3.21^{a} ±0.14	94.54^{e} ±27.39
	16	67.86^{a} ±0.65	10.75^{fg} ± 0.27	42.63^{f} ±0.31	0.22^{d} ± 0.01	2.95^{b} ± 0.19	119.26^{e} ±9.12
	19	66.68^{b} ± 1.31	10.55^{g} ± 0.38	45.86^{d} ± 0.59	0.45^{b} ± 0.06	2.00^{d} ± 0.14	357.03^{c} ±50.29
20	13	61.52^{d} ± 0.14	13.65^{d} ± 0.14	43.48^{e} ±0.20	0.25^{d} ± 0.02	2.37^{c} ±0.09	235.44^{d} ± 5.97
	16	62.43^{c} ±0.49	13.35^{e} ± 0.13	48.06^{b} ± 0.18	0.32^{c} ±0.02	1.97^{d} ± 0.13	245.83^{d} ± 39.61
	19	57.74^{f} ±0.99	13.79^{d} ± 0.40	47.04^{c} ±0.76	0.34^{c} ±0.01	1.83^{e} ±0.09	432.55^{b} ± 56.25
30	13	57.62^{f} ±0.34	15.42^{a} ± 0.16	45.76^{d} ± 0.13	0.33^{c} ±0.09	1.59^{f} ±0.08	326.57^{c} ±30.26
	16	57.23^{f} ±1.06	14.79^{b} ± 0.33	49.96^{a} ±1.14	0.40^{b} ± 0.06	1.53^{f} ±0.11	401.23^{b} ± 70.09
	19	57.57^{f} ±1.06	14.51^{c} ±0.33	48.61^{b} ± 1.14	0.62^{a} ±0.07	1.50^{f} ±0.09	502.59^{a} ±71.27

$^{a-d}$ values are mean ± SD of triplicate measurements with different lowercase superscript letters in each column are significantly different ($p \leq 0.05$).
Technical terms of color parameters in CIELAB system and shows as L*, a* and b*.

Expansion ratio and bulk density

The expansion ratio was related to the bulk density expressed as a quality index of the degree of puffing in the finished product. In general, a decrease in the expansion ratio is associated with increasing bulk density. In this study, it was found that the feed moisture content and PF had significant effects on the expansion ratio and bulk density of the product, as shown in Table 1. The highest PF (30%) resulted in a significant ($p \leq 0.05$) decrease in the expansion ratio and a significant increase in the bulk density. The highest levels of PF and feed moisture content resulted in the highest density (0.62 g/cm^3) and lowest expansion ratio (1.50). It is possible that the high fiber content of the PF had an effect on the reduction in size and the number of internal air cells due to the premature rupture of air cells or the inhibition of starch matrix puffing during the extrusion process (Parada et al., 2011; Robin et al., 2012). A similar observation was reported for the fortification of bean flour with cornstarch-based extruded snacks (Anton et al., 2009). In addition the feed moisture content increased from 13% to 19%, the expansion ratio decreased while the bulk density increased. High moisture content may cause a change in the low melt elasticity in starch-based material and decrease the shear rate of the feed material flow during extrusion, resulting in the extrudates shrinking after they come out of the die.

Texture hardness

The hardness test was used to assess the texture characteristics of the ring-shaped snacks. Both the PF and feed moisture content significantly affected the hardness of extrudates (94.54–502.59 N, Table 1), with increases causing an increase in the hardness. The change in hardness was probably the result of the presence of fiber in the PF and the higher moisture contents. Fiber could increase the hardness because of possible inhibition of swollen starch and an increase in the cell wall thickness affecting the loss porosity of the extrudates (Yanniotis et al., 2007). In addition, the higher moisture content resulted in a harder extruded snack, since under a high feed moisture content, the elasticity of the dough through plasticization of the melt and the vapor pressure were low (Stojceska et al., 2009) resulting in a reduced expansion ratio and increased hardness of the extrudates.

Chemical properties of extrudates

Total phenolic content (TPC)

The TPC values of the flour mixture (control) before extrusion and of the extrudates are presented in Table 2. The TPC was significantly affected by the PF and feed moisture content. The TPC of extrudates with different PF contents were significantly ($p \leq 0.05$) lower than those of the non-extruded flour mixtures. These effects occurred due to extrusion cooking, which resulted in the loss of bioactive compounds such as carotenoids and phenolic compound (Viscidi et al., 2004; Shih et al., 2009). However, the other variables in the process conditions, such as the feeding rate, screw speed and barrel temperature, were also influenced. In this study, the TPC in the extrudates was in the range 6.41–63.24 mg GAE/100 g sample, db. The higher the PF and the lower the feed moisture content, the higher the TPC retained in the extrudates. Therefore, the highest and the lowest TPC values were found at 30% PF and 13% feed moisture content and at 10% PF and 19% feed moisture, respectively. With all PF contents, the highest TPC in extrudates was observed under the lowest feed moisture content (13%). This was perhaps due to breakdown and release from the cell wall matrix of raw material during the harsh thermal processing, that is, from high temperature, high pressure and high shear (Brennan et al., 2011). In this study, the decreased 13% feed moisture content induced high temperature pressure conditions causing greater release of phenolic compounds from the cell wall matrix.

Antioxidant activity

The DPPH radical scavenging activity, ABTS radical cation scavenging activity and ferric reducing antioxidant power (FRAP) are some of the most frequently used assays in the determination of antioxidant activity in extrudates (Reis and Abu-Ghannam, 2014; Chalermchaiwat et al., 2015; Wang et al., 2018; Zhang et al., 2018). The antioxidant activities of the non-extruded flour mixtures before extrusion were higher than those of the extruded snack as shown in Table 2. The extrusion process decreased all antioxidant properties of all analyzed samples perhaps as a result of the reduction in the TPC during this process, as the phenolic contents have a high positive correlation with antioxidant activity (Yang et al., 2010). Moreover, decreasing the feed moisture content from 19% to 13% with all PF contents resulted in significant increases in the DPPH, ABTS and FRAP values. The thermal processing conditions (as found in extrusion cooking under high temperature in combination with a low moisture content) could also favor the formation of brown-colored compounds from the Maillard browning reaction, which enhances antioxidant activity (Sharma et al., 2012). This Maillard reaction product may provide the higher levels of antioxidant activity observed at 13% feed moisture content with all levels of PF contents. According to Soison et al. (2014) decreasing the feed moisture content from 16% to 10% at the same screw speeds resulted in an increase in the DPPH and FRAP values in extrudates from purple-flesh sweet potato flour. In

Table 2
Total phenolic content (TPC) and antioxidant activities of extruded snacks as affected by pumpkin flour (PF) content and feed moisture content.

PF (%)	Feed moisture content (%)	TPC (mg GAE/100 g sample, db)	DPPH (mg GAE/100 g sample, db)	ABTS (mg GAE/100 g sample, db)	FRAP (mg $FeSO_4$/100 g sample, db)
Control 1 [A]		30.90^{e} ± 0.95	15.65^{e} ± 0.02	20.87^{f} ± 0.24	605.39^{e} ± 30.66
10	13	25.43^{f} ± 0.34	14.99^{e} ± 2.31	19.73^{g} ± 0.71	602.45^{e} ± 19.44
	16	14.39^{h} ± 0.12	9.49^{g} ± 0.55	13.98^{i} ± 0.55	370.91^{g} ± 15.86
	19	6.41^{j} ± 0.29	5.97^{h} ± 1.88	7.01^{k} ± 0.10	141.22^{h} ± 16.39
Control 2 [A]		43.95^{c} ± 0.21	25.16^{b} ± 0.21	26.21^{c} ± 0.19	949.91^{c} ± 57.45
20	13	35.68^{d} ± 0.62	22.45^{c} ± 2.19	25.15^{d} ± 0.06	846.24^{d} ± 23.73
	16	21.63^{g} ± 0.55	15.86^{f} ± 0.61	19.30^{g} ± 0.38	490.29^{f} ± 0.75
	19	11.09^{i} ± 0.04	7.80^{gh} ± 0.95	9.75^{j} ± 0.97	335.70^{g} ± 15.90
Control 3 [A]		63.24^{a} ± 0.49	33.03^{a} ± 0.13	29.97^{a} ± 0.20	1835.47^{a} ± 11.98
30	13	53.69^{b} ± 3.70	21.56^{c} ± 0.72	28.93^{b} ± 0.21	1095.22^{b} ± 29.81
	16	34.92^{d} ± 0.34	18.42^{d} ± 0.19	23.68^{e} ± 0.30	1043.89^{b} ± 60.46
	19	23.05^{fg} ± 0.33	11.58^{f} ± 0.29	16.68^{h} ± 0.24	512.54^{f} ± 12.53

DPPH = 2,2-diphenyl-1-picprylhydrazyl; ABTS = 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP = ferric reducing antioxidant power; db = dry basis.
[a–j] values are mean ± SD of triplicate measurements with different lowercase superscript letters in each column are significantly different ($p \leq 0.05$).
[A] Control 1, Control 2 and Control 3 contain PF at 10%, 20% and 30%, respectively, as non-extruded flour.

addition, the increase of PF significantly increased the antioxidant activity (DPPH, ABTS and FRAP) of the extruded snacks because pumpkin is a good source of β-carotene and phenolic compounds (Promsakha na Sakon Nakhon et al., 2017) which impacted on the enhanced nutritional quality of snack.

Sensory evaluation

Sensory acceptability of extrudates using a 9-point hedonic scale was significantly affected by the PF and feed moisture content as presented in Table 3. Increasing the PF content from 10% to 30% along with increasing the feed moisture content (13%–19%) tended to result in low liking scores in all sensory attributes. These extruded snacks were unsatisfactory, especially at 30% PF, because of their uneven shape, small diameter and very hard texture. The highest liking scores of all sensory attributes of extrudates were obtained with extrudates using 10% PF at 13% feed moisture content, except for the taste attribute. The taste attributes had a low preference because of the uncoated flavor of this extruded snack.

Optimization and verification

RSM was applied to the optimized formula for development of a highly nutritious and acceptable extruded snack for consumers. RSM was used to investigate the relationships between the independent variables PF (X_1) and the feed moisture content (X_2) with the dependent variables TPC (Y_1) and sensory attributes including appearance (Y_2) and hardness (Y_3) using a second-order polynomial model (Table 4). These three parameters were chosen because this study has made an attempt to produce healthy snacks and consumer acceptance. Therefore, the antioxidant activity and sensory evaluation were used to obtain the optimum region in this study. The TPC variable was investigated in order to be the most important factor in terms of health benefit. It also acts as an antioxidant. The appearance and hardness variables of sensory attributes are important characteristics of uncoated-flavoring snack for consumer acceptance. From the regression models, R^2 values of appearance (0.65) and hardness (0.72) were high (Table 4), whereas those of odor (0.14), taste (0.10) and overall liking (0.50) were low. The lack of fit was determined and found that TPC and the appearance and hardness sensory attributes models were not significant ($p > 0.05$), showing that these models were valid for the present work.

The superimposed optimization area was obtained from the contour plot regions of the optimum formulation based on the PF and feed moisture content, as shown in Fig. 1(A–D). To identify the optimum conditions suitable for producing a nutritious and acceptable snack, the TPC and acceptable snack sensory values were considered. In this study, the contour plot regions of TPC and the sensory attributes of appearance and hardness are shown in Fig. 1(A–C). The TPC value was assumed to indicate the nutritional value of the extruded snack because the TPC had a positive correlation with the antioxidant activities, DPPH ($r = 0.980$), ABTS ($r = 0.927$) and FRAP ($r = 0.990$). The hedonic scores of appearance and hardness were used to represent acceptable sensory attributes for consumer acceptance because these attributes were the important attributes of uncoat-flavored snack. Coated, flavored snacks in the market are more acceptable than uncoated, flavored snacks, especially with regard to color, odor and taste (Pavithra et al., 2011).

The critical boundaries of the three contour plot regions to determine the optimum area were obtained from a TPC of ≥20 mg GAE/100 g sample (this range had high levels of phenolic compounds) and liking scores of appearance and hardness of ≥6.5 (that is, slightly liked on a 9-point hedonic scale), as shown in Fig. 1D. Therefore, the superimposed area revealed the optimum extrusion formulation of 10–13% PF and 13–14% feed moisture content as shown by the shaded area in Fig. 1D. Within this optimum range, the extrudate would have a TPC of 20.4–28.1 mg GAE/100 g sample, an appearance score of 6.5–7.1 (slightly like to moderately like) and a hardness score of 7.1–7.8 (moderately like to like very much).

The verification of the selected conditions was performed for confirmation of the validity and suitability of the model equations to predict optimum response values. The predicted values derived from the model equations and the experimental values obtained under the selected conditions from the optimum area of the RSM as

Table 3
Sensory evaluation of extruded snacks as affected by pumpkin flour (PF) content and feed moisture content.

PF (%)	Feed moisture content (%)	Sensory attribute				
		Appearance	Odor	Taste	Hardness	Overall liking
10	13	$7.1^{a} \pm 1.1$	$6.3^{a} \pm 1.2$	$5.6^{e} \pm 1.7$	$7.4^{a} \pm 1.1$	$7.1^{a} \pm 1.0$
	16	$6.8^{a} \pm 1.3$	$6.2^{a} \pm 1.2$	$5.4^{d} \pm 1.8$	$6.2^{b} \pm 1.7$	$6.3^{b} \pm 1.3$
	19	$5.2^{bcd} \pm 1.8$	$5.6^{bc} \pm 1.1$	$4.1^{b} \pm 1.8$	$3.0^{d} \pm 1.3$	$3.8^{c} \pm 1.5$
20	13	$5.3^{bc} \pm 1.6$	$5.6^{b} \pm 1.5$	$4.8^{d} \pm 1.8$	$6.2^{b} \pm 1.5$	$5.7^{c} \pm 1.5$
	16	$4.9^{cde} \pm 1.6$	$5.3^{bcd} \pm 1.5$	$4.2^{c} \pm 1.6$	$4.1^{c} \pm 1.6$	$4.3^{e} \pm 1.5$
	19	$4.7^{de} \pm 1.7$	$5.2^{cd} \pm 1.6$	$3.7^{c} \pm 1.8$	$2.8^{d} \pm 1.6$	$3.3^{f} \pm 1.5$
30	13	$5.2^{bcd} \pm 1.8$	$5.4^{bcd} \pm 1.7$	$4.7^{c} \pm 1.9$	$6.0^{b} \pm 1.6$	$5.5^{c} \pm 1.7$
	16	$5.5^{b} \pm 2.0$	$5.2^{bcd} \pm 1.6$	$4.6^{a} \pm 1.9$	$4.6^{c} \pm 1.8$	$4.8^{d} \pm 1.8$
	19	$4.6^{e} \pm 1.7$	$5.0^{d} \pm 1.6$	$4.1^{b} \pm 1.8$	$2.9^{d} \pm 1.6$	$3.4^{f} \pm 1.6$

[a-d] Means ± SD of triplicate measurements with different superscripted letters in each column are significantly different ($p \leq 0.05$).

Table 4
Predictive regression models for total phenolic content (TPC) and sensory evaluation (appearance and hardness attribute) of extruded snacks.

Dependent variable	Predictive model [a]	R^2	Lack of fit (p-value)
TPC	$142.947 + 20.284X_1 - 14.781X_2 + 5.107X_1^2 - 1.763X_1X_2 + 0.427X_2^2$	0.99	>0.05
Sensory attribute			
Appearance	$5.041 - 0.522\ X_1 + 0.931\ X_2 + 0.0074\ X_1^2 + 0.0103\ X_1X_2 - 0.041\ X_2^2$	0.65	>0.05
Hardness	$14.180 - 0.493\ X_1 + 0.01\ X_2 - 0.007\ X_1^2 + 0.03\ X_1X_2 - 0.028\ X_2^2$	0.72	>0.05

X_1 = pumpkin flour (%), X_2 = feed moisture content (%).
R^2 = correlation of determination.
[a] Second-order polynomial regression model: $Y_i = \beta 0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_1X_2 + \beta_4X_1^2 + \beta_5X_2^2$.

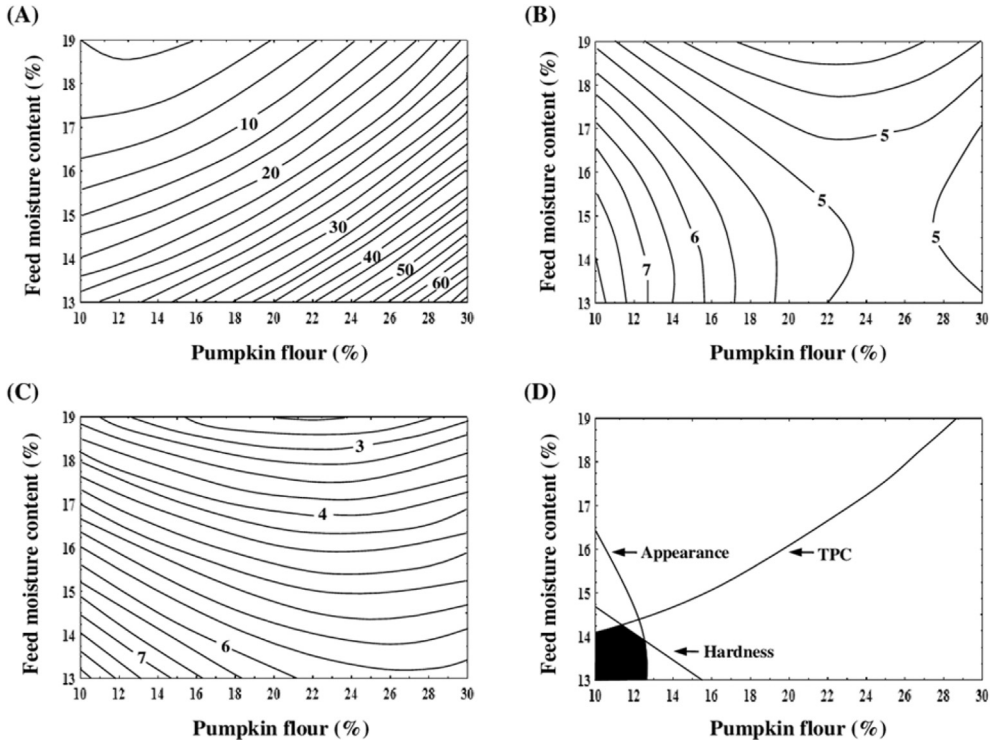


Fig. 1. Response surface methodology contour plots of: (A) total phenolic content (TPC; milligrams gallic acid equivalents/100 g sample on a dry basis); (B) appearance sensory attribute based on a 9-point hedonic scale; (C) hardness sensory attribute based on a 9-point hedonic scale; (D) optimization area (shaded) after overlapping critical boundaries of three contour plot regions of TPC (≥20 mg GAE/100 g sample, db), appearance and hardness at attribute scores ≥ 6.5.

shown in Table 5. The model suitability was determined using the root mean squared error (RMSE). The RMSE values of the TPC, appearance attribute and hardness attribute were 1.25, 0.14 and 0.47, respectively. Therefore, the regression model could be used to predict reasonably well the TPC and sensory scores of appearance and hardness attributes.

The physical properties, TPC, antioxidant activity and sensory properties of extruded snacks made from pumpkin-germinated brown rice flour using an extrusion process were significantly affected by the PF and feed moisture content. Increasing the PF content significantly increased the bulk density, hardness, TPC and antioxidant activity, but decreased the expansion ratio of the extruded snack. A decrease in the feed moisture content from 19% to 13% resulted in increased TPC and antioxidant activity of the extrudates. RSM was successfully used to optimize the formulation of GBRF and PF for developing the extruded snack using the TPC and the liking scores of appearance and hardness. The optimum formula of pumpkin-germinated brown rice flour extrudates was 10–13% and 13–14% feed moisture content. The extrudates had TPC values of 20–60 mg/100 g sample, an appearance score of 6.5–7.1 and a hardness score of 7.1–7.8. Therefore, the GBRF and PF could be useful main ingredients for developing healthy extruded snacks. Further studies should be performed using coated flavorings to encourage consumer acceptability.

Table 5
Experimental and predicted values of extruded snack under selected conditions for verification of optimized region.

Properties and extrusion formulation	Predicted[a]	Experimental[b]	RMSE
TPC (mgGAE/100g sample)			
10% PF and 13% feed moisture content	25.4	24.96 ± 0.82	1.25
10% PF and 14% feed moisture content	20.4	21.63 ± 1.23	
13% PF and 13% feed moisture content	28.1	26.42 ± 1.56	
Sensory attribute: Appearance			
10% PF and 13% feed moisture content	7.1	6.9 ± 1.1	0.14
10% PF and 14% feed moisture content	7.0	7.1 ± 1.1	
13% PF and 13% feed moisture content	6.5	6.5 ± 1.0	
Sensory attribute: Hardness			
10% PF and 13% feed moisture content	7.8	7.1 ± 1.3	0.47
10% PF and 14% feed moisture content	7.4	7.0 ± 1.3	
13% PF and 13% feed moisture content	7.1	7.0 ± 1.1	

TPC = total phenolic content; PF = pumpkin flour; RMSE = Root mean squared error between experimental and predicted value; TPC measured as milligrams of gallic acid equivalents/100 g on a dry basis.
[a] Values derived from predictive regression model (Table 4).
[b] Mean ± SD of triplicate measurements.

Conflict of interest

The authors declare that there are no conflicts of interest.

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