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Optimisation of extrusion conditions for production of antioxidant-rich extruded breakfast cereals from purple sweet potato (*Ipomoea batatas* L.) and red rice using response surface methodology

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Abstract

Purple sweet potato powder (PSPP) is rich in anthocyanins and other polyphenols that provide excellent antioxidant and other biological activities with potential health benefits. In the present work, the response surface methodology (RSM) was used to optimise the extrusion processing conditions to develop healthy breakfast cereals. The independent variables studied included barrel temperature, screw speed, and feed moisture. The linear terms of barrel temperature and feed moisture content were found to be the significant ($p < 0.05$) factors affecting the product's functional and physicochemical properties. The expansion property of extrudate significantly ($p < 0.001$) increased at low temperature, high screw speed, and low feed moisture. The recommended optimum extrusion conditions of barrel temperature, screw speed, and feed moisture content were at 157.0°C, 126.0 rpm, and 13.0%, respectively; and under these optimum conditions, significantly high retention (75.0%) of anthocyanin content was detected. Furthermore, scanning electron micrographs depicted that the optimised breakfast cereals had a better cell structure with smoother and thinner cell walls than the non-optimised samples.

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Introduction

Sweet potato (*Ipomoea batatas* L.) is a dicotyledonous crop, belonging to the Convolvulaceae family. The annual global production of sweet potato is around 90 million metric tonnes in 2020, and it is the sixth most important food crop in the world after rice, wheat, maize, cassava, and potatoes. Sweet potato is a tuber crop that is rich in nutritional values including carbohydrates (70 - 83 g/100 g dry weight (DW)), dietary fibres (5.8 - 12.2 g/100 g DW), anthocyanins (7477 - 34569 mg/kg DW), phenolic acids (1.8 - 7.4 mg gallic acid equivalent/g DW), carotenoids (0.8 - 42 mg/100 g DW), vitamins (4.5 - 109.2 mg/100 g DW), and minerals (1.2 - 4.1 g/100 g DW). However, nutritional composition and bioactive compound variations occur due to different factors such as genotype, tuber colour, geography, maturity, and harvesting (Alam, 2021). The health benefit of purple sweet potato (PSP) has attracted the attention of researchers in the past years, owing to the presence of anthocyanin and other phenolic compounds which contribute to several biological activities such as free radical scavenging, antimutagenicity, antidiabetic, antitumor, and antihypertensive (Fan *et al.*, 2008; Senevirathna *et al.*, 2021). Blending purple sweet potato powder (PSPP) with major cereals cultivated worldwide, such as rice and corn, is expected to produce colourful enriched PSPP-based breakfast cereal of various forms and shapes with excellent antioxidant properties, especially if whole red rice is used. Whole red rice is considered another potential source of phytochemicals besides containing a substantial amount of fibre (Samyor *et al.*, 2018; Wang *et al.*, 2020; Yamuangmorn *et al.*, 2021).

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Extrusion is an efficient and versatile technology, widely used in producing a variety of food products such as breakfast cereals, savoury snacks, pre-cooked flours, and cereal-based foods (Dalbhagat *et al*., 2019; Sahu and Patel, 2020). The effects of extrusion parameters such as barrel temperature, feed moisture, feed rate, and screw speed on the quality of food products have been reported (Alam *et al*., 2016; Natabirwa *et al*., 2018; Jalgaonkar *et al*., 2019). Samyor *et al.* (2018) reported that physicochemical and phytochemical properties of extrudates were significantly affected by barrel temperature, screw speed, and feed moisture content. Response surface methodology (RSM) has been applied for optimisation of the operating condition of extruders for several food products such as extruded rice starch-pea protein snacks (Philipp *et al*., 2017), ready-to-eat expanded snacks (Lotfi Shirazi *et al*., 2020), and Roba1 bean extrudate (Natabirwa *et al*., 2018).

However, not many studies have been reported on the effect of extrusion parameters on the physicochemical, textural, and functional properties of PSP-based extruded breakfast cereal. Therefore, the objective of the present work was to investigate the effects of the aforementioned extrusion process parameters on the physicochemical, textural, and functional properties of PSPP-based extruded puffed breakfast cereals using RSM with Box-Behnken design.

Materials and methods

Materials

Freshly harvested PSP tubers were obtained from a local farm in Selangor, Malaysia. Whole red rice (WRR) and cornflour (CF) were purchased from a local shop in Selangor, Malaysia. The Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), and gallic acid (98%) were purchased from Merck (Darmstadt, Germany). TPTZ (2,4,6-tripyridyl-s-triazine) and Trolox were purchased from Acros Organics (Geel, Belgium). Other solvents and chemicals used in the present work were of analytical grade, and purchased from Fisher Scientific (Leicestershire, UK).

Sample preparation

The preparation of PSPP was according to Senevirathna *et al*. (2021). Briefly, unpeeled PSP tubers (~4 kg) were washed thoroughly, weighed, and cut into cubes of approximately 3 × 3 × 1.5 cm (length × width × height) using a stainless-steel knife. The PSP cubes were steamed at 100°C for 30 min in a stainless-steel steam cooker, and cooled to room temperature before mashed into a purée, using a bowl cutter (Mainca CM-21, Spain) at low speed for 10 min. After 1 min of mashing time, 0.6% citric acid powder was added to the purée. The PSP purée was passed through a pre-heated double drum dryer (R. Simon Dryers Ltd., Nottingham, England). The steam pressure was set at 500 kPa, and the drum rotation speed was 3 rpm. Then, the dried flakes were milled using a high-speed stainless-steel grinder (IKA M20, IKA Labor-Technik, Staufan, Germany) for 20 s, and sieved through a 425-µm mesh screen to obtain the PSPP. WRR was milled with an electric hammer mill, and passed through a 400-µm sieve to obtain whole red rice flour (WRRF). The PSPP and WRRF were stored at 4°C in laminated aluminium foil bags before use. The range of main ingredients used for the formulation of breakfast cereals were selected based on the optimised formulation, as reported in our previous work (Senevirathna *et al*., 2022). The dry ingredients were PSPP (55.00%), WRRF (15.00%), and CF (30.00%). A mixture of 0.5 kg of dough from each batch was pre-mixed in a commercial food mixer (1000W 'Chef Premier' Mixer, Kenwood, Britain) for 1 min. Then, a specific amount of water (calculated beforehand) was added into the flour mixture while mixing to reach the targeted moisture content (known as feed moisture) of 13.00,15.50, and 18.00%, whereupon the mixing was further continued for another 5 min. The flour mixtures were packaged in polypropylene bags, and tempered in a chiller (4 ± 2°C) for 24 h to ensure a uniform hydration of the flour before extrusion.

Extrusion cooking

The extrusion cooking was performed in a single screw extruder (Stand Alone Extruder 16/25D, Brabender GmbH and Co. KG, Germany) with a compression ratio of 3:1. The flour mixtures were extruded at different die temperatures (140.0 - 180.0°C), screw speeds (100.0 - 140.0 rpm), and feed moistures (13.0 - 18.0%) based on the Box-Behnken design (BBD) at a fixed feed rate of 45.00 rpm, using the 3.00-mm die. A three-blade cutter operated at 130 rpm was used to form ball-shaped extrudates. The extrudates, referred to as breakfast cereals, were dried in a drying cabinet (EDO-10, EURO ASIA) at 55 ± 2°C to reduce the moisture content to below 5%. The breakfast cereals were packed in laminated aluminium foil bags, and stored at 4 ± 2°C for further analyses.

Physicochemical and textural properties of samples

Textural properties of extrudates

A texture analyser (TA-XT2i® Texture Analyser, UK) fitted with a P/50 (50-mm diameter, cylinder aluminium) probe was used to determine the texture characteristics of breakfast cereals. An average of 20 randomly selected sample measurements were taken (Oliveira *et al*., 2018; Pardhi *et al*., 2019). The bowl-life texture was determined according to Oliveira *et al*. (2017), with slight modifications. Briefly, breakfast cereals were immersed in whole milk (3% fat) at 5°C for 3 min, and drained in a sieve for approximately 10 s. The hardness and crispness of the extrudates were measured according to Oliveira *et al*., 2018; Pardhi *et al*., 2019.

Scanning electron microscopy (SEM)

Samples of breakfast cereals were cut using a razor blade, and coated with a thin layer of gold before being examined with field-emission scanning electron microscopy (FESEM, FEI Nova NanoSEM 230, USA), using an operating voltage of 5 kV. Samples were photographed at a working distance of 4.80 - 5.60 mm (Philipp *et al*., 2017).

Sectional expansion index (SEI)

The sectional expansion index of breakfast cereals was determined according to Oliveira *et al.* (2018).

Bulk density (BD)

The bulk density of breakfast cereals was determined by a seed displacement method, according to Oliveira *et al*. (2017).

Determination of colour (L, a, b*, hue angle, and chroma)*

A chromameter (CR-410, Konica, Minolta, Japan) was employed to determine the colour attributes of expanded breakfast cereals, according to Senevirathna *et al.* (2021).

Water absorption capacity and water solubility index

The water absorption capacity (WAC) and the water solubility index (WSI) of breakfast cereals were determined according to Senevirathna *et al.* (2021), with slight modifications. Eqs. 1 and 2 were used to calculate the WAC and WSI of the extrudates, respectively:

$$\text{WSI} = \frac{\text{residue supernatant dry weight}}{\text{dried powder weight}} \times 100 \tag{Eq. 1}$$

$$\text{WAC} = \frac{\text{weight of precipitate}}{\text{dried powder weight} - \text{residue supernatant dry weight}} \tag{Eq. 2}$$

Determination of antioxidant activity

Extraction of antioxidants

Extraction of antioxidants from extruded breakfast cereals was carried out according to Yang *et al*. (2010), with slight modifications. In brief, 2 g of powdered extrudates were mixed with 10 mL of 80% methanol in 50-mL centrifuge tubes. The mixture was vortexed for 30 s, and shaken for 2 h at room temperature (28 ± 2°C). Then, the tubes were centrifuged at 3,000 *g* for 10 min. The supernatant was collected and stored at -20°C in capped tubes for further analyses.

High-performance liquid chromatography (HPLC)

Powdered extrudates (2 g) were mixed with 30 mL of 80% methanol and 10 mL of 6 mol/L HCl in a 50-mL graduated tube. The tube was screw-capped and heated in a water bath at 90°C for 90 min. The tube was cooled in an ice bath, and the volume was made up to 50 mL using 80% methanol. The supernatant was collected after centrifugation for 15 min at 3,000 *g*. Standards of peonidin and cyanidin at 0.2 - 0.0125 mg/mL were also acid hydrolysed in the same manner. Anthocyanins were separated by reversed-phase HPLC using a Waters 2695 HPLC system with an RP-18 LiChrospher column of 250 × 4.6 mm and 5-µm particle size (Merck, Darmstadt, Germany). The eluents used were formic acid in water (A), 5% (v/v) and methanol 100% (B). The solvent gradient used was: 0 - 15 min, 15 - 35% B; 15 - 30 min, 35 - 60% B; 30 - 40 min, 60 - 80% B; and 40 - 45 min, 80 - 15% B. The flow rate was 1.0 mL/min, and the injection volume was 20 µL (Senevirathna *et al*., 2021).

Determination of total anthocyanin content

The TAC of breakfast cereals was determined by the pH differential method, according to Jiang *et al.* (2019), with slight modifications.

Determination of total flavonoid content (TFC)

A method described by Yea *et al.* (2019) with slight modifications was used to determine the TFC of breakfast cereals.

Determination of total phenolic content

The TPC of breakfast cereals was determined by a Folin-Ciocalteu assay according to Singleton *et al*. (1999), with slight modifications.

Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity of breakfast cereals was determined according to Brand-Williams *et al*. (1995), with slight modifications.

Determination of ferric reducing antioxidant power (FRAP)

The FRAP of breakfast cereals were determined according to Benzie and Strain (1996), with slight modifications.

Experimental design and data analysis

The quadratic polynomial equations generated by the Design-Expert (version 11.1.2.0, State-Ease, Minneapolis, MN) software with Box-Behnken design were used to optimise the parameters of the breakfast cereal extruder process as shown in Eq. 3:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_1 X_3 + \beta_6 X_2 X_3 + \beta_7 X_1^2 + \beta_8 X_2^2 + \beta_9 X_3^2 \qquad \text{(Eq. 3)}$$

where, Y = response calculated by the models; β_0, β_1, β_2, β_3, β_4, β_5, β_6, β_7, β_8, and β_9 = regression coefficients; and X_1, X_2, and X_3 = independent variables.

The statistical significance and quality of the fit for each equation were determined by analysis of variance (ANOVA). Post analysis confirmation test of Design-Expert software was used to evaluate the validity of the fitted models. All the measurements were taken in triplicates, and results were presented as mean ± standard deviation.

Results and discussion

Fitting for the best model

The ANOVA and multiple regression analysis techniques were used to test the statistical significance of response variables. The best-fitted models were selected based on low standard deviation, insignificant lack of fit, high predicted R^2, and low predicted sum of squares. The experimentally observed responses of extruded breakfast cereals are given in Table 1. Linear and second-order polynomial models with interaction terms were used to determine the goodness of fit for the model. The best-fitted models for the experimented parameters are shown in Table 2. Extrudates were significantly different in colour, texture, expansion, and other functional properties.

Effect of processing parameters on physicochemical and textural properties of breakfast cereals

Bulk density (BD)

The BD of an extruded product indicates the magnitude of expansion, and it is very important in puffed breakfast cereals. The BD of the extrudates was significantly affected by the independent variables of this extrusion experiment (Table 1). As shown in Table 2, the R^2 value (0.98) was very high along with a non-significant lack-of-fit, which confirmed that the developed model correctly predicted the relationship among BD and independent variables. The coded version of the reduced multiple regression model is given in Table 2. In addition, Figure 1a shows the relationship between barrel temperature (BT), screw speed (SS), and moisture content (MC), with the BD of breakfast cereals.

The increase in temperature and moisture content increased BD. However, an increase in SS decreased BD. The interaction effect of BT with MC was negative on BD, but the quadratic effect of SS was significantly positive on BD. Natabirwa *et al.* (2018) observed similar findings in their study on pea extrudates, where they reported that high BD at increased feed moisture was due to low mechanical shear in the extruder, caused by the lubricating effect of water in low expansion. The high BD at low SS (Figure 1a) at all temperatures and feed moisture contents might have been due to low shear and decreased starch gelatinisation, thus resulting in less expansion of breakfast cereals caused by low vapour pressure at the die (Hagenimana *et al*., 2006; Natabirwa *et al*., 2018).

Sectional expansion index (SEI)

The Pearson's correlations (*r*) analysis demonstrated that SEI and BD had a high negative correlation (r = -0.90), thus indicating that a highly

Table 1. Effect of extruder process parameters on the textural, physicochemical, and functional properties of extrudates.

Run No.	Response variables					
	SEI	BD (g/cm^3)	Hardness (kg)	Crispness (kg.s)	WSI (%)	WAC (g/g)
1	2.61 ± 0.12^{de}	0.55 ± 0.01^{c}	4.06 ± 0.60^{g}	36.21 ± 5.76^{fg}	15.22 ± 0.48^{fg}	7.04 ± 0.05^{def}
2	3.23 ± 0.16^{a}	0.31 ± 0.00^{i}	3.77 ± 0.64^{g}	33.65 ± 5.41^{g}	22.86 ± 0.16^{a}	5.79 ± 0.26^{g}
3	2.73 ± 0.18^{cd}	0.54 ± 0.02^{cd}	4.97 ± 0.79^{ef}	41.50 ± 6.25^{cdef}	11.41 ± 0.21^{h}	8.34 ± 0.14^{ab}
4	3.30 ± 0.18^{a}	0.33 ± 0.00^{i}	4.22 ± 0.72^{fg}	39.99 ± 6.39^{efg}	19.65 ± 0.47^{bcd}	6.98 ± 0.14^{ef}
5	2.78 ± 0.09^{c}	0.51 ± 0.01^{e}	5.09 ± 0.77^{de}	47.72 ± 8.19^{bcd}	20.74 ± 1.43^{ab}	5.78 ± 0.15^{g}
6	2.55 ± 0.18^{ef}	0.67 ± 0.02^{a}	4.92 ± 0.76^{ef}	42.42 ± 7.00^{bcdef}	11.93 ± 0.14^{h}	8.58 ± 0.09^{a}
7	2.72 ± 0.08^{cd}	0.48 ± 0.01^{f}	5.73 ± 0.61^{bcde}	48.92 ± 7.73^{bc}	16.44 ± 1.52^{ef}	7.41 ± 0.21^{cde}
8	2.69 ± 0.11^{cd}	0.45 ± 0.01^{gh}	5.28 ± 0.82^{cde}	44.68 ± 6.08^{bcde}	16.69 ± 1.51^{ef}	7.30 ± 0.35^{def}
9	2.78 ± 0.10^{c}	0.47 ± 0.01^{fg}	5.39 ± 0.52^{cde}	48.04 ± 8.36^{bcd}	17.36 ± 0.65^{def}	7.15 ± 0.08^{def}
10	3.09 ± 0.12^{b}	0.32 ± 0.01^{i}	5.85 ± 0.80^{bcd}	41.01 ± 6.52^{defg}	20.53 ± 0.19^{abc}	6.22 ± 0.23^{g}
11	2.71 ± 0.20^{cd}	0.52 ± 0.01^{de}	5.08 ± 0.73^{de}	48.77 ± 7.41^{bc}	13.71 ± 0.42^{gh}	8.25 ± 0.14^{ab}
12	2.47 ± 0.08^{f}	0.65 ± 0.01^{b}	7.51 ± 1.11^{a}	60.43 ± 5.84^{a}	18.03 ± 0.85^{cde}	6.86 ± 0.07^{f}
13	2.44 ± 0.07^{f}	0.56 ± 0.02^{c}	6.03 ± 0.98^{bc}	49.60 ± 7.56^{b}	16.09 ± 0.25^{efg}	7.88 ± 0.12^{bc}
14	2.73 ± 0.05^{cd}	0.43 ± 0.00^{h}	7.32 ± 0.77^{a}	49.53 ± 7.18^{b}	20.27 ± 1.28^{bc}	6.16 ± 0.19^{g}
15	2.78 ± 0.08^{c}	0.48 ± 0.01^{f}	6.41 ± 0.96^{b}	45.42 ± 7.36^{bcde}	17.26 ± 0.60^{def}	7.56 ± 0.07^{cd}

Run No.	L	a*	Hue	TAC (mg/100 g)	DPPH (µmol TE/g)	TPC (mg GAE/g)
1	33.62 ± 0.16^{f}	20.90 ± 0.10^{f}	0.84 ± 0.08^{cde}	37.79 ± 0.94^{cd}	12.97 ± 0.51^{bc}	4.88 ± 0.06^{f}
2	37.08 ± 0.65^{de}	23.00 ± 0.63^{cd}	-0.38 ± 0.10^{gh}	43.49 ± 1.52^{ab}	14.11 ± 0.38^{ab}	5.53 ± 0.04^{b}
3	31.18 ± 0.50^{g}	19.43 ± 0.40^{g}	1.12 ± 0.18^{bc}	32.03 ± 0.68^{f}	11.16 ± 0.23^{de}	4.40 ± 0.04^{h}
4	33.32 ± 0.44^{f}	22.41 ± 0.41^{de}	0.74 ± 0.18^{de}	35.74 ± 0.85^{de}	12.23 ± 0.42^{cd}	4.67 ± 0.05^{g}
5	38.17 ± 0.27^{abc}	23.51 ± 0.20^{bc}	-0.94 ± 0.12^{i}	42.82 ± 0.97^{ab}	14.09 ± 0.25^{ab}	5.31 ± 0.01^{cd}
6	33.23 ± 0.27^{f}	20.88 ± 0.18^{f}	0.97 ± 0.21^{cd}	29.68 ± 0.61^{f}	10.64 ± 0.63^{e}	4.03 ± 0.02^{i}
7	36.44 ± 0.21^{e}	22.97 ± 0.27^{cd}	-0.56 ± 0.05^{h}	40.39 ± 1.93^{bc}	13.40 ± 0.17^{abc}	5.09 ± 0.08^{e}
8	36.27 ± 0.46^{e}	23.52 ± 0.40^{bc}	-0.15 ± 0.06^{g}	38.64 ± 0.80^{cd}	13.51 ± 0.13^{abc}	4.77 ± 0.04^{fg}
9	37.41 ± 0.34^{cd}	24.22 ± 0.16^{b}	-0.21 ± 0.09^{g}	40.87 ± 1.80^{bc}	13.63 ± 0.26^{ab}	5.13 ± 0.06^{de}
10	38.92 ± 0.62^{a}	24.26 ± 0.13^{b}	-1.55 ± 0.17^{j}	45.00 ± 1.72^{a}	14.35 ± 0.26^{ab}	5.51 ± 0.08^{b}
11	33.86 ± 0.32^{f}	21.91 ± 0.26^{e}	0.59 ± 0.21^{ef}	32.76 ± 0.35^{ef}	11.11 ± 0.30^{de}	4.42 ± 0.12^{h}
12	38.56 ± 0.30^{ab}	22.54 ± 0.21^{de}	0.27 ± 0.08^{f}	37.94 ± 0.53^{cd}	13.93 ± 0.17^{ab}	5.42 ± 0.07^{bc}
13	36.98 ± 0.45^{de}	21.78 ± 0.51^{e}	1.33 ± 0.14^{b}	36.64 ± 0.33^{d}	12.13 ± 0.29^{cd}	4.68 ± 0.03^{g}
14	38.83 ± 0.62^{a}	25.20 ± 0.47^{a}	0.56 ± 0.23^{ef}	45.82 ± 0.84^{a}	14.36 ± 1.30^{a}	5.89 ± 0.08^{a}
15	37.86 ± 0.34^{bcd}	22.80 ± 0.50^{cd}	2.59 ± 0.19^{a}	36.16 ± 0.94^{d}	13.05 ± 0.24^{abc}	5.11 ± 0.06^{e}

Means in the same column followed by different lowercase superscripts are significantly different at $p < 0.05$. BD: bulk density, SEI: sectional expansion index, WSI: water solubility index, WAC: water absorption capacity, TAC: total anthocyanin content, TPC: total phenolic content, and DPPH: radical scavenging activity.

Table 2. Regression coefficient and predicted coded model for the experimental data of the breakfast cereals.

Parameter	**Predicted coded model**	**R^2 value**	***p*-value (model)**
BD	= 0.46 + 0.05 A** - 0.09 B** + 0.09 C** - 0.027 AC* +0.04 B^2*	0.98	< 0.0001
Hardness	= 5.44 + 1.28 A** - 0.003 B - 0.13 C - 0.62 AC*	0.91	< 0.0001
Crispness	= 45.13 + 6.82 A** - 1.56 B + 1.30 C - 4.46 AB* + 3.26BC*	0.88	0.0007
SEI	= 2.77 - 0.18 A** + 0.18 B** - 0.18 C** - 0.09 AB*	0.92	< 0.0001
L	= 36.11 + 2.13 A** + 0.05 B - 2.22 C** + 1.01 AC*	0.95	< 0.0001
a*	= 23.04 + 0.82 A* + 0.44 B - 1.50 C** - 0.78 A^2*	0.87	0.0002
b*	= 0.12 + 0.13 A + 0.06 B + 0.30 C* + 0.23 AB* + 0.46A^2*	0.82	0.0034
Hue	= -0.26 + 0.30 A + 0.15 B + 0.79 C* + 0.60 AB* + 1.15A^2*	0.84	0.0022
Chroma	= 23.04 + 0.83 A* + 0.45 B - 1.50 C** - 0.78 A^2*	0.87	0.0002
TAC	= 39.70 + 0.94 A + 0.18 B - 5.75 C** - 2.46 B^2*	0.93	< 0.0001
DPPH	= 13.49 + 0.38 A* - 0.11 B - 1.48 C** - 0.43 B^2* - 0.53C^2*	0.94	< 0.0001
TPC	= 4.99 + 0.20 A* + 0.008 B - 0.59 C**	0.87	< 0.0001
WAC	= 7.15 + 0.04 A + 0.09 B + 1.14 C*	0.93	< 0.0001
WSI	= 16.77 + 0.31 A + 0.65 B* - 3.91 C** - 1.30 AB* +1.82 AC** + 0.83 A^2*	0.98	< 0.0001

A: barrel temperature; B: screw speed; C: moisture content. *Significant at 0.05 level, **Significant at 0.001 level. BD: bulk density, SEI: sectional expansion index, WSI: water solubility index, WAC: water absorption capacity, TAC: total anthocyanin content, TPC: total phenolic content, and DPPH: radical scavenging activity.

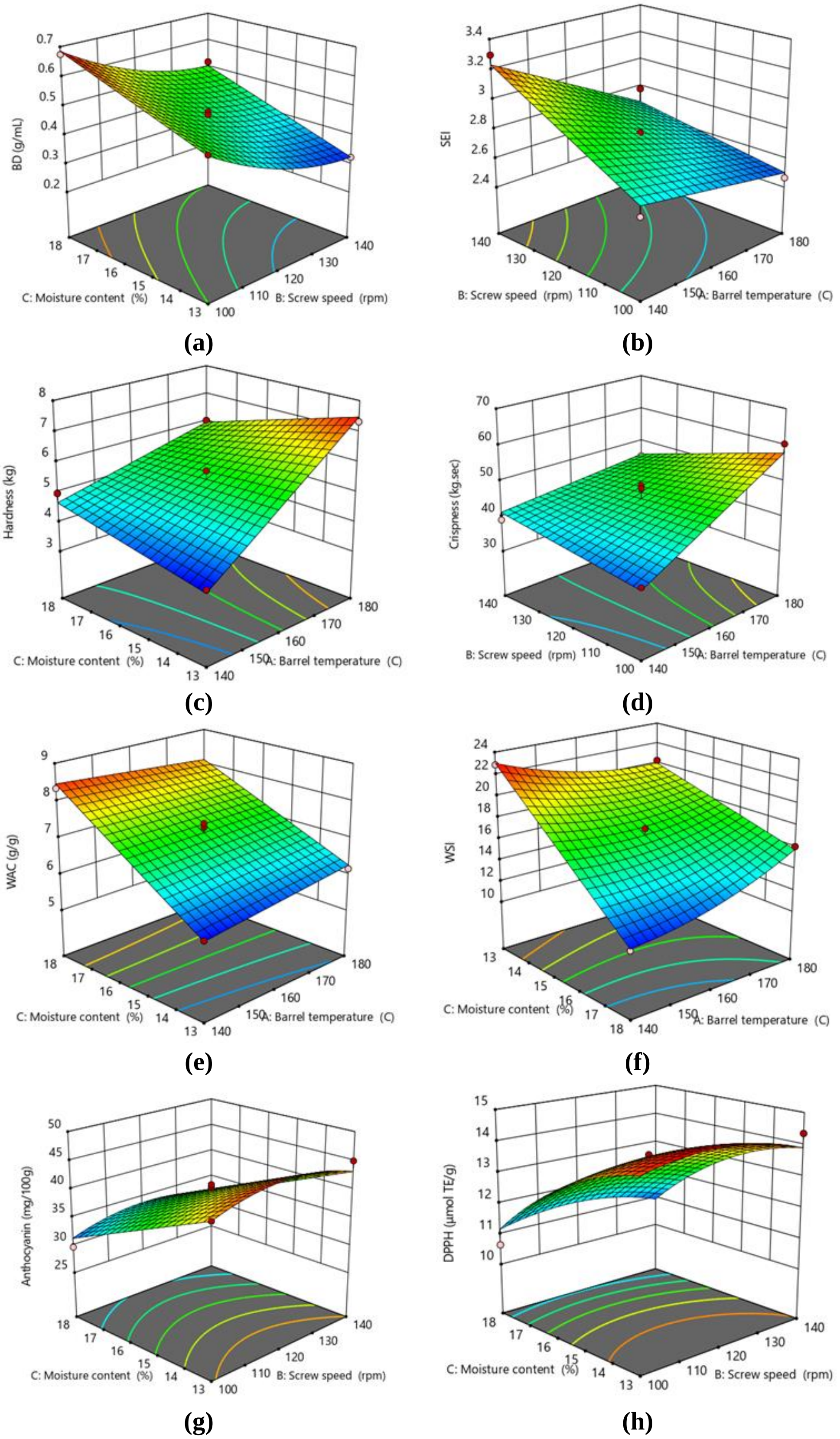


Figure 1. Effect of processing parameters on **(a)** bulk density, **(b)** sectional expansion index, **(c)** hardness, **(d)** crispness, **(e)** WAC, **(f)** WSI, **(g)** total anthocyanin content, and **(h)** DPPH radical scavenging activity.

expanded product and a less dense product was produced. The effect of the extrusion parameters on the SEI of the extrudates is shown in Table 1. As shown in Table 2, the ANOVA for the reduced model showed a highly significant p-value ($p < 0.0001$) and non-significant lack-of-fit along with high R^2 value (0.92), thus suggesting that this model was appropriate for inferring the experimental data.

Table 2 also shows that the linear terms A and C, and the interaction effect of AB had a significant negative effect on SEI. A positive linear coefficient of "B″ contributed to the increase in SEI. Figure 1b shows the response surface plots for SEI against the extrusion parameters. The decrease in SEI with increasing barrel temperature could be attributed to the weakening of starch structure due to higher dextrinisation at high temperatures (Lotfi Shirazi *et al*., 2020; Mendonça *et al*., 2000). The low SEI at high moisture content may reduce the elasticity of dough, as a result of plasticisation of the melt during extrusion (Natabirwa *et al.*, 2018). Lotfi Shirazi *et al.* (2020) mentioned that high moisture acts as a lubricant, and reduces the effect of barrel temperature, thus resulting in a less expanded product due to the dropping of starch gelatinisation. The higher SEI at high screw speed could possibly be due to a rise in vapour pressure of the die, which resulted in bubble growth (Natabirwa *et al.*, 2018). Therefore, the current findings suggested that low temperature, high screw speed, and low moisture contents are vital to obtain high expanded extruded PSP breakfast cereals.

Hardness (HD)

Multiple regression analysis (Table 2) indicated that the second-order polynomial model was highly significant ($p < 0.0001$), along with high R^2 (0.91) and insignificant lack-of-fit. This implied that the model developed effectively described the relationship between hardness and the independent variables studied.

The effect of independent variables on the hardness is presented in Figure 1c. The equation shown in Table 2 indicates that barrel temperature had a significantly ($p < 0.001$) positive effect on the hardness of extrudates. However, the negative coefficients of the interaction of "AC" suggested that their interaction reduced the hardness of the product. A low hardness value is desirable for extruded products (Mendonça *et al.*, 2000). Basilio-Atencio *et al.* (2020) observed an increment in hardness when the barrel temperature was increased to 175°C, which was in agreement with our findings. The high-temperature extrusion weakened the starch structure due to higher dextrinisation and restricting of bubble growth, thus resulting in a more rigid texture with a denser structure (Mendonça *et al.,* 2000).

Crispness (CR)

The reduced regression model of extrudates demonstrated that crispness was significant, with high R^2 value (0.88) and non-significant lack-of-fit, thus indicating the model's suitability to explain the experimental data (Table 2). As presented in Figure 1d, the barrel temperature showed a significantly positive ($p < 0.001$) relationship with crispness. Nevertheless, barrel temperature and screw speed interaction had a significant ($p < 0.05$) negative effect on the crispness of extrudates. Meanwhile, the positive significant coefficient of the interaction of "BC" indicated that the interaction of terms increased the crispness of the product. Natabirwa *et al.* (2018) also found a positive relationship between die temperature and crispness of extruded Roba1. High positive correlation ($r = 0.82$) between CR and HD observed in the present work could have been due to the restriction of bubble growth at high temperature, due to higher dextrinisation of starch. The formation of a more significant number of tiny bubbles in extrudates may result in high CR and HD at higher extrusion temperatures. Furthermore, this can be confirmed by a significant negative correlation ($r = -0.60$) between SEI and CR, whereby a less expanded product could result in higher CR.

Colour

Table 1 presents the effect of extrusion variables on colour attributes (hue, L*, and a*) of breakfast cereals. Polynomial equations for all the colour attributes (L*, a*, b*, hue, and chroma) showed a significant p-value ($p < 0.01$), non-significant lack-of-fit, along with high R^2, which confirmed that the reduced models interpret the experimental data reliably, as shown in Table 2. The L* value refers to lightness of the product. The result indicated that the "A" and "C" significantly ($p < 0.001$) positively and negatively affected the lightness of the extrudates, respectively, but the screw speed did not show a significant effect on any colour attributes. The "AC" interaction increased the L* value of the product. The a* value refers to the redness of the product, and this value was significantly affected with a change of main effects "A" and "C", as well as the quadratic term of temperature. Ilo *et al*. (1999) also found that increasing barrel temperature increased the colour attribute due to the increased rate of browning reaction, which was compatible with our findings. A chroma gives the colour intensity of the extrudates. The hue and chroma values of the extrudates were significantly affected by the independent variables, as illustrated in Table 1. Nayak *et al.* (2011) mentioned that the colour changes of extrudates were due to the degradation of anthocyanins and the presence of Maillard reaction products during the high-temperature extrusion processing.

Water solubility index (WSI) and water absorption capacity (WAC)

Table 1 presents the variation in WSI and WAC of extrudates, as affected by the independent variables. The regression analysis results (Table 2) confirmed that both models described the design space precisely. Both models were highly significant ($p < 0.0001$) and showed non-significant lack-of-fit ($p > 0.05$) with higher R^2 values.

As presented in the contour plot Figure 1e and Table 2, significant positive coefficient of "C" illustrated that WAC was positively related to the feed moisture content. This was due to the high level of moisture that acted as a lubricant, decreased starch degradation, and increased WAC of extrudates (Pardhi *et al.*, 2019).

WSI often expresses the amount of soluble compound released from the starch during extrusion cooking (Alam *et al.*, 2016). It was evident that the WSI of the extrudate was significantly affected by screw speed ($p < 0.05$) and moisture content ($p < 0.001$) (Figure 1f). As shown in Table 2, the interaction of "AC" and the quadratic effect of "A" increased the number of water-soluble products, whereas the interaction effect of "AB" decreased WSI. The combination of low moisture and harsh conditions might have increased the degradation of starch granules, thus resulting in increased WSI of the product (Alam *et al.,* 2016; Basilio-Atencio *et al.*, 2020).

Effect of processing parameters on antioxidant properties of breakfast cereals

Total anthocyanin content

The TAC of breakfast cereals ranged from 29.68 ± 0.61 - 45.82 ± 0.84 mg/100 g (Table 1). Table 2 illustrates the regression analysis results, and confirms that the model accurately described the design space. The model had higher R^2 values and non-significant lack-of-fit ($p > 0.05$), and was highly significant ($p < 0.0001$). As shown in Figure 1g and Table 2, MC and quadratic terms of SS had significant ($p < 0.001$ and $p < 0.05$) negative effect on TAC. Initially, the flour mixture had a TAC of 59.62 ± 1.15 mg/100 g, but after extrusion, the TAC of the extrudates decreased significantly ($p < 0.05$). The loss of anthocyanins might have resulted from the decomposition and degradation of anthocyanins at high temperatures during extrusion processing. However, in the present work, variation in BT did not result in significant changes in the TAC. Similarly, Hu *et al.* (2018) also reported that the extrusion process significantly reduced the anthocyanins in black rice.

In addition, an increase in the feed moisture resulted in a significant ($p < 0.001$) decrease in anthocyanins in extrudates. Menchaca-Armenta *et al.* (2020) also reported a similar trend in nixtamalised blue corn extrudates. It was suggested that at high MC, starch in the ingredients gelatinised to form a paste, thus resulting in a decrease in flow rate during extrusion, hence causing longer exposure to high mechanical shear and temperature, leading to a high degradation of anthocyanins. In addition, the high feed moisture induces self-association of the anthocyanins to form high molecular weight compounds, which decreases the extractability and quantification.

Total phenolic content

The TPC of extrudates varied between 4.40 ± 0.04 - 5.89 ± 0.08 mg GAE/g (Table 1), and was significantly lower than the flour mixture (before extrusion) (6.47 ± 0.17 mg GAE/g). As shown in Table 2, the model was significant ($p < 0.0001$) with high R^2 and non-significant lack-of-fit ($p > 0.05$). BT had a significant positive effect on TPC. An increase in MC significantly ($p < 0.001$) decreased the TPC of extrudates. However, the effect of SS on TPC was found to be insignificant. It was observed that an increase in BT, increased the TPC of extrudates. The increase in TPC for the extrudates at low feed moisture content and higher barrel temperature might have been due to the release of polyphenolic compounds from the ingredients used in the extrudates under extreme conditions (Soison *et al.*, 2014), or the production of new polymeric compounds due to Maillard reactions during extrusion (Peksa *et al.*, 2016). A similar findings was observed by Natabirwa *et al*. (2018) and Soison *et al.* (2014) in their study on Roba1 bean extrudate and PSP extrudate, respectively.

DPPH radical scavenging activity

The antioxidant activities of breakfast cereals varied from 10.64 ± 0.63 - 14.36 ± 1.30 µmol TE/g (Table 1), and this value was significantly lower than the antioxidant activities of the flour mixture (17.63 ± 0.58 µmol TE/g). The lower antioxidant activity of the extrudates when compared with that of the raw formulation could probably be due to the decomposition of polyphenolic compounds at high barrel temperature during extrusion (Soison *et al.*, 2014). However, Maillard reactions that occurred at the same time generated new polymeric compounds, which improved the antioxidant activities of the extrudates (Peksa *et al.*, 2016). Hence, the generation of new compounds balanced the loss of natural antioxidant compounds present in extrudates (Nayak *et al.*, 2011). Therefore, this might explain the reason for the satisfactory retention of antioxidant properties in PSP breakfast cereals after extrusion. Similar observations were reported by Nayak *et al.* (2011) and Soison *et al.* (2014) in their studies on purple potato, and yellow pea extrudate and PSP extrudate, respectively.

The ANOVA and regression of data observed in the present work showed that there was a highly significant p-value ($p < 0.0001$), along with a non-significant lack-of-fit and high R^2 value (0.94) on DPPH. Positive significant coefficients of BT suggested the increase in the DPPH value of extrudates. However, an increase in the feed MC during extrusion cooking significantly ($p < 0.001$) decreased the antioxidant activities of extrudates (Figure 1h). The quadratic terms of SS and feed MC were significantly negative for the antioxidant activity of extrudates. Like the effect of feed MC on TPC, the high temperature and low feed moisture provided severe extrusion conditions, thus resulting in the increased extractable phenolic compound from ingredients used in the formulation, or production of new polymeric compounds due to Maillard reactions (Peksa *et al.*, 2016).

Correlation analyses

The Pearson's correlation analysis (r) illustrated that there was a highly positive correlation between DPPH, TAC, and TPC of breakfast cereals. These results demonstrated that the anthocyanins and the phenolic compounds were mainly responsible for the total antioxidant capacity of extrudates. A significant and very strong correlation between TAC and TPC ($r = 0.92$) confirmed the correlation of flavonoids with the phenolic compounds in most foods (Nayak *et al.*, 2011). Additionally, a highly significant negative correlation between BD and SEI ($r = -0.90$) illustrated that the higher the SEI, the lower the BD of extrudates. In their study on soybean-rice ready-to-eat snacks, Alam *et al.* (2016) also reported a similar relationship between SEL and BD. Furthermore, WSI was positively correlated ($p < 0.05$) with SEI, chroma, TAC, DPPH, and TPC, while negatively correlated ($p < 0.05$) with WAC and BD of PSP breakfast cereals. Yadav *et al*. (2014) also reported that the expansion index was similarly correlated with BD, WSI, and WAC of pearl millet-based extruded snacks.

Optimisation

Numerical and multiple optimisation analysis methods of Design-Expert (version 11.1.2.0, State-Ease, Minneapolis, MN) software were used to determine the optimum processing conditions. Pre-determined goals for responses were used to determine the breakfast cereals with optimum physicochemical, textural, and functional properties as presented in Table 3. A commercial extruded puffed breakfast cereal was used as the reference sample to set the target range for WAC, WSI, hardness, and crispness. In this experiment, the overall desirability value was 0.806, which was considered very good. Amini Sarteshnizi *et al.* (2015) stated that if the desirability of a product ranged from 0.8 to 1, it could be conceded that the quality of the product is excellent. The coordinates corresponding to the optimum desirability of the product were at 157.23°C, 125.69 rpm, and 13.00% for BT, SS, and MC, respectively.

Verification of the model

Verification of the model was performed using a post-analysis confirmation test in Design-Expert software. First, the extruder was run under the optimum processing condition. Thereafter, the experimental values were compared with the predicted values. The experimental values were reasonably close to the predicted values, with no significant difference ($p > 0.05$); hence, these results

Table 3. Optimum values for dependent and independent variables of extruded breakfast cereals.

Variable	Goal	Experimental range		Optimum value	Desirability
		Lower limit	**Upper limit**		
A: Barrel temperature (°C)	range	140.00	180.00	157.00	
B: Screw speed (rpm)	range	100.00	140.00	126.00	
C: Moisture content (%)	range	13.00	18.00	13.00	
Response				**Predicted value**	
Hardness (kg)	minimise	3.77	7.51	5.31	0.806
Crispness (kg.s)	maximise	33.65	60.43	41.69	
BD (g/cm^3)	minimise	0.31	0.66	0.34	
SEI	maximise	2.44	3.30	3.03	
L	maximise	31.18	38.92	38.19	
a*	maximise	19.43	25.20	24.53	
Hue	minimise	-1.55	2.59	-1.05	
Chroma	maximise	19.43	25.20	24.54	
TAC (mg/100 g)	maximise	29.68	45.82	45.17	
DPPH (µmol TE/g)	maximise	10.74	14.36	14.32	
TPC (mg GAE/g)	maximise	3.05	5.90	5.55	
WAC (%)	minimise	5.63	8.67	6.04	
WSI (%)	range	11.28	33.50	21.14	

BD: bulk density, HD: hardness, SEI: sectional expansion index, WSI: water solubility index, WAC: water absorption capacity, TAC: total anthocyanin content, TPC: total phenolic content, and DPPH: radical scavenging activity.

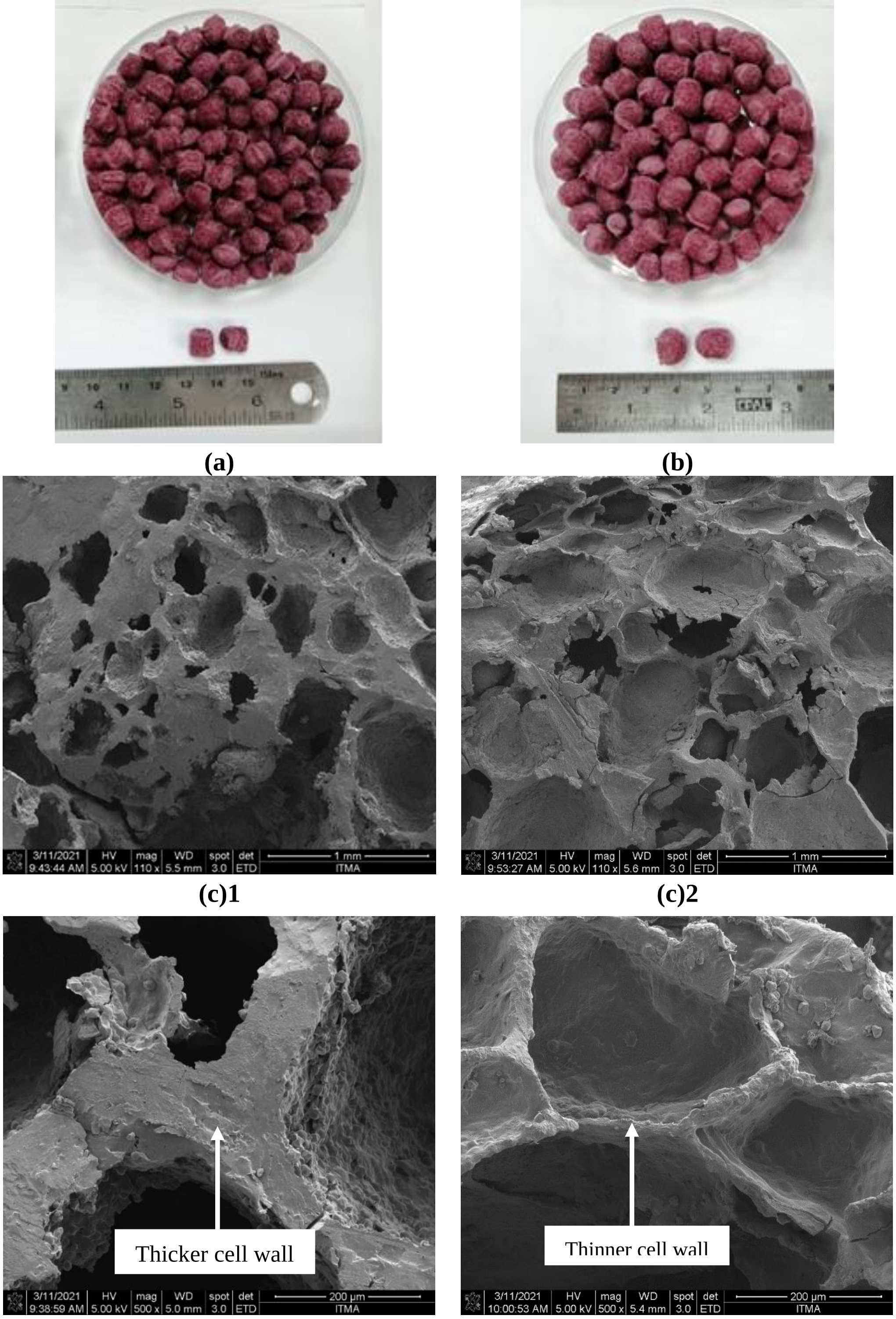
confirmed the suitability and adequacy of the model used (Hagar *et al*., 2021).

Appearance and microstructure of optimised breakfast cereal

Under the optimum conditions used in the present work, a significant improvement in expansion was observed as compared to that of non-optimised samples, previously reported by the same research group (Senevirathna *et al*., 2022), as illustrated in Figures 2a and 2b. Furthermore, as shown in the scanning electron micrographs (Figure 2c and 2d), the optimised samples depicted a better cell structure, with smoother and thin cell walls than the non-optimised samples. Lotfi Shirazi *et al.* (2020) stated that the microstructure of the extruded product is related to the property of expansion. Under the optimum condition studied, there was less moisture than in the non-optimised formulation. Thus, high feed MC acted as a lubricant, decreasing the extrusion temperature and starch gelatinisation during extrusion, resulting in the collapse of the cell structure, and leading to the formation of extrudates with high cell wall thickness and small cell size. These findings were comparable to the work of on extruded rice starch-pea protein snacks (Philipp *et al.*, 2017), ready-to-eat expanded snacks (Lotfi Shirazi *et al.*, 2020), and Roba1 bean extrudate (Natabirwa *et al.*, 2018).

Quantification of breakfast cereal anthocyanins using HPLC

The HPLC chromatogram of optimised breakfast cereals is shown in Figure 3. Acid hydrolysis of anthocyanins allows for precise quantification of anthocyanidins, as there are no co-elution compounds available for HPLC quantification of PSP anthocyanins. Fan *et al.* (2008) reported that the PSP anthocyanins are based on acylated forms of cyanidin and peonidin. In the present work, it was



(a) **(b)**

(c)1 **(c)2**

(d)1 **(d)2**

Figure 2. Appearance of breakfast cereals before **(a)** and after **(b)** optimisation. Scanning electron micrographs of extrudates at 110× **(c)** and 500× **(d)** magnification. 1: Before optimisation; 2: After optimisation.

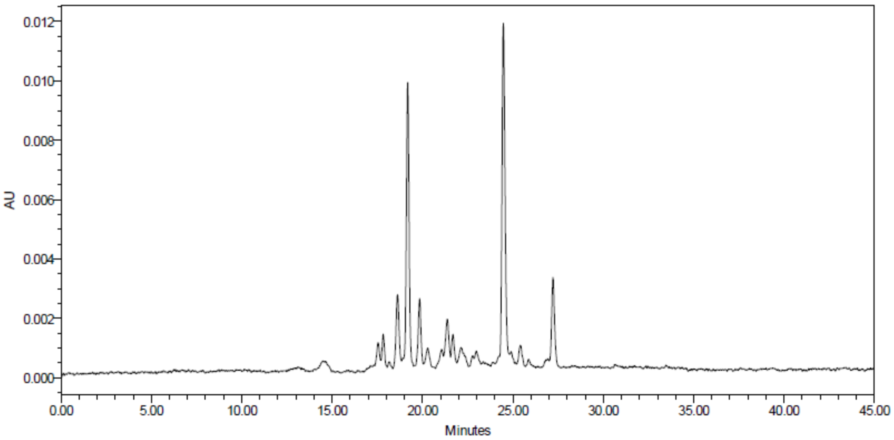


Figure 3. HPLC chromatogram for anthocyanidin in optimised breakfast cereals.

found that cyanidin-based anthocyanin (40.67 ± 2.87 mg/100 g) content was higher than the peonidin-based anthocyanins (36.06 ± 3.38 mg/100 g) of PSP breakfast cereals. Furthermore, significantly high TAC was observed in the optimised breakfast cereals (76.73 mg/100 g), compared to the non-optimised samples (66.35 mg/100 g). This could probably be due to the protective effect of optimum extrusion conditions against the degradation of anthocyanins. These findings implied that the optimisation of the extruder's parameters is essential for the production of healthy extruded products containing a high level of anthocyanins.

Texture measurement after immersion in milk (bowl-life)

The bowl-life of PSP breakfast cereal was compared to a similar commercial sample. Bowl-life crispness and hardness of breakfast cereal were 23.85 ± 3.41 kg.s and 4.71 ± 0.70 kg, respectively, and they were significantly lower than those of dry samples. Oliveira *et al*. (2018) also observed loss of crispness in enriched breakfast cereals after soaking in milk, and this was due to changes in the microstructure of breakfast cereals, as uptake of milk. However, the loss of crispness in PSP breakfast cereals after immersion in whole milk was significantly lower than for the commercial samples (44.73%). Therefore, developed product can be consumed with whole milk as similar to the commercial samples.

Conclusion

RSM with BBD was successfully used to model and assess the effects of three extruders' variables (BT, SS, and MC) on the textural, physicochemical, and functional properties of extruded PSP breakfast cereals. Results indicated that all models were significant ($p < 0.05$) for all the studied response variables with high coefficients of determination ($R^2 > 0.82$), along with a non-significant lack-of-fit. It was found that higher expanded product could be produced at low temperature, high screw speed, and low moisture content. Furthermore, increasing the feed moisture resulted in significantly ($p < 0.001$) lower anthocyanins in extrudates. However, processing at high temperature and low feed MC positively affected the TPC and antioxidant activity of the final product. Under optimised extrusion conditions, significantly high retention (75.0%) of anthocyanin content was detected in the final extruded product. Therefore, the optimum processing condition of BT at 157°C, SS at 126 rpm, and feed MC of 13% successfully produced a naturally brightly purplish-red coloured antioxidant-rich extruded product, with better expansion and an overall desirability value of 0.81. Anthocyanins and phenolic compounds could have been responsible for the total antioxidant capacity of extrudates. The TAC, antioxidant activity, and TPC of breakfast cereals were 76.73 mg/100 g, 14.29 µmol TE/g, and 5.54 mg GAE/g, respectively. The brightly purplish-red colour was indicated by the L, a*, b* of 37.85, 25.06, and -0.30, respectively, and the final product showed better texture and expansion, as indicated by crispness and SEI of 43.15 kg.s and 3.00, respectively. Finally, the anthocyanins of healthy breakfast cereals were quantified using HPLC, where cyanidin-based anthocyanin (40.67 ± 2.87 mg/100 g) content was higher than the peonidin-based anthocyanins (36.06 ± 3.38 mg/100 g).

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