Effect of extrusion processing conditions on the techno-functional, antioxidant, textural properties and storage stability of wholegrain-based breakfast cereal incorporated with Indian horse chestnut flour

Farhana Mehraj Allai[1,2], Z.R.A.A. Azad[1]*, B.N. Dar[2]*, Khalid Gul[3]*

[1]*Department of Post-Harvest Engineering and Technology, Faculty of Agricultural Science, Aligarh Muslim University, Uttar Pradesh, India;* [2]*Department of Food Technology, Islamic University of Science and Technology, Awantipora, India;* [3]*Department of Food Process Engineering, National Institute of Technology, Rourkela, India*

*Corresponding Authors: Z.R.A.A. Azad, Department of Post-Harvest Engineering and Technology, Faculty of Agricultural Science, Aligarh Muslim University, Uttar Pradesh, India. Email: zrazad@gmail.com; B.N. Dar, Department of Food Technology, Islamic University of Science and Technology, Awantipora, India. Email: darnabi@gmail.com; and Khalid Gul, Department of Food Process Engineering, National Institute of Technology, Rourkela, India. Email: gulmk@nitrkl.ac.in

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Abstract

The central composite rotatable design was used to plan the experiments using feed moisture (FM) content, barrel temperature (BT), screw speed (SS), and concentration of Indian horse chestnut flour (IHCF) as process variables varied between 10–18%, 70–150°C, 290–380 rpm and 1.75–4.75%, respectively. Surface mechanical energy, water holding capacity, swelling volume, piece density, longitudinal expansion, water activity and colour attributes were used as response variables. The whole grain flours added with 2.5% IHCF and extruded at 12% FM, 130°C BT and 380 rpm SS produced optimum quality breakfast cereals with 0.713 desirability. Extrudates packed in aluminum pouches were found to be suitable packaging materials and were shelf-stable for 6 months with better quality retention.

Keywords: extrusion cooking, fibre, horse chestnut, packaging, shelf-life, whole grains

Introduction

Over the decades, people across the world have struggled to ensure a healthy and delicious diet. For this purpose, producers had to adopt different techniques aimed at making food products more appealing and tastier with increased nutritional value and organoleptic attributes (Nkhata *et al.*, 2018). The traditional cooking method improves the bioavailability and bioaccessibility of nutrients but at the same time causes significant loss of macro- as well as micronutrients (Kamau *et al.*, 2020). Nutrient utilization can be increased by processing methods such as extrusion cooking.

Extrusion cooking is the most popular food processing technique in which the ingredients are subjected to amalgamation of temperature, internal pressure, moisture and shear forces, leading to the modification and disruption of molecular structure and texture resulting in the development of an ample range of food products such as breakfast cereals, pasta, snacks, baby foods and texturised meat substitutes (Espinosa-Ramirez *et al.*, 2021). It is also used to overcome the limitations associated with the processing of traditional cereal-based food products by enhancing its functional, physicochemical and shelf stability (Jabeen *et al.*, 2021). The extrusion process improves the food quality by reducing the deterioration of essential nutrients by thermal treatment while increasing the palatability, digestibility by starch gelatinization, modifying the molecular structure of the protein, and inhibiting unacceptable compounds including antinutritional factors and enzymes (Pasqualone *et al.*, 2020).

Ready-to-eat products such as breakfast cereals are most commonly consumed in the morning as the first meal of the day (Oliveira *et al.*, 2017b). Traditionally, breakfast cereals are prepared from refined cereal flours due to their high availability, ease of processing, low cost, and their bland taste. Technically, refined flours are nutritionally poor in micro- and macronutrients and contain only the digestible carbohydrate, that is, starch in comparison to wholegrain flours (Oliveira *et al.*, 2017a). In food industry, cereals are predominantly used as the base ingredient for the development of extruded products, but the trend is now changing and shifting towards the healthy ingredients due to the increased incidences of chronic diseases (Naseer *et al.*, 2021). As such, addition of different whole grains such as whole wheat flour (WWF), whole corn flour (WCF) and whole barley flour (WBF) in breakfast cereals has a promising scope for enhancing the potential health benefits and market acceptability of cereal-based products. Wholegrain flours are rich sources of antioxidant activity, bioactive compounds, vitamins, minerals, and dietary fibres such as cellulose, hemi-cellulose, lignin, β-glucan and resistant starch (Allai *et al.*, 2021). Several researchers have prepared extruded products from non-conventional seeds and evaluated their antioxidant and phytochemical properties (Beigh *et al.*, 2019; Jabeen *et al.*, 2021). Indian horse chestnut is an underutilised nut that has not been used previously with wholegrain flours and can, therefore, be explored for the development of healthy, sugar-free breakfast cereal.

Indian horse chestnut (*Aesculus indica*) provides an excellent opportunity for the pharmaceutical and food industries for having unique medicinal properties that include anti-obesity, anti-fever, antiviral, anti-inflammatory and anti-oedemic (Ahmad and Gani, 2021). In India, horse chestnut remains unrecognised and is mainly consumed by cattles and wild animals and considered as waste (Mishra *et al.*, 2018). The seeds are poisonous and bitter in taste, if consumed without processing (raw), because of the presence of anti-nutrients such as tannins and saponins. So, the seeds must be kept under running water in order to eliminate the bitterness and grind to fine flour. Indian horse chestnut flour (IHCF) is simply added with other ingredients, partially replacing the wheat flour, to make wide range of products such as halwa (porridge), chapatti (flat bread), pasta and noodles (Rafiq *et al.*, 2021). Horse chestnut could be utilised as a nutritional supplement due to their high levels of dietary fibre, starch, minerals, vitamins, polyphenols, flavonoids and other bioactive compounds such as triterpenoids, epicatechin and kaempferol (Idris *et al.*, 2020).

Due to its nutritional quality, IHCF may be considered as a potential non-conventional alternative to conventional flours. Very limited research has been done regarding IHCF, and so far, no research has been conducted on the use of WWF, WBF, WCF and IHCF for the development of breakfast cereal.

Therefore, it is necessary to characterise the wholegrain flours and IHCF to analyse their possible utilisation in food industry and also to optimise the processing conditions, that is, feed moisture content, barrel temperature and screw speed and IHCF for the development of breakfast cereals. In this research, the physicochemical and functional properties, colour, texture and storage stability of the developed extrudates were studied.

Materials and Methods

Wholegrain flours

Whole grain wheat (SW-2) and whole white corn (DT-2) were purchased from Sher-e-Kashmir University of Agricultural Sciences and Technology (SKUAST), Shalimar, Jammu and Kashmir, (J&K), and whole grain barley (PL-807) was obtained from Kargil, India. The whole grains were cleaned to eliminate the impurities. After removing the impurities, the whole grains were milled to obtain flour. The whole grain flours were then packed and stored at −21°C for further use. Indian horse chestnut seeds (*Aesculus indica*) were manually harvested from local areas of Shalimar, J&K.

Preparation of Indian horse chestnut flour

The preparation of IHCF is done according to the method described in our previous research (Allai *et al.*, 2022a).

Conception of experimental design

Response surface methodology (RSM) consists of different experimental designs based on statistical calculations and mathematical models on raw experimental data, to describe the empirical association between two or more independent variables, including design factors (x_i) and design response (y_i) that are required to be optimised through prediction approach (Moussaoui *et al.*, 2021). Statistical Software Design-Expert-12 (Stat-Ease Inc. Minneapolis, MN, USA) was applied to determine the influence of process parameters on response variables by using the second-order polynomial model as shown in the below equation. The adjusted and predicted R-square was in a reasonable agreement for all the models as presented in Table 1.

$$y_i = b_0 + \sum_{i=1}^{4} b_i X_i + \sum_{i=1}^{4} b_{ii} X_{ii}^2 + \sum_{i=1}^{4} \sum_{i=1}^{4} b_{ij} X_i X_j$$

Table 1. ANOVA for the fit of data to response surface models.

Dependent variables	Models	R^2	Adjusted R^2	Predicted R^2	Adequate precision	CV (%)	P	Lack of fit
SME	$SME = 129.88 + 1.99x_1 - 4.54x_2 - 9.17x_3 + 5.69 x_4 + 2.07x_1^2 + 1.18x_4^2$	0.97	0.95	0.90	23.97	1.91	<0.05	NS
PD (g/ml)	$PD = 6.34 + 0.0996x_1 + 0.803x_2 - 0.1388x_3 - 0.407x_4 - 0.210.6x_2x_4 - 0.0594x_3x_4 - 0.5430\ x_1^2 - 0.186x_2^2$	0.99	0.99	0.97	50.10	1.59	<0.05	NS
LEI	$LEI = 0.286 - 0.011x_1 - 0.010x_2 + 0.0133x_3 + 0.0285x_4$	0.79	0.76	0.72	18.67	5.58	<0.05	NS
a_w	$a_w = 0.3033 + 0.042x_2 - 0.01x_3$	0.93	0.87	0.79	16.27	4.77	<0.05	NS
WHC (g/g)	$WHC = 5.57 + 0.182x_1 + 0.662x_2 + 0.464x_3 - 0.275x_4 - 0.266x_2{}^*x_3 + 0.11x_1^2 + 0.252x_2^2 + 0.412x_3{}^2 - 0.134x_4{}^2$	0.96	0.93	0.83	22.87	4.00	<0.05	NS
SV (g/ml)	$SV = 4.81 + 0.093x_1 + 0.264x_2 + 0.205x_3 - 0.154x_4 - 0.121x_2x_3 - 0.045x_2^2 + 0.471x_3^2 - 0.215x_4^2$	0.98	0.97	0.93	49.20	1.99	<0.05	NS
$L*$	$L* = 66.94 - 0.623x_2 - 0.717x_3 + 1.40x_3x_4 + 0.833x_1^2 + 0.905x_4^2$	0.85	0.71	0.56	9.54	1.68	<0.05	NS
$a*$	$a* = 4.26 - 0.124x_1 + 0.572x_2 + 0.227x_3 + 0.052x_4 + 0.045x_2x_3 + 0.077x_1^2 + 0.166x_2^2 + 0.234x_3^2 + 0.036x_4^2$	0.99	0.98	0.95	38.40	0.44	<0.05	NS
$b*$	$b* = 22.64 + 1.15x_2 + 1.08x_3$	0.77	0.74	0.65	15.83	3.75	<0.05	NS

$a*$, redness; a_w, water activity; $b*$, yellowness; CV, coefficient of variance; $L*$, luminosity; LEI, longitudinal expansion index; NS, non-significant; PD, piece density; SME, surface mechanical energy; SV, swelling volume; WHC, water holding capacity.

Table 2. Coded levels and experimental ranges of the independent variables generated using central composite rotatable design.

Numerical variable	Symbol	Coded variable levels				
		−2	−1	0	1	2
Indian horse chestnut flour (%)	X_1	1.75	2.5	3.25	4	4.75
Feed moisture content (%)	X_2	10	12	14	16	18
Temperature (°C)	X_3	70	90	110	130	150
Screw speed (rpm)	X_4	290	320	350	380	410

Where y_i is the design response, and x_i is the independent factors, that is, X_1, X_2, X_3 and X_4 represent feed composition (IHCF), feed moisture content, barrel temperature and screw speed, respectively, and the coefficients of regression for constant, linear, quadratic and interaction regression terms were indicated by b_0, b_i, b_{ii} and b_{ij}, respectively.

In this study, the percentage of WBF and WWF was kept constant (10%) throughout the experiment. In the control, WCF accounted for the remaining 80% of the feed formulation. In the feed mixture, IHCF was used to replace WCF. The central composite rotatable design was used to investigate the effect of the four independent variables. X_1, X_2, X_3 and X_4 at each five levels are shown in Table 2. The experiment runs along with actual and coded levels for the independent variables are presented in Table 3.

Extrusion cooking

The breakfast cereals were prepared in a co-rotating twin-screw extruder (Basic Technology Pvt. Ltd., Kolkata, India) with a die width of 3.0 mm and a length to diameter ratio 8:1. The temperatures of the first, second and third zones were maintained at 40°C, 60°C and 80°C, respectively, throughout the experiment, while the fourth zone varied according to the design of experiment. The extrusion process was carried out using different feed

Table 3. Effect of independent variables on techno-functional characteristics of extrudates.

Runs	Independent variables				Physical Properties				Functional		Colour		
	IHCF (%)	Feed moisture (%)	Barrel temperature (ºC)	Screw speed (rpm)	SME (Wh/Kg)	PD (g/ml)	LEI (%)	a_w	WHC (g/g)	SV (mL/g)	*L**	*a**	*b**
1	2.5(−1)	12(−1)	90(−1)	320(−1)	142.2	4.86	0.28	0.27	5.04	4.48	71	4.1	20.17
2	4(+1)	12(−1)	90(−1)	320(−1)	145.56	5.24	0.22	0.29	5.56	4.92	71.64	3.8	19.64
3	2.5(−1)	16(+1)	90(−1)	320(−1)	130.5	7.09	0.25	0.36	7	5.23	69.75	5.3	23.13
4	4(+1)	16(+1)	90(−1)	320(−1)	137.4	7.23	0.23	0.37	7.11	5.46	70.33	4.7	23
5	2.5(−1)	12(−1)	130(+1)	320(−1)	120.7	4.72	0.32	0.24	6.41	5.06	64.71	4.47	22.67
6	4(+1)	12(−1)	130(+1)	320(−1)	124.3	5.01	0.3	0.25	6.78	5.35	66.92	4.22	22
7	2.5(−1)	16(+1)	130(+1)	320(−1)	110.6	6.83	0.28	0.34	7.34	5.37	67.41	5.74	25.19
8	4(+1)	16(+1)	130(+1)	320(−1)	114.5	7	0.25	0.36	7.55	5.55	66	5.53	24.31
9	2.5(−1)	12(−1)	90(−1)	380(+1)	149.8	4.57	0.33	0.27	4.22	4.3	68.74	4.22	20.69
10	4(+1)	12(−1)	90(−1)	380(+1)	152.6	4.83	0.32	0.3	5.04	4.36	69.32	4.13	18.02
11	2.5(−1)	16(+1)	90(−1)	380(+1)	142.4	5.95	0.3	0.36	6.34	5.08	66.23	5.37	23
12	4(+1)	16(+1)	90(−1)	380(+1)	145.3	6.11	0.28	0.35	6.88	5.17	67	5.05	23.15
13	2.5(−1)	12(−1)	130(+1)	380(+1)	133.4	4.24	0.35	0.24	6	4.96	69.7	4.53	23
14	4(+1)	12(−1)	130(+1)	380(+1)	139.5	4.44	0.33	0.26	6.12	5.11	70	4.44	22.78
15	2.5(−1)	16(+1)	130(+1)	380(+1)	121.5	5.41	0.33	0.34	6.66	5.21	68.36	5.78	25
16	4(+1)	16(+1)	130(+1)	380(+1)	127.4	5.56	0.31	0.36	6.97	5.29	68	5.51	24.82
17	1.75(−2)	14(0)	110(0)	350(0)	134.2	4.03	0.3	0.31	5.46	4.79	68.92	4.73	23.03
18	4.75(+2)	14(0)	110(0)	350(0)	140.3	4.35	0.26	0.31	6.15	5.15	70.65	4.3	22.4
19	3.25 (0)	10 (−2)	110(0)	350(0)	135.6	4.11	0.29	0.24	5.07	4	66.14	3.7	21.65
20	3.25(0)	18(+2)	110(0)	350(0)	120.3	7.12	0.27	0.39	7.68	5.26	63.13	6.04	24.12
21	3.25(0)	14(0)	70(−2)	350(0)	146.55	6.54	0.28	0.33	5.89	6.19	67.42	4.66	21.88
22	3.25(0)	14(0)	150(+2)	350(0)	113.4	6.21	0.31	0.3	8.14	7.2	65.26	5.62	25.33
23	3.25(0)	14(0)	110(0)	290 (−2)	121.06	6.94	0.23	0.31	5.34	4.39	70.15	4.33	23.21
24	3.25(0)	14(0)	110(0)	410 (+2)	146.3	5.49	0.32	0.33	4.31	3.51	70	4.37	20.01
25	3.25(0)	14(0)	110(0)	350(0)	132.5	6.33	0.29	0.32	5.57	4.88	69	4.3	23.43
26	3.25(0)	14(0)	110(0)	350(0)	130.2	6.4	0.26	0.29	5.61	4.8	67.22	4.28	23
27	3.25(0)	14(0)	110(0)	350(0)	127.3	6.32	0.25	0.33	5.5	4.71	65.22	4.32	22.65
28	3.25(0)	14(0)	110(0)	350(0)	125.2	6.41	0.27	0.31	5.46	4.79	68.42	4.25	22.13
29	3.25(0)	14(0)	110(0)	350(0)	130.5	6.3	0.28	0.29	5.87	4.85	66.7	4.2	22.87
30	3.25(0)	14(0)	110(0)	350(0)	133.6	6.29	0.29	0.28	5.41	4.81	65.1	4.22	23

a*, redness; a_w, water activity; *b**, yellowness; IHCF, Indian horse chestnut flour; coded values are in parenthesis; *L**, luminosity; LEI, longitudinal expansion index; PD, piece density; SME, surface mechanical energy; SV, swelling volume; WHC, water holding capacity.

compositions and process parameters as given in Table 3. The amount of water to be added before the extrusion was calculated in order to adjust the feed moisture content. A homogeneous flour mixture was obtained by mixing properly for 20 min, and water was added to the feed. The prepared feed mixtures were allowed to equilibrate for 12 h prior to extrusion to stabilise the moisture content. The prepared samples were then collected and cooled to

ambient temperature, dried and packed in low-density polyethylene (LDPE) bags for further analysis.

Surface mechanical energy

Surface mechanical energy (SME) is the amount of energy given to extrudates for the conversion of starch.

SME was calculated using the following formula (Oliveira *et al*., 2017a), the motor torque was recorded for each treatment displayed in the monitor panel.

$$\text{SME (Wh/Kg)} = \frac{\text{Screw speed (rpm)} \times \text{motor power (kW)} \times \text{torque (\%)}}{\text{Maximum screw speed (rpm)} \times \text{mass flow rate}\left(\dfrac{\text{kg}}{\text{h}}\right) \times 100}$$

Where motor power = 4000 W, and maximum screw speed = 682 rpm.

Techno-functional parameters

Physical properties
Piece density (PD)
Piece density of breakfast cereals was measured according to the protocol followed by Seker (2005). A 250 ml graduated cylinder was filled with 4 g of the sample. The cylinder was filled by adding mustard seeds. The extrudates were then removed, and the volume of leftover mustard seeds was measured and noted. Piece density was calculated as follows:

$$\text{PD(g/ml)} = \frac{\text{Mass of extrudates}}{\text{Volume of extrudates}} \times 100$$

Longitudinal expansion index (LEI)
The LEI of the extruded products was determined by the method explained in by Alvarez- Alvarez-Martinez *et al*. (1988). It is the ratio of the velocity of expanded extrudates to the velocity in the die orifice, which is indicated as follows:

$$\text{LER (\%)} = \left[\frac{\rho_d}{\rho_e}\right]\left[\frac{1}{\text{SER}}\right]\left[\frac{1-M_d}{1-M_e}\right]$$

Where, ρ_d is the density of melt behind the die (ρ_d= 1400 kg/m^3), and ρ_e is the extrudate density. M_e is the moisture content of extrudates, and the moisture content of the melt (M_d) was measured by drying 2–3 g of the samples in an oven at 105°C until constant weight was achieved.

Water activity (a_w)
Water activity is a dimensionless number representing the ratio of vapour pressure of the sample to that of pure water at a given temperature. a_w of flour and extrudates was measured using a water activity meter (Novasina AG CH-8853, Lachen). 1.0 g of flour was kept in the sample cup of water activity meter. The lid was closed and the sample was allowed to equilibrate, read as a_w.

Colour analysis
The CIELAB space parameters of extrudates were analysed with a Hunter Lab colorimeter (CR 300, Konica Minolta, Japan). *L**, *a** and *b** values represent lightness or darkness, redness and blueness or yellowness, respectively. Each value is an average of five different independent measurements.

Functional parameters

Swelling volume (SV) and water holding capacity (WHC)
SV and WHC of extrudates were determined by following the method by Espinosa-Ramirez *et al*. (2021). WHC measures the amount of retained water in the extrudates without undergoing any stress, and SV was defined as the ratio of total volume of swollen extrudates to the weight of solids.

Optimal point and validation
Optimization of independent variables was done through the highest desirability function in order to validate the developed models, that is, by comparing the predicted and actual values. The optimal conditions considered for numerical optimisation was minimum piece density and maximum longitudinal expansion (LE), SME, swelling volume (SV) and WHC. The developed extrudates were evaluated for all the selected parameters, and percentage prediction error was measured as follows (Scheuer *et al*., 2016).

$$\text{Prediction error (\%)} = \frac{\text{Actual value} - \text{predicted value}}{\text{Predicted value}} \times 100$$

Texture analysis
The texture of extrudates was investigated using a TA-HD Plus texture analyser (Stable Micro System, Godalming, Surrey, UK) equipped with five blade Kramer shear cell (Oliveira *et al*., 2017b) fitted with a 50 kg load cell. The compression test was done by arranging the sample on a single-layer bed, and the test was carried out at a pre-test speed of 1.0 mm/s, test speed of 2 mm/s and post-test speed of 10.00 mm/s. The trigger type used was button type, and the samples were compressed to 50% of the original height. The software recorded the number of peaks produced from the force deformation curve, resulting in the wall fracture of the sample, which represented the crispiness (Np) (Dogan and Kokini, 2007). Hardness was measured as the maximum force (N) required for rupturing the sample. Also, average crushing force (C_f) and crispiness work (C_w) were measured using the below equations (Igual *et al*., 2020):

$$\text{Np} = \frac{\text{n}}{\text{d}}$$

Where, n = number of peaks
D = distance travelled by the probe

$$Cw = \frac{S}{n}$$

Where, S = Area under deformation curve (upto 50% deformation)

$$Cf = \frac{S}{d}$$

Total phenolic content

The procedure by Allai *et al*. (2022b) was used to calculate the total phenolic content in the extrudates. The extraction process was carried out using methanol as solvent.1 g of extrudate was homogenised in 30 ml of methanol. The mixture was placed into an ultrasonic water bath for 15 min. Then the homogenate was left undisturbed for 12 h. The resulting mixture was centrifuged for 10 min at 3500 rpm. 0.5 ml of the extract was taken after centrifugation and blended with 2.5 ml of Folin−Ciocalteu reagent and incubated at room temperature for 5 min. The mixture was then mixed with 2 ml of 7.5% Na_2CO_3 and kept aside for 1 h at 25°C. After 1-s reaction time, the absorbance at 750 nm was measured using a spectrophotometer. Gallic acid was used to make a calibration curve, and the total phenolic content was expressed as mg GAE/100 g of dry sample.

Antioxidant activity

The antioxidant activity of the samples was estimated by the DPPH radical scavenging assay according to the method by Zhang *et al*. (2018) with a slight modification. Methanol was used to prepare 0.1 mM of DPPH solution. Extraction of different concentration was made, followed by the addition of 5 ml of DPPH solution and was mixed properly. The mixture was left undisturbed for about 45 min in dark at 25°C, and absorbance of the sample at 517 nm was measured. DPPH radical scavenging activity was calculated using the following equation:

$$\%\,Inhibition = \frac{A_{control} - A_{sample} \times 100}{A_{sample}}$$

Where, $A_{control}$ and A_{sample} are the absorbance values of the control and the sample, respectively.

Techno-functional properties of flours

Bulk density (BD)

BD was measured by filling pre-weighed sample in a 100 ml graduated cylinder. The base of cylinder was gently tapped till a constant volume was achieved (Adeloye *et al*., 2020). BD was calculated by using mass/volume relationship as given below:

$$Bulk\ density \left(\frac{g}{mlL}\right) = \frac{weight\ of\ sample\ (g)}{volume\ of\ sample\ after\ tapping\ (ml)}$$

Swelling capacity (g/ml)

Swelling capacity was determined by following the method of Okaka and Potter (1977) with some modifications. A 100 ml graduated cylinder was taken and filled with sample to the 10 ml mark. Distilled water was added to adjust the total volume to 50 ml. The top of the measuring cylinder was covered tightly, and the solution was mixed by inverting the cylinder. After 120 s, the suspension was again inverted and allowed to rest for 30 min. The final volume occupied by the sample was noted after 30 min.

Shelf-life studies

The optimised product was packed in aluminum-laminated (AL) pouches and LDPE, and stored at 25°C for 6 months. The stored samples were analysed for water activity, moisture, hardness, crispiness, free fatty acids (FFAs), peroxide value (PV), total plate count and overall acceptability.

Moisture (%)

Moisture content was determined by the Oven Method, as per the protocol described by AOAC (2005).

Free fatty acid (%)

FFA was estimated by the standard procedure of AOAC (1973), with some slight modifications. 5 g of sample was placed in a 250 ml flask, and to this 50 ml of benzene was added. The mixture was kept undisturbed for 30 min for extraction of FFA. Then, 5 ml of the extract was taken to which 10 ml of alcohol, 5 ml of benzene and two drops of phenolphthalein indicator were added. It was then titrated against 0.02 N KOH till the light pink colour disappeared. FFA was expressed as the percentage of oleic acid, and was calculated using the following formula,

$$FFA\,(\%\,of\,oleic\,acid) = \frac{282 \times 0.02\,N\,KOH \times ml\,of\,alkali\,used \times dilution\,factor}{1000 \times weight\,of\,sample\,taken} \times 100$$

Peroxide value (%)

30 ml of acetic acid chloroform solution and 0.5 ml of potassium iodide was added to 5 g of sample, and the mixture was allowed to stand for 60 s with occasional shaking. 30 ml of distilled water was added to the mixture. This was then titrated against 0.1 N sodium thiosulphate solution with constant swirling till the yellow colour disappeared. 0.5 ml of starch solution as indicator

was added and titrated until the blue colour disappeared. Blank was also determined. PV was expressed as meq/kg and was calculated using the following formula (Tatledgis *et al*., 1960):

$$PV = \frac{Titre \times N \times 100}{weight\ of\ the\ sample\ (g)}$$

Where, titre = sample reading–blank reading
N = normality of sodium thiosulphate solution

Microbiological analysis

Total plate count
The total plate count of samples was carried out after every 1 month of storage interval, up to 6 months. Samples were evaluated for total fungi and bacteria by using the standard serial dilution method. 10 g of grounded sample was dissolved in 90 ml of water and stirred for 5 min. After stirring, the aliquots were serially diluted; 1 ml in 9 ml of sterile saline was prepared in test tubes, and 0.1 ml of dilution was transferred aseptically on sterile plates using nutrient agar as media. The plates were rotated gently for uniform spread of the inoculum before the media solidified. The plates were then incubated at 37°C for 3–7 days. The number of colonies developed on each plate of different dilutions was counted using the digital colony counter and is calculated as:

$$CFU/g = \frac{Number\ of\ colonies \times dilution\ factor}{volume\ of\ sample\ used\ (ml)}$$

Sensory analysis
Twenty-five consumers who take breakfast cereal daily (15 females and 10 males) were selected randomly. Samples were served to the consumers in a three-digit coded manner and were carried out on a nine-point hedonic scale (1 meant 'dislike very much,' and 9 denoted 'like very much'). Consumers were guided to rinse their mouths after the consumption of every different treatment to determine different attributes (texture, taste and overall quality) of the extrudates. Overall acceptability of breakfast cereals was evaluated as the average score of sensory attributes determined**.** The purchase intent of each sample was questioned using a five-point scale (5 = certainly would buy, 3 = might or might not buy, 1 = definitely would not buy) to complement the acceptance results.

The acceptability index (AI) was calculated using the following formula

$$AI\ (\%) = \frac{A}{B} \times 100$$

Where, A and B represent the average score given to the product and the maximum score obtained for the product, respectively. A product that obtains an AI score of 70% is considered a good product (Gusmao *et al*., 2019).

Statistical Analysis

SPSS (Version 20) statistical software was used to analyse the data acquired throughout the studies. The results were expressed as mean ± standard deviation. Duncan's multiple range test and analysis of variance (ANOVA) were used to find a significant difference at ($P < 0.05$), among the means of samples.

Results and Discussion

A fit of models

ANOVA was used to select the appropriate models for different dependent responses. Statistics of fit summary suggested quadratic models for SME, PD, WHC, SV, L^*, and a^*; and linear models for LEI, a_w and b^*. The regression models were significant ($P < 0.05$) with a high coefficient of determination ($R^2 = 0.99–0.77$) for all the responses. These dependent variables were significantly influenced by feed composition, feed moisture content, barrel temperature and screw speed. The predicted and adjusted R^2 were observed to be in reasonable agreement, and the regression models showed less than 1.91–7.84% of coefficient of variation (CV), suggesting high precision and reproducibility of obtained results.

Surface mechanical energy

SME is the key parameter that determines the molecular degradation or breakdown of ingredients received during the extrusion process (Lee *et al*., 2022). Higher SME indicates a rapid conversion of starch, resulting in an increased puffing of extruded products (Jabeen *et al*., 2021). The values of SME varied between 110 and 152 Wh/kg for wholegrain breakfast cereals enriched with IHCF (Table 3). The fitted regression analysis exhibited quadratic coefficients of feed composition, feed moisture content, barrel temperature and screw speed in extrudates (Table 1).

The regression analysis showed that barrel temperature had the most dominant effect on SME (Table 1) among all parameters. Table 1 shows ANOVA results for the fit of data to response surface models. It was noticed that the feed moisture content (X_2) and barrel temperature (X_3) were inversely proportional to SME. With an increment in X_2 and X_3, a reduction in SME was recorded, whereas

higher feed composition (X_1) and screw speed (X_4) showed increased SME values. An increase in feed composition along with an increasing SME was mainly due to the increased starch content of IHCF (Meuser *et al.*, 1990). Furthermore, the presence of fibre in wholegrain flours increases SME as high fibre content has more water binding affinity and could dilute the concentration of starch in the mixture (Singha *et al.*, 2018). Feng and Lee (2014) reported that an increase in feed moisture content causes a reduction in the viscosity of feed ingredients. Viscosity is temperature dependent, and reduction of barrel temperature can reduce the gelatinization of starch, resulting in enhanced viscosity (Karkle *et al.*, 2012). Thus, increasing barrel temperature causes a reduction in viscosity leading to a decrease in torque and SME values (Kesre and Masatcioglu, 2022). Kaur *et al.* (2015) described that with an increment in screw speed, SME progressively enhanced due to the high shear force and less residence time that induces viscosity, starch conversion and high SME.

The CV assessed the relative dispersion of the experimental points, that is, 1.91% for developed breakfast cereals (Table 1). R^2, a coefficient of determination, indicated that the correlation among the selected processing conditions for extrudates was good, which showed a value of 0.90.

Piece density (PD)

The PD for developed extrudates was observed to be in the range of 4.03–7.23 g/ml (Table 3). The fitted model for PD is shown in Table 1. In Table 1, the model terms X_1, X_2, X_3, X_4, X_2X_4 X_3X_4, X_1^2, and X_2^2 were significant at $P < 0.01$, whereas the model terms X_1X_2, and X_2X_3 were non-significant. As evidenced in Table 3, the feed composition and feed moisture content showed a positive linear effect, whereas the barrel temperature and the screw speed exhibited negative linear effect on the PD of extrudates. RSM (Figure 1) illustrates the effect of independent parameters on PD. .

The increase in PD of extrudates with the increased feed composition might be due to the presence of fibre in IHCF that enhances the extrudate density. The fibre tends to breakdown the air cells, which reduces extensibility and expansion, resulting in higher density of extrudates with less porous structure (Dos Santos *et al.*, 2019). This suggests its suitability and applicability for the development of food products. High feed moisture content also decreases frictional force between screw and mixture leading to reduced expansion and enhanced density (Bisharat *et al.*, 2013). The extrusion process does not evaporate whole moisture content at the exit die point. So, the retention of some of the water content makes the product denser with decreased puffing (Asare *et al.*, 2004). An increase in barrel temperature and screw speed causes a reduction in the density of the extruded products. This might be because of the higher barrel temperature that enhances the degree of starch gelatinization and also the generated superheated vapours that produce a more expanded structure with lighter weight products (Samray *et al.*, 2019). Additionally, an increased shear rate disintegrates the structure of macromolecules of proteins and starches that subsequently weaken the structure, leading to a reduced density with increased screw speed (Bisharat *et al.*, 2013). Figure 1 showed the
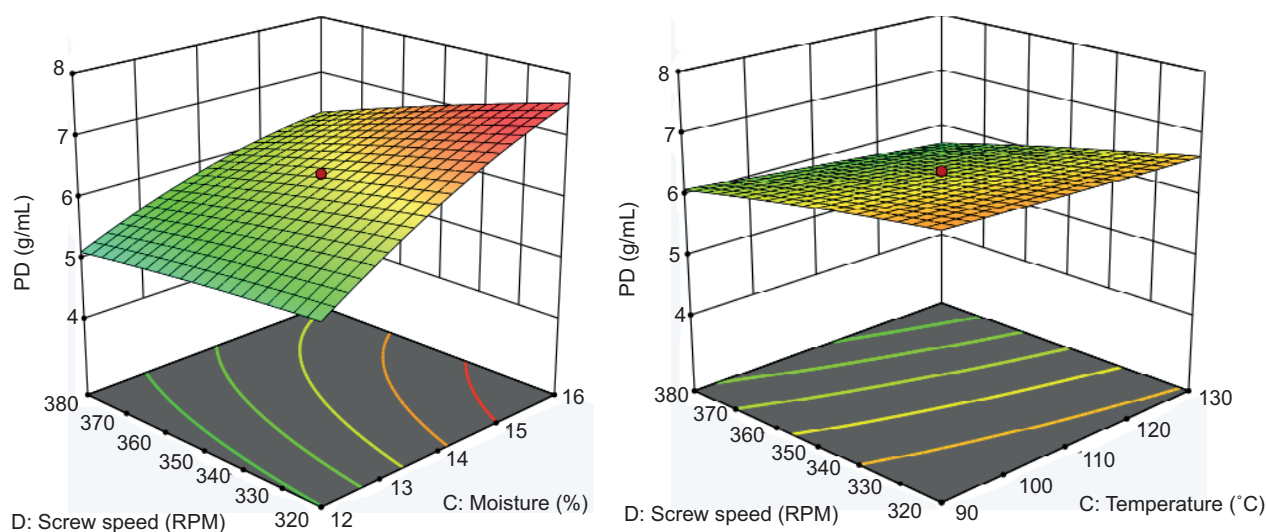

Figure 1. Response surface plots of piece density as a function of whole wheat flour, corn flour, barley flour and Indian horse chestnut flour.

negative interaction effect of X_2X_4 and X_3X_4. Similar results for the interaction terms have been reported by Jabeen *et al.* (2021) in corn-water chestnut extrudates and by Pansawat *et al.* (2008) in fish rice–based snacks.

Longitudinal expansion index

LEI values of extrudates varied from 0.22–0.35 (Table 3). The regression analysis revealed that the feed composition and feed moisture content had a significantly (P < 0.05) negative linear effect (Table 1). This might be due to the enhanced water content in the melt that would soften the molecular structure of amylopectin, reducing its elasticity and thus decreasing the longitudinal expansion (Alvarez-Martinez *et al.*, 1988). It can be well described by the fact that low moisture content promotes drag forces that enhance the die pressure, leading to more expansion of the developed products (Kaur *et al.*, 2022). Higher feed moisture content induces a lubrication effect, decreasing the internal barrel temperature as well as the shear rate of the extruder. As a result, a reduction in cooking of the ingredient and expansion can take place (da Silva *et al.*, 2014). The addition of IHCF caused a negative effect on the product expansion rate as the presence of fibre in the feed composition binds with some of the water content in the matrix and acts as an interference factor, thus decreasing its availability for longitudinal expansion (Witczak *et al.*, 2021). The LEI values increased significantly as the barrel temperature enhanced (Table 3). A similar trend was also observed for the screw speed response. The highest LEI value (3.5) was observed at the highest barrel temperature (130°C), screw speed (380 rpm) and at the lowest feed moisture content (12%) and feed composition (2.5%) (Table 3). The increased temperature inside the barrel causes superheating, which implies a higher degree of protein cooking

and starch gelatinization, which may enhance the expansion of extrudates (Yadav *et al.*, 2021). As a result of the increase in speed, less residence time for material was observed, leading to less degradation of the material and higher expansion of extruded products (Kaur *et al.*, 2015).

Water activity (a_w)

The a_w of the extrudates prepared from blend of wholegrain flours and IHCF varied from 0.24 to 0.39 (Table 3). The higher values of a_w accelerated the rate of reaction in the food products (Shah *et al.*, 2017) that determined the shelf life of foods. Fit statistics summary suggested a quadratic model for a_w among all parameters. The feed moisture content had a significantly dominant effect (P < 0.01) on water activity. The impact of the feed moisture content on a_w is presented by the 3-D surface plots in Figures 2A,B. Figure 2A showed a straight line with respect to temperature (110°C) along the axis of feed moisture content (14%). A similar observation is shown in Figure 2B, where the variation of a_w with respect to the feed moisture content (14%) also presents a straight line along the axis of screw speed (350 rpm). Generally, water activity above 0.7 promotes microbial load (Zhou *et al.*, 2021). Thus, from our study, all the extruded samples prepared under different treatment conditions fell in the safe category of shelf-stable products.

Colour analysis

The colour values of extruded products are displayed in Table 3. The regression analysis for *L**, *a** and *b** values of extrudates are presented in Table 1. The regression
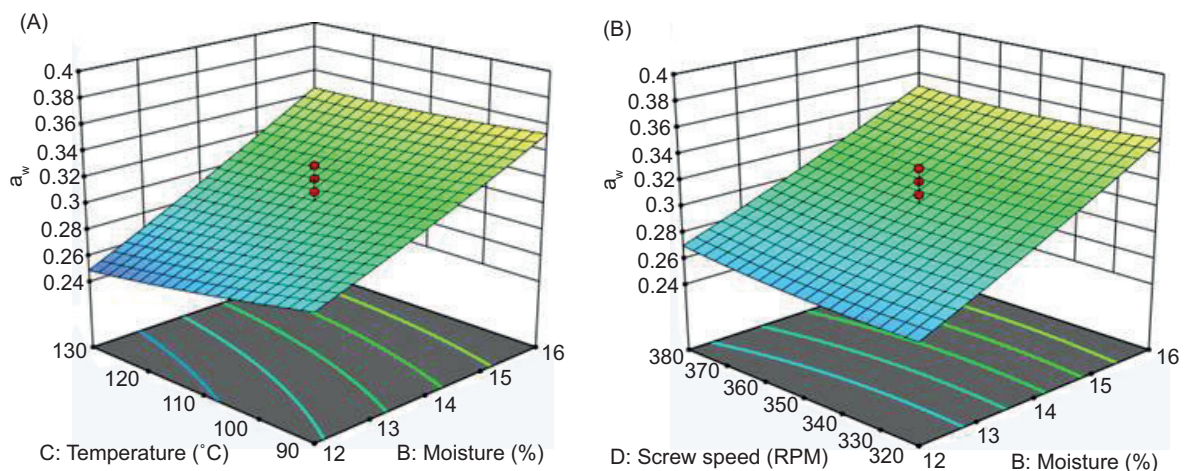

Figure 2. Response surface plots of water activity as a function of whole wheat flour, corn flour, barley flour and Indian horse chestnut flour.

equation for luminosity (L^*) (Table 1) showed significantly negative($P < 0.01$) linear and quadratic effects of feed moisture content (X_2) and barrel temperature (X_3). It was observed that the barrel temperature and the feed moisture content had the most predominant effect that influenced all the three colour coordinates. The L^*, a^* and b^*values of extrudates developed by different processing conditions were in the range of 63.13–71.64, 3.7–6.04 and 18.02–25.33, respectively. Generally, extrusion cooking, that is, increasing feed moisture content and barrel temperature caused reduction in the luminosity (L^*) and increments in a^* and b^* values (Table 3). The presence of higher water content leads to the development of extrudates with dense air cells, packed together, which enhances the absorption of light and decreases the luminosity (Nakhon *et al.*, 2018). Another parameter that affects the colour values is the increased barrel temperature that may cause loss of pigments, that is, non-enzymatic browning reactions such as caramelization and Maillard reaction, which can occur during the extrusion process and change the colour of the ingredients (Zhang *et al.*, 2020), leading to a decline in the L^* value and a subsequent increase in the a^* and b^* values (Jabeen *et al.*, 2022). During the extrusion, the degradation of pigment could also be used to measure the process intensity with regard to chemical and nutritional changes (Devrajan *et al.*, 2018). It is also an essential quality parameter as it indicates the degree of cooking as well as the level of chemical reactions that occur during extrusion process.

Water holding capacity and swelling volume

The extrusion enhances the water holding and binding capacity of the extrudates (Espinosa-Ramirez *et al.*, 2021). WHC of extrudates varied between 4.22 and 8.14 g/g (Table 3). The impact of independent variables on WHC was measured using ANOVA. The fitted model showed a highly significant linear, quadratic as well as interaction effect on WHC at $P \leq 0.01$, as shown in Table 1. The positive effect of the feed composition content, moisture content and the barrel temperature indicates that WHC increases with an increase in IHCF, moisture content and temperature, while the negative coefficient for the screw speed showed that an increase in shear rate causes a decrease in WHC. The presence of fibre and starch in feed composition also led to a higher WHC. Fibre-rich flours have a higher WHC and can be utilised as functional ingredients to modify the texture and viscosity and also avoid syneresis in food products (Repo-Carrasco-Valencia *et al.*, 2009). The proteins present in wholegrain flours and IHCF may be improved during extrusion treatment, by dissociation and unfolding of molecules that enhance the exposure of hydrophilic sites due to the variation in macromolecular

structure (Wang *et al.*, 2019a). The increased moisture content and temperature might increase the gelatinization of starch, where the granules of starch are disintegrated and more water remains bound to it, resulting in enhanced WHC (Wang *et al.*, 2019b). A high shear rate causes disruption of molecular structure, which leads to increase in solubility and decrease in WHC of extruded snacks (Ek *et al.*, 2021).

The regression model elucidates positive linear as well as quadratic terms for X_1, X_2, and X_3, while X_4 shows a negative effect on SV. Figure 3 shows that the variation of SV with respect to feed moisture content is curvilinear along the axis of barrel temperature. There was a positive correlation between SV and WHC of extrudates ($r = 0.98$). The higher SV in extrudates has been ascribed to the changes in the fibre and starch fractions. This could lead to an increased change in the fibre integrity, resulting in a higher SV for the prepared extrudates (Espinosa-Ramirez *et al.*, 2021). The negative interactive effect ($P < 0.05$) of moisture content and barrel temperature (X_2X_3) suggested that the moisture content (X_2) had a dominant effect over barrel temperature (X_3).

Optimization and model validation

The highest desirability (0.713) was obtained on the basis of optimal solutions, suggesting that an IHCF substitution of 2.5%, a feed moisture content of 12%, a barrel temperature of 130°C, and a screw speed of 380 rpm were the optimum criteria to develop good quality sugar-free breakfast cereals with higher SME, LEI, WHC, SV, and low water activity and PD values (Figure 4). Table 4 depicts the optimum conditions applied for the optimisation of independent variables. The values for predicted responses were found to be similar to the actual values with less than 4.5% variation.
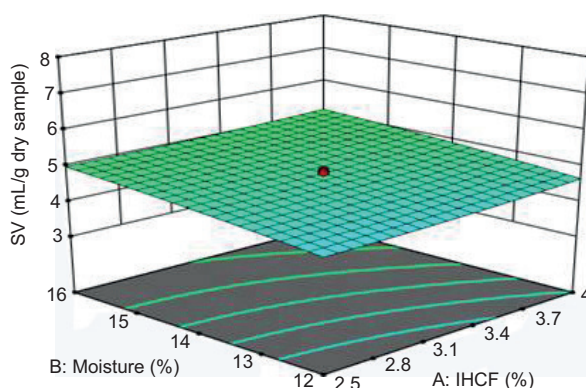

Figure 3. Response surface plots of swelling volume as a function of whole wheat flour, corn flour, barley flour and Indian horse chestnut flour.

Techno-functional properties of wholegrain flours, IHCF and optimised extrudates

The functional properties of wholegrain flours and IHCF are depicted in Table 5. The functional attributes define how a food material interacts with other food ingredients. It also determines its suitability for end use. Thus, flour with good functional characteristics can be easily substituted for other foods and will yield good quality
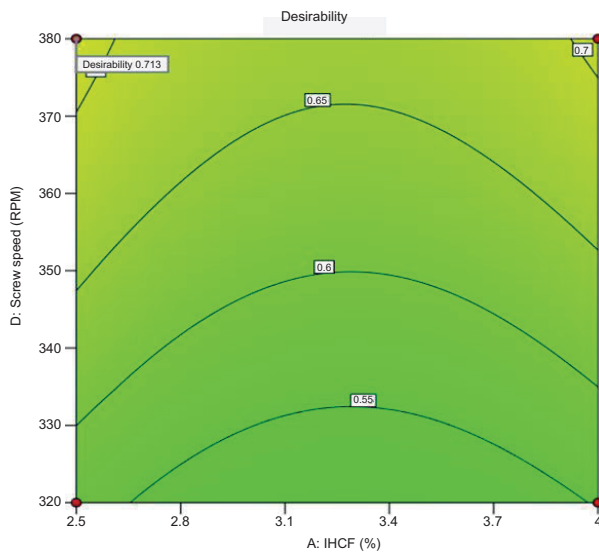
with acceptable end products. The BD of flour was similar in all flours, with a BD of 0.774 ± 0.52 in WWF, 0.722 ± 0.44 in WCF, 0.44 ± 0.26 in WBF and 0.6 ± 0.58 in IHCF. These results are similar to those reported by Tangariya and Srivastava (2022) for WWF, Adedeji and Tadawus (2019) for corn flour, Hamdani *et al.* (2014) for WBF and Shafi *et al.* (2016) for horse chestnut flour. The higher BD helps to enhance the weight of flour-supplemented foods without affecting the volume and the flours also favour their suitability in processing of different food products, while low BD helps in the preparation of complementary foods (Awuchi *et al.*, 2019). WWF had slightly higher swelling power (0.85 ± 0.45 g/ml) than WCF (0.79 ± 0.43 g/ml), WBF (0.7 ± 0.33 g/ml) and IHCF (0.66 ± 0.27 g/ml) (Adegunwa *et al.*, 2014; Chaudhary *et al.*, 2018). WWF was characterised by a higher hydration capacity and hydration index (6.72 g/g and 3.36 g/g, respectively) with relative to WCF (5.19 g/g and 2.5 g/g), WBF (4.6 g/g and 2.3 g/g) and IHCF (2.33 g/g and 1.16 g/g). These findings are consistent with the literature (Adegunwa *et al.*, 2014; Boucheham *et al.*, 2019; Rafiq *et al.*, 2021). The differences in functional properties might be due to the variation in the particle size of the flour, their variety and the milling process (Das *et al.*, 2019).

Table 5 depicts the functional properties of optimised product (extrudates). A significantly higher (P < 0.05) BD (4.95), hydration capacity (6.03), hydration index (3.015) and swelling power (0.91) were observed in the developed extrudates than native wholegrain flours and IHCF. The increased capacity of extruded flours to hold water

Figure 4.   Desirability plot.

Table 4. Numerical optimisation.

Variable	Goal	Range		Importance	Values		Variation level (%)
		Lower limit	Upper limit		Actual	Predicted	
IHCF	Is in range	2.5	4	3	-	-	-
Feed moisture	Is in range	12	16	3	-	-	-
Barrel temperature	Is in range	90	130	3	-	-	-
Screw speed	Is in range	320	380	3	-	-	-
Responses							
SME	Maximise	110.6	152.6	3	133.0	134.14	0.84
PD	Minimise	4.03	7.23	3	4.17	4.24	1.65
LEI	Maximise	0.22	0.35	3	0.33	0.34	2.9
a_w	Minimise	0.24	0.39	3	0.22	0.23	4.3
WHC	Maximise	4.22	8.14	3	5.68	5.77	1.55
SV	Is in range	3.51	7.2	3	4.8	5.0	4
L^*	Is in range	63.13	71.64	3	67.45	69.06	2.33
a^*	Is in range	3.7	6.04	3	4.33	4.5	3.7
b^*	Is in range	18.02	25.33	3	21.92	22.03	0.5

a^*, redness; a_w, water activity; b^*, yellowness; L^*, luminosity; LEI, longitudinal expansion index; PD, piece density; SME, surface mechanical energy; SV, swelling volume; WHC, water holding capacity.

Table 5. Functional, colour and textural attributes of individual flour, their blends and optimised extrudates.

Parameters	WWF	WBF	WCF	IHCF	Blend	Optimised extrudate
Techno-functional characteristics						
Bulk density (g/ml)	0.77 ± 0.52^b	0.44 ± 0.58^f	0.722 ± 0.26^c	0.6 ± 0.58^e	0.69 ± 0.37^d	4.95 ± 0.32^a
Swelling power (g/ml)	0.85 ± 0.45^c	0.7 ± 0.33^e	0.79 ± 0.43^d	0.66 ± 0.27^f	0.85 ± 0.31^b	0.91 ± 0.52^a
Hydration capacity (g/g)	6.72 ± 0.25^a	4.6 ± 0.55^e	5.19 ± 0.18^d	2.33 ± 0.10^f	5.24 ± 0.05^c	6.03 ± 0.44^b
Hydration index	3.36 ± 0.03^a	2.3 ± 0.05^e	2.5 ± 0.06^d	1.16 ± 0.11^f	2.62 ± 0.06^c	3.01 ± 0.13^b
Colour attributes						
L^*	79.14 ± 0.22^c	72.35 ± 0.47^e	83.83 ± 0.1^b	90.27 ± 0.06^a	77.34 ± 0.35^d	69.7 ± 0.32^f
a^*	3.09 ± 0.04^d	4.29 ± 0.07^c	1.09 ± 0.1^e	3.09 ± 0.06^d	4.33 ± 0.17^b	4.53 ± 0.23^a
b^*	15.65 ± 0.20^c	16.04 ± 0.17^b	12.70 ± 0.3^f	12.97 ± 0.06^e	13.21 ± 0.65^d	23 ± 0.17^a
Textural properties						
Hardness (N)	-	-	-	-	-	199.29 ± 0.34
Crispiness	-	-	-	-	-	56 ± 0.27
Crispiness work	-	-	-	-	-	1.86 ± 0.47
Average crushing force	-	-	-	-	-	5.1 ± 0.31
Total phenolic and antioxidant content						
Total phenolic content (mg GAE/g)	29.38 ± 0.33^b	20.02 ± 0.46^c	39.78 ± 0.21^a	10.66 ± 0.17^e	17.24 ± 0.02^d	5.87 ± 0.14^f
Antioxidant activity (%)	25.72 ± 2.11^f	68.21 ± 1.77^b	70.31 ± 2.52^a	63.55 ± 0.32^c	42.77 ± 2.53^d	18.33 ± 0.42^e

WWF, whole wheat flour; WBF, whole barley flour; WCF, whole corn flour; IHCF, Indian horse chestnut flour; WWF:WBF:IHCF:WCF 10:10:2.5:77.5, blended flour. Values are mean ± SD. Values with different superscripts within same column differ significantly ($P < 0.05$).

content upon rehydration as compared to raw flours might be due to the disintegration of the starch granules or due to the molecular disruption as a result of the shear stress and the thermal extrusion process (Martínez *et al.*, 2014).

The phenolic compounds act as antioxidants and perform an essential role in stabilising the free radicals. The total phenolic content and the antioxidant activity (DPPH radical scavenging) of raw flours, their blends and optimised extrudates are presented in Table 5. WCF had the highest total phenolic content and antioxidant activity among the flours, with a total phenolic and antioxidant contents of 39.78 mg GAE/g and 70.31, respectively (Lopez-Martinez *et al.*, 2009; Oboh *et al.*, 2010). The total phenolic content of WWF, WBF, IHCF and their blends was 29.38, 20.02, 10.66 and 17.24 mg GAE/g, respectively (Abozed *et al.*, 2014; Baba *et al.*, 2016; Shafi *et al.*, 2016; Zengin *et al.*, 2017). The antioxidant activities of WWF, WBF, IHCF and their blend were 25.72 (%), 68.21 (%), 63.55 (%) and 42.77 (%), respectively (Abozed *et al.*, 2014; Horvat *et al.*, 2020). After extrusion, the total phenolic and antioxidant content of optimised extrudates were reduced as compared to raw flours (Table 5). The reduction in the total phenolic content and antioxidant activity after extrusion might be attributed to the changes in the molecular structure of bioactive compounds, leading to the reduction in extraction efficiency and chemical reactivity due

to the development of polymerised products (Pandey *et al.*, 2021).

The colour characteristics of WWF, corn flour, WBF, IHCF and optimised extrudates are presented in Table 5. Generally, extrusion reduces the luminosity (L^*) of flour with an increase in a^* and b^* attributes. The results of this work are in accordance with the previous literature reports and can be mostly ascribed to the degradation of pigments and Maillard reaction during extrusion processes (Brahma *et al.*, 2016; Jafari *et al.*, 2017). For example, carotenoids present in whole grains are heat unstable pigments that are lost into colourless compounds during extrusion (Kadian *et al.*, 2013). Thus, after extrusion, the colour developed may influence the extrudate acceptability of these flours.

Textural analysis

Textural parameters of extruded products depend largely on the ingredient composition and their blend. It is an essential physical property of extrudates. The texture parameters for optimised extrudates are shown in Table 5. The hardness, crispiness, crispiness work (C_w) and average crushing force (C_f) values of optimised IHCF blended with wholegrain flours produced at minimum feed moisture content (12%) and highest barrel

temperature (130°C) and screw speed (380 rpm) were found to be 199.29 N, 56, 1.86 and 5.1, respectively. Crispiness work (C_w) can be inferred as the work that is needed to break one pore or group of pores, and it is also the sensory attribute of fracturability. Average crushing force (C_f) is defined as the force required to compress a solid substance and depends mostly on the sensory perception such as hardness during chewing (Igual et al., 2020). Lower moisture content (12%) had a positive effect on the hardness and crispiness of the extrudates as low water content increases the SME and viscosity, producing soft extrudates with higher crispiness. Kesre *et al.* (2022) reported that higher screw speed and barrel temperature exhibited a inverse relationship with hardness and direct relationship with expansion and crispiness. The expanded extrudates are more puffed with thinner cell walls that results in the easy crushing of extrudates under compression (Yao *et al.*, 2006). Furthermore, the availability of low residual moisture content during extrusion cooking and the increment in the degree of gelatinization and superheating of water might favour more expansion with softer texture and also improves the crispiness of extruded products (Guha and Ali, 2006; Wang *et al.*, 2005). The results of our work are in agreement with the published literature, which explains that the crispiness of extrudates has a positive and strong correlation with expansion.

Storage studies

The shelf-life stability of breakfast cereals indicates that the storage days and the packaging materials had a considerable influence on the FFA, PV, moisture content, water activity, hardness, crispiness and overall acceptability. The moisture content of the samples packed in LDPE and AL pouches was initially 3.04% that enhanced to 5.12 and 3.7%, respectively, during 180 days of storage (Figure 5A). The temperature, relative humidity (storage environment) and hygroscopic nature of extruded products, as well as the nature of packaging material, increase the moisture content during storage (Sharma *et al.*, 2004). The extrudates packed in LDPE showed increased moisture content compared to AL pouches due to its lower barrier characteristic. Jan *et al.* (2017) also reported the quick gain of moisture content in gluten-free extrudates packed in AL and LDPE packages. Water activity of extrudates was observed to be increased from 0.3 to 0.55 and 0.42 when packed in LDPE and AL pouches, respectively (Figure 5B) (Hussain *et al.*, 2017).

The FFA change was noticed in the extrudates with storage period in both the packaging materials. The change in LDPE was higher (0.04–0.32%) as compared to AL pouches (0.04–0.21%) (Figure 5C). These values were well within the acceptable range of 0.5% as reported by

Food Safety and Standards Authority of India (FSSAI) for dehydrated snacks. The increase in FFA of extrudates packed in AL pouches was non-significant for 120 days. The samples packed in LDPE had a significant effect that might be due to the property of AL pouches that acts a barrier for the transfer of light, which is mainly responsible for the rancidity of product. Furthermore, the lipid hydrolysis leads to the disintegration of long-chain FFA into single fatty acids (Syed *et al.*, 2019) and also higher permeability to oxygen and water vapour resulting in higher values of FFA in LDPE as compared to AL pouches. Also, mold growth was absent during the entire period of storage for all the samples either packed in LDPE or AL pouches. The reason for low total plate count could be attributed to the low moisture level. The total plate count was too low to count (less than 25 colonies/plate) up to 180 days of storage period. Jabeen *et al.* (2021) also reported similar results in total plate count of corn-based extrudates enriched with water chestnut during storage period.

Peroxide value indicates the amount of rancidity because of the oxidation process during storage. It can be observed from Figure 5D that the PV of the breakfast cereals increased significantly (P < 0.05) from 0.28 to 0.59 meq/100 g in extrudates packed in LDPE and 0.47 meq/100 g for those packed in AL pouches after 180 days of storage. The increase in PV is due to the poor oxygen and moisture barrier property of LDPE in comparison to that of AL pouches (Raleng *et al.*, 2019). The PV in this study was found to be under the permissible range (<10 meq/kg) given by FAO.

The hardness (Figure 5E) of the breakfast cereals increased from 199.29 to 217.56 N for the extruded product packed in LDPE and 199.29 to 208.56N for the extrudates packed in AL pouches. The packaging materials significantly affected (P < 0.05) the hardness of the extrudates during the storage period of 180 days. The hardness values increased due to the increment in water content of the extruded products, thereby modifying the balance in bonds in starch granules. However, there was a non-significant change during the initial months of storage. A similar trend was reported by Badding-Smithey *et al.* (1995) for beef-based extrudates enriched with carrot pomace powder. The crispiness of the extrudates packed in LDPE and AL pouches reduced from 56 to 51.32 and 56 to 55.32, respectively (Figure 5F). The crispiness of dehydrated foods like snacks, breakfast cereals, chips and crackers is desirable, but the excess absorption of moisture in samples causes sogginess and finally the rejection of extrudates (Dar *et al.*, 2014). During storage, absorption of moisture could be due to the packaging material and storage conditions (Badding-Smithey *et al.*, 1995). Moisture gain reduces the storage shelf life and stability of the product.
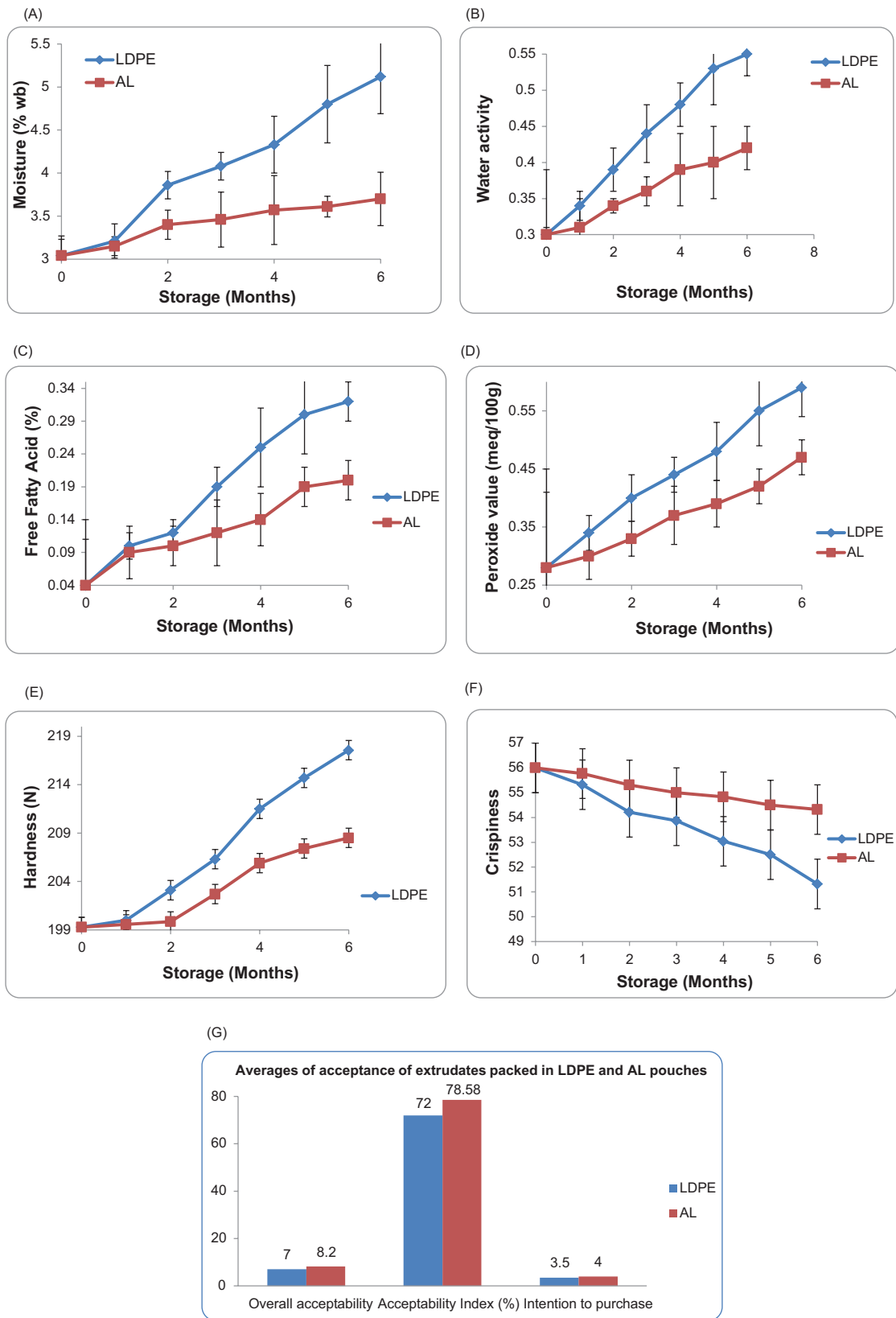
Figure 5. **Effect of storage period and packaging material on (A) Moisture content, (B) Water activity, (C) Free fatty acids, (D) Peroxide value, (E) Hardness, (F) Crispiness and (G) Over all acceptability of optimised extrudates packed in LDPE and AL pouches.**

The mean values for sensory attributes are presented in Figure 5G. The optimised samples packed in LDPE and AL pouches showed good scores in colour, texture, taste and overall acceptability. The overall acceptability for the samples packed in LDPE and AL pouches ranged from 7.0 to 8.2, representing 'like slightly' to 'like very much' in terms of the hedonic scale. The test for an index of purchase indicates the probable buying of a product. Samples packed in LDPE and AL pouches showed non-significant variations, and the panelist suggested that the products are recommended to buy (4 scores). The acceptability index (AI) was evaluated based on the mean scores given by the judges, where the samples packed in AL pouches reported the highest AI (78.58%), while samples packed in LDPE reported AI above 70% (Figure 5G). A product having at least 70% of approval is considered to be acceptable (Dutcosky, 2011).

Conclusion

Extrusion cooking is a versatile technology used to produce ingredients with improved functional and textural properties from whole grains and non-conventional flour. This study exhibited that RSM was used to optimise the process conditions and feed composition for the development of fibre-rich wholegrain-based extrudates using IHCF. Furthermore, the incorporation of IHCF into wholegrain breakfast cereals could broaden consumer acceptability for a better phytochemical profile and hydration rate. Results obtained from this study revealed that independent variables such as feed composition, moisture content, barrel temperature and screw speed had a considerable effect on all the dependent responses. The optimal conditions for the preparation of IHCF incorporated wholegrain-based breakfast cereals were 2.5% IHCF, 12% feed moisture content, 130°C temperature and screw speed of 380 rpm. The hardness and crispiness of the optimised extrudates were found to be 199.29 (N) and 56, respectively, which reported that extrudates enriched with IHCF at 2.5% level improves textural attributes. The antioxidant and total phenolic contents of optimised breakfast cereal were 30.36 (%) and 5.03 (mg GAE/g), respectively, which indicated that breakfast cereal enriched with WWF (10%), WBF (10%), WCF (77.5%) and IHCF (2.5%) level improve the phytochemical profile for the consumer's daily diet. The shelf-life studies depicted that the quality and overall acceptability of extruded products could be preserved safely up to 6 months in aluminum pouches under room temperature (25°C) without any deterioration.

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