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Optimization of bioactive peptides production in multigrain flakes and their antioxidative and inflammatory activity

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Abstract

The objective of this study was to optimize the extrusion processing for production of maximum peptides spectra in multigrain flakes. The results showed that maximum peptides spectra were produced at extrusion condition of 160 °C temperature and 100 rpm screw speed and minimum peptides spectra were observed at 120 °C temperature and 300 rpm. Among proximal composition, highest changes were observed in fat content and thus, significantly affected the atherogenicity and thrombogenicity indices of products. The products with higher peptides spectra (16) showed higher 2,2-diphenyl-1-picrylhydrazyl (DPPH) (48.63%), ferric reducing antioxidant power (FRAP) (48.63 ± 4.81 mmol Fe+2/g protein) and superoxide radical-scavenging activity (SRSA) (39.05 ± 4.51%). Similarly, higher peptides spectra product showed higher angiotensin converting enzyme (ACE) (86.01 ± 5.22%) inhibition and inhibition of protein denaturation (IP) (38.12 ± 2.89%). Thus optimization of extrusion process for bioactive peptides can be used for production of biological active products and healthily production system.

Keywords: extruded products; optimization; bioactive peptides; antioxidants; anti-inflammatory properties.

Practical Application: Food processing and healthy products are one of the major concerns regarding consumer choices. In this study, we optimize the extrusion process for production of bioactive peptides and evaluated for antioxidant and anti-inflammatory properties. This will help the processors to optimize extrusion processing for production of functional foods.

1 Introduction

Functional foods have been become a driving force for global health and wellness market and food industry has made it a major focus for such products and product development. The development of functional products is a complex process with integrative and innovative approach (Khan et al., 2013). The particular peptide sequences in proteins are responsible for execution of biological function until it has become functional. Thus, the parent proteins are cleaved to release such peptides that become biological functional. Such proteins not only fulfill the nutritional requirement but also have a crucial role in other physiological functions and human health (Rizzello et al., 2016). The bioactive peptides are widely used in functional foods for optimal health, nutraceutical and pharmaceuticals for disease management, cosmetic and food additives. In designer foods, they have functional role as solubilizer, emulsifying, water holder, and foaming former (Görgüç et al., 2020). It has wide spectrum of functional activities like antioxidant, antimicrobial, angiotensin-I-converting enzyme, antithrombotic, antihypertensive, anti-obesity, and hypercholesterolemic owing to modulation or improvement of the physiological functions of human body (Erdmann et al., 2008). The bioactive peptides act as modifier to reduce the risk of much illness. They act like drugs and hormones (Sánchez & Vázquez, 2017). Production of biological active product and application of the bioactive peptides into different food formulations has been a recent trend of functional food development. The bioactive peptides from food have gained huge attention being used as functional ingredient bioactive food and nutraceutical. The bioactive peptides that were produced naturally by hydrolysis in gastrointestinal tract are being produced in food production process to enhance their biological functions. Thus, cooking, ripening and fermentation were the processes helping in hydrolysis and production of peptides (Abdel-Hamid et al., 2017). Consequently, most studies are focusing on processing and production of bioactive peptides in food products and their practical application for medical biochemistry (Fu et al., 2019). Many studies have established that extrusion processing widely used for production of ready-to-eat foods that exhibit nutraceutical potential due to the bioactive peptides produced during thermal processing (Paula et al., 2022). In this study, we optimize first time the spectra of bioactive peptides produced during thermal extrusion and evaluated their antioxidant and anti-inflammatory potential.

2 Material and methods

In this study, multigrain flakes products were developed from cereals. The products were evaluated for their proximate analysis and antioxidants effect. The descriptions of material and analytical method involved during this research are discussed below.

2.1 Procurement of raw materials

Extruded foods made from wheat, corn, barley, millet and rice that were procured from local market of Faisalabad, Pakistan.

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2.2 Product development

Multigrain flakes {barley (20%), millet (20%), rice (20%), corn (20%) and wheat (20%)} were developed by adding 3.0% sugar, 0.05% salt, water and 0.5% oil to avoid clumping into flours accordingly and subjected to thermal extrusion for cooking and die formation.

2.3 Optimization of extrusion process for maximum production of peptides spectra

Box Behnken design, response surface methodology (RSM) was used to optimize the extrusion process for the maximum production of bioactive peptides, antioxidant and anti-inflammatory activity. In the barrel, the temperature of first three zones was same but last zone was cooled with the help of air. The extrusion optimization was done using feed flow rate of 10-30 rpm, screw speed of 100-300 rpm with extrusion temperature of 120-160 °C and feed flow rate of 15-35 kg/h. They were wrapped in polyethylene bags and placed at room temperature for further analysis and usage. Following optimization treatments were used as presented in Table 1.

3 Physico-chemical characterization

3.1 Proximate analysis

Proximate analysis of multigrain flakes were performed through previously described method of Association of Official Analytical Chemists (1997).

3.2 Extraction of proteins/peptides

The proteins/peptides were extracted using modified method (Török et al., 2015). The ground samples of 15 mg of flakes were mixed with 1 mL of 50 mM sodium phosphate buffer (pH 7.0) containing 1% Sodium dodecyl sulfate. The aliquots were stirred for 10 min and then the centrifuged at 8000 rpm for 10 min. The supernatant were gentility removed and pellets were again dissolved into 1 mL of same buffer and sonicated under amplitude of 90% for 2 min conditions using 50 kHz frequency (ultrasonicator, Hielscher, Germany) for extraction of insoluble proteins. Sonicated samples were centrifuged under given conditions and filtrates were extracted. The both fractions of soluble and insoluble proteins were mixed and filtered using 0.22 μm pore size syringe filter. The samples were stored at -80 °C for further analysis.

Table 1. Independent variables for optimization process using Box-Buhnken design.

Sr #	Temperature (°C)	Moisture (%)	Screw speed (rpm)	Feed flow rate (kg/hr)
1	140	15	100	15
2	120	10	200	25
3	140	10	200	15
4	160	15	200	35
5	160	15	300	25
6	120	15	100	25
7	140	15	200	25
8	140	15	300	15
9	140	15	100	35
10	160	10	200	25
11	160	15	200	15
12	140	10	200	35
13	160	20	200	25
14	120	15	200	35
15	140	15	200	25
16	140	20	200	35
17	140	15	200	25
18	140	20	300	25
19	140	15	200	25
20	140	10	100	25
21	120	15	300	25
22	140	10	300	25
23	140	15	300	35
24	140	20	200	15
25	120	15	200	15
26	140	15	200	25
27	160	15	100	25
28	120	20	200	25
29	140	20	100	25

3.3 Chromatographic analysis by reversed-phase HPLC

HPLC analysis will be carried out using reverse phase-HPLC method. A LiChrospher column C18 with (Merck Millipore Corporation, Billerica, MA, USA) connected to model HPLC Alliance 2690 unit (Waters, Milford, MA, USA) and Uv-vis diode array detector were used. The sample was separated on ZORBAX Eclipse XDB-Phenyl at 30 °C maintaining flow rate of 1 mL/min. The protein/peptides spectra were assessed using linear gradient of acetonitrile from 5.0 to 42.5% in the presence of 0.1% trichloric acetic acid. A photodiode detector was used for detection using 215 nm wavelengths.

3.4 Index of atherogenicity (AI) and thrombogenicity (TI)

In the first step, the extracted fatty acids were converted to methyl ester of fatty acid though the procedure adopted from (Liu, 1994). These esters were subjected to analyze though gas chromatography and the quantified according to method of (Ahmad et al., 2020). The peak area and retention time was used to calculate the quantify and composition determination of fatty acids using Varian Chem Station software. Using fatty acids, lipid indices for health such as AI and index of thrombogenicity (TI) of developed products were determined using following equations (Equations 1-2) as cited by Ulbricht & Southgate (1991).

$$AI = \frac{C12:0 + \left(4\,X\,C14:0\right) + C16:0}{\Sigma\, MUFA + \Sigma\, \varepsilon - 6 + \Sigma\, \varepsilon - 3} \quad (1)$$

$$TI = \frac{\left(C14:0 + C16:0 + C18:0\right)}{\left[\left(0.5\ \mathrm{x}\ \Sigma\, MUFA\right) + \left(0.5\,X\,\Sigma\, \varepsilon - 6\right) + \left(3\ \mathrm{x}\ \Sigma\varepsilon - 3\right) + \left(\Sigma\, \varepsilon - 3\right)/\left(\Sigma\, \varepsilon - 6\right)\right]} \quad (2)$$

In Equation 1, C12 is lauric acid, C14:0 is myristic acid and C16:0 is palmitic acid. The "∑" is sum of mono-unsaturated fatty acid (MUFA) and ∑ω-6 is the sum of omega-6 polyunsaturated fatty acids (PUFA) and ∑ω-3 is sum of omega-3 PUFA.

4 Biochemical characteristics

The antioxidant and anti-inflammatory activity of products containing bioactive peptides was determined using following methods.

4.1 The total protein concentration

The total protein concentration of extracted proteins/peptides from flakes samples was measured using the DC Protein Assay kit though a modified Lowry method (Virtanen et al., 2007). Bovine serum albumin (BSA) was used as standard. Prior to each assay, the sample protein concentration was diluted with phosphate buffer (PBS) using basic dilution factor method to achieve minimum concentration of 1.2 mg/mL of samples.

4.2 Anti-inflammatory activity

The *in vitro* studies, the anti-inflammatory activity of the products was assessed though inhibition of albumin denaturation, xanthine oxidase activity inhibition, angiotensin converting enzyme (ACE) II Inhibition according to following methods given below.

Inhibition of protein denaturation

The method of Kumari et al. (2015) was adopted with slight modification for determination of anti-inflammatory activity of products (Kumari et al., 2015). The product sample extract having concentration of 0.1% each (1.0 mg/mL), and positive standards (ibuprofen and diclofenac) were prepared. A reaction vessel consisting of 0.2 mL of egg albumin, 1.4 mL of PBS, and 1.0 mL of sample extract was prepared for each mixture. The distilled water was used as negative control instead of sample extracts. The prepared mixtures were incubated for 15 min at 37 °C and further subjected to heat treatment for 5 min at 70 °C. The mixtures were cooled and spectrophotometric absorbance was recorded at 660 nm. The % inhibition was calculated by following Equation 3:

$$\text{Denaturation inhibition}\left(\%\right) = 1 - \frac{\text{Absorbance of sample}}{\textit{Absorbance of negative control}}\,X\,100 \quad (3)$$

Control while Diprotin A in same buffer was considered as positive control.

Angiotensin Converting Enzyme (ACE) inhibition

The assay for determination of ACE-inhibitory activity was carried out using method of Hall et al. (2018) with little modification (Hall et al., 2018). The principle of assay is to hydrolyze Hippuryl-His-Leu (HHL) substrate into product hippuric acid (HA) and histidyl-leucine. A standard curve of HA was obtained using a series of HA dilution (0.01-0.16 mg/mL) from stock solution in 0.5 mL 0.1 M sodium borate buffer of pH 8.3 with 0.3 M NaCl. The above aliquat was vortexed with quinolone (0.6 mL) for 10 s and subsequently benzene sulfonyl chloride (0.2 mL) was mixed for 20 s and incubated at 30 °C for 30 min in darkness. Finally, 3.7 mL ethanol was added and further incubated for 30 min under same conditions. The volume of 0.2 mL was taken and absorbance was recorded at 492 nm. For experiment, the sample (20 µL) was dissolved with already incubated 50 µL of 5 mM HHL in 0.1 M sodium borate buffer having pH 8.3 containing 0.3 M NaCl was at 37 °C for 5 min. Then the mixture was incubated with 100 mU/mL (10 µL) of ACE to initiate reaction and incubated at 37 °C for 30 min. Finally the reaction was stopped by adding 100 µL of HCl and the 500 µL volume was made by addition of sodium borate buffer. The spectrophotometric absorbances were recorded to measure the realize of HA by ACE accordingly (Li et al., 2005) and % inhibitory activity of ACE was calculated using following Equation 4.

$$\text{ACE inhibitory activity}\left(\%\right) = \frac{B - A}{B - C}\,X\,100 \quad (4)$$

4.3 Antioxidant activity

Ferric Reducing Antioxidant Power Assay (FRAP) assay

FRAP assay was used to calculate total antioxidant activity of protein/peptides extracts according to previously described method (Zahin et al., 2010) with slight modification. The FRAP reagent was composed of acetate buffer (0.3 M), 1-10-Phenanthroline,

2,4,6-tris (2-pyridyl)-s-triazine (TPTZ, 0.01 M) pH 3.6 maintained by 0.04 M HCl and mixed with 0.02 M $FeCl_3.6H_2O$ having pH 3.6 at the ratio of 5 by 1. The protein/peptides extract @ 2 mg/mL was used for analysis. Absorbance of the samples, standards and blank solution was measured as 593 nm. Glutathione (GSH) reacted with FRAP reagent was used as positive control. Linear equation using 0.03-0.9 µmol/mL concentration of FeSO4.7H2O was derived and the results are presented in mmole of Fe2+ reduced per g protein.

Superoxide Radical Scavenging Activity (SRSA)

To determine the SRSA of the samples, a reagent buffer of Tris-HCl buffer (0.05 M) of pH 8.3 containing 0.001 M EDTA was used. A sample of 80 µL volume having 1 mg/mL concentration was used for SRSA while buffer alone was used as blank. Glutathione was used in same buffer as positive control. The aliquots of above volumes were prepared and freshly prepared 40 µL pyrogallol (0.0015 M) in 0.01 M HCL was added and the spectrophotometric absorbances were recorded immediately at 420 nm accordingly. The SRSA activity was calculated according to following Equation 5:

$$\text{SRSA}(\%) = \frac{(\text{Abs of blank} - \text{Abs of sample})}{\text{Abs of blank}} \times 100 \quad (5)$$

Where Abs is absorbance values at 420 nm.

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DDPH) radical assay

The antioxidant capacity of product protein/peptides was evaluated using DPPH assay using method of Alara et al. (2019). Absorbance was taken at 517 nm against ascorbic acid as standard and 100% methanol as blank. Free radical inhibition potential of the test samples was calculated using following Equation 6.

$$\% \text{ Inhibition} = \frac{(\text{OD of control sample} - \text{OD of test sample})}{\text{OD of control sample}} \times 100 \quad (6)$$

5 Statistical analysis

For the optimization of extrusion conditions, Box–Behnken designs for response surface methodology (RSM) was used though Design-Expert Version 12, Stat Ease. The results were analyzed using the coefficient of determination (R^2), analysis of variance (ANOVA) and statistical and response plots. Considering all the linear terms, square terms and linear by linear interaction items, the quadratic response model was used.

6 Results

6.1 Bioactive peptides spectra

Bioactive peptides and its production in food product development is a recent innovation in food industry and derived the attention of manufacturer for functional foods. We first time optimized the extrusion process for the production of maximum spectra of bioactive peptides. To study the combined effect of independent factors temperature, screw speed, moisture and feed flow rate, 29 experiments were performed for different combinations using statistically designed experiments. A wide range of peptides spectra were obtained under different combination as presented in contour graph (Figure 1). It was observed that temperature and screw speed significantly affects the production of peptides spectra. The maximum peptides spectra (16) were produced at extrusion condition of 160 °C and 100 rpm. At same temperature and screw speed of 200 and 300 rpm generates 15 and 14 peptides spectra. However; the feed flow rate and moisture did not influence the production of peptides spectra significantly. Similarly at temperature of 140 °C and 100 rpm, there were 12 peptides spectra and at same temperature for 200 and 300 rpm, 10 and 9 peptides spectra were observed in HPLC chromatograms. Similarly at 120 °C and 100 rpm, 10 peptides spectra was observed on chromatogram while 8 and 7 peptides spectra was produced at same temperature and 200 and 300 rpm respectively.

6.2 Proximate nutrients composition

The 29 products were evaluated for their proximate analysis, antioxidant and anti-inflammatory properties. The results of proximate analysis are presented in Table 2. The proximate results showed that all independent factors did not affect significantly the proteins content at 120 °C. However, proteins content significantly changed at 140 °C, 160 °C. The screw speed also affected significantly. While feed flow rate and moisture content did not influenced significantly the proximal composition. The highest proteins content (15.55 ± 1.23%) was observed at 120 °C and 300 rpm. The lowest proteins content (14.43 ± 1.17%) was observed at 160 °C and 100 rpm. Highest changes were observed in fat contents and it was observed that temperature and screw speed significantly change the lipid contents. Highest fat content (7.80 ± 0.25%) were observed at 120 °C and 300 rpm while lowest fat content (5.91 ± 0.16%) were observed at 160 °C and 100 rpm. Dietary fiber did not change significantly at 120 and 140 °C however, significantly changes

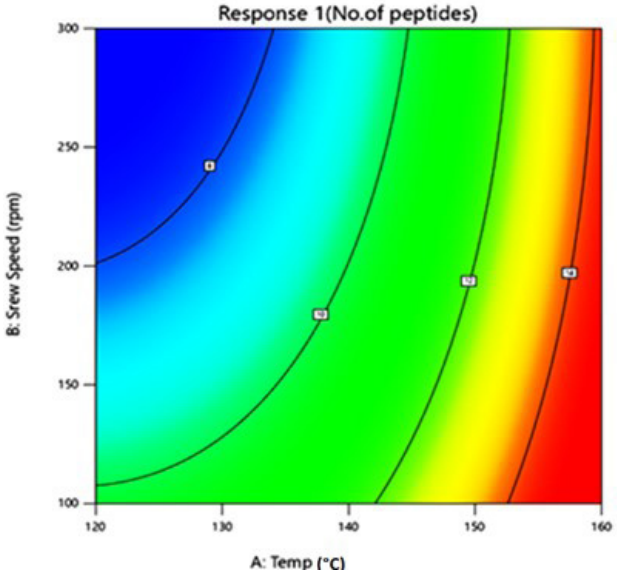

Figure 1. Number of peptides spectra obtained under different variable combination as presented in contour graph.

Table 2. Proximal compositions of products.

Sr #	Independent variables				Dependent variables				
	Temperature (°C)	Moisture (%)	Screw speed (rpm)	Feed flow rate (kg/hr)	Protein (%)	Fat (%)	Dietary Fiber %	Ash (%)	Moisture (%)
1	140	15	100	15	15.20 ± 1.16^{c}	6.73 ± 0.13^{f}	12.90 ± 0.83^{bc}	1.38 ± 0.02^{b}	11.53 ± 0.30^{ab}
2	120	10	200	25	15.43 ± 1.17^{ab}	7.60 ± 0.15^{b}	14.50 ± 1.24^{a}	1.10 ± 0.06^{c}	11.70 ± 0.41a
3	140	10	200	15	15.28 ± 1.10^{b}	6.90 ± 0.12^{e}	12.95 ± 1.14^{bc}	1.42 ± 0.09^{b}	11.50 ± 0.18^{ab}
4	160	15	200	35	14.76 ± 1.11^{b}	6.20 ± 0.20^{h}	12.65 ± 1.31^{c}	1.65 ± 0.04^{a}	11.33 ± 0.19^{c}
5	160	15	300	25	15.06 ± 1.10^{d}	6.43 ± 0.09^{g}	12.55 ± 1.27^{c}	1.55 ± 0.05^{ab}	11.23 ± 0.22^{cd}
6	120	15	100	25	15.49 ± 1.29^{a}	7.45 ± 0.16^{c}	14.40 ± 1.13^{a}	1.18 ± 0.03^{c}	11.73 ± 0.28^{a}
7	140	15	200	25	15.30 ± 1.12^{b}	6.93 ± 0.15^{e}	12.99 ± 1.16^{bc}	1.40 ± 0.01^{b}	11.55 ± 0.40^{ab}
8	140	15	300	15	15.25 ± 1.18^{bc}	7.20 ± 0.13^{d}	12.92 ± 1.22^{bc}	1.41 ± 0.02^{b}	11.52 ± 0.15^{ab}
9	140	15	100	35	15.14 ± 1.17^{cd}	6.70 ± 0.10^{f}	12.84 ± 1.19^{bc}	1.39 ± 0.08^{b}	11.51 ± 0.20^{ab}
10	160	10	200	25	14.80 ± 1.15^{e}	6.16 ± 0.18^{h}	12.60 ± 1.29^{c}	1.63 ± 0.03^{a}	11.30 ± 0.23^{c}
11	160	15	200	15	14.79 ± 1.19^{e}	6.19 ± 0.23^{h}	12.52 ± 1.32^{c}	1.67 ± 0.04a	11.45 ± 0.17^{b}
12	140	10	200	35	15.35 ± 1.10^{ab}	6.91 ± 0.12^{e}	13.03 ± 1.15^{bc}	1.40 ± 0.07^{b}	11.56 ± 0.29^{ab}
13	160	20	200	25	14.71 ± 1.26^{e}	6.23 ± 0.10^{h}	12.56 ± 1.11^{c}	1.68 ± 0.11^{a}	11.28 ± 0.14^{c}
14	120	15	200	35	15.41 ± 1.19^{ab}	7.62 ± 0.09^{b}	14.45 ± 1.25^{a}	1.16 ± 0.05^{c}	11.75 ± 0.11^{a}
15	140	15	200	25	15.29 ± 1.16^{b}	6.92 ± 0.20^{e}	13.10 ± 1.19^{b}	1.39 ± 0.06^{b}	11.49 ± 0.13^{b}
16	140	20	200	35	15.28 ± 1.14^{b}	6.94 ± 0.8b^{e}	12.88 ± 1.35^{bc}	1.42 ± 0.08^{b}	11.42 ± 0.24^{b}
17	140	15	200	25	15.23 ± 1.13^{bc}	6.89 ± 0.16^{e}	13.01 ± 1.29^{bc}	1.45 ± 0.02^{b}	11.40 ± 0.28^{b}
18	140	20	300	25	15.22 ± 1.22^{bc}	7.22 ± 0.13^{d}	12.94 ± 1.21^{bc}	1.43 ± 0.09^{b}	11.38 ± 0.09^{bc}
19	140	15	200	25	15.27 ± 1.18^{b}	6.79 ± 0.18^{ef}	13.05 ± 1.19^{bc}	1.37 ± 0.06^{bc}	11.54 ± 0.35^{ab}
20	140	10	100	25	15.39 ± 1.20^{ab}	6.67 ± 0.15^{f}	12.91 ± 1.22^{bc}	1.38 ± 0.03^{b}	11.34 ± 0.24^{bc}
21	120	15	300	25	15.55 ± 1.23^{a}	7.80 ± 0.25^{a}	14.54 ± 1.13^{a}	1.12 ± 0.07^{c}	11.77 ± 0.40^{a}
22	140	10	300	25	15.21 ± 1.15^{bc}	7.18 ± 0.10^{d}	13.01 ± 1.15^{bc}	1.31 ± 0.02^{bc}	11.25 ± 0.16^{cd}
23	140	15	300	35	15.33 ± 1.19^{ab}	7.21 ± 0.20^{d}	12.98 ± 1.09^{bc}	1.30 ± 0.05^{bc}	11.26 ± 0.13^{cd}
24	140	20	200	15	15.23 ± 1.21^{bc}	6.84 ± 0.23^{ef}	13.15 ± 1.29^{b}	1.41 ± 0.05^{b}	11.24 ± 0.30^{cd}
25	120	15	200	15	15.46 ± 1.22^{a}	7.63 ± 0.51^{b}	14.43 ± 1.11^{a}	1.18 ± 0.07^{c}	11.68 ± 0.18^{a}
26	140	15	200	25	15.15 ± 1.09^{b}	6.85 ± 0.11^{ef}	13.18 ± 1.10^{b}	1.44 ± 0.04^{b}	11.22 ± 0.25^{cd}
27	160	15	100	25	14.43 ± 1.17^{f}	5.91 ± 0.16^{i}	12.62 ± 1.20^{c}	1.60 ± 0.06^{a}	11.20 ± 0.22^{cd}
28	120	20	200	25	15.40 ± 1.16^{ab}	7.65 ± 0.13^{b}	14.42 ± 1.26^{a}	1.22 ± 0.02^{c}	11.72 ± 0.18^{a}
29	140	20	100	25	15.31 ± 1.24^{b}	6.71 ± 0.21^{f}	13.19 ± 1.20^{b}	1.38 ± 0.10^{b}	11.18 ± 0.30^{d}

The superscript alphabets on values show significantly difference within column.

were observed at 160 °C while the other parameter did not had significantly effect. Similarly, significantly changes were in ash and temperature solemnly had effects. Highest ash content (1.68 ± 0.11%) was observed at 160 °C, 20% moisture, 200 rpm and 25% feed flow rate while lowest ash content (10 ± 0.06) were observed 120 °C, 10% moisture, 200 rpm and 25% feed flow rate. Highest moisture content (11.77 ± 0.40%) was observed at 120 °C, 15% moisture, 300 rpm and 25% feed flow rate while lowest ash content (11.18 ± 0.03%) were observed 140 °C, 20% moisture, 100 rpm and 25% feed flow rate.

6.3 Atherogenicity and thrombogenicity indices

The effect of independent variables on atherogenicity index (AI), thrombogenicity index (TI) is presented in Figure 2. The interactive impact of temperature, screw speed, moisture contents and feed flow rate showed that only temperature and screw speed significantly change the AI and TI while other variable did not had significant influence. The highest AI (0.33 ± 0.08) and TI (0.46 ± 0.05) were observed at 160 °C, 20% moisture, 100 rpm and 25% feed flow rate. While the lowest AI (0.06 ± 0.01) and TI (0.10 ± 0.03) were observed at 120 °C, 15% moisture, 300 rpm and 25% feed flow rate.

6.4 Antioxidant activity

The products were evaluated for antioxidant potential in the term of DPPH, FRAP and SRSA. The result of DPPH, FRAP and SRSA are presented as response surface graph in Figure 3. It was observed that DPPH significantly changed with changing in temperature and screw speed. The DPPH in 29 products ranged from 15.69 to 48.63%. The highest and lowest DPPH were observed at 120 °C, 15% moisture, 100 rpm and 25% feed flow rate and 160 °C, 15% moisture, 300 rpm and 25% feed flow rate, respectively.

Similarly, the highest (48.63 ± 4.81 mmol Fe+2/g protein) and lowest FRAP (0.05 + 0.09 mmol Fe+2/g protein) values were observed at 160 °C, 15% moisture, 300 rpm and 25% feed flow rate and 120 °C, 20% moisture, 200 rpm and 25% feed flow rate, respectively. Similarly, the highest (39.05 ± 4.51%) and lowest SRSA (9.8 ± 1.93%) values were observed at 160 °C, 15% moisture, 300 rpm and 25% feed flow rate and 120 °C, 15% moisture, 100 rpm and 25% feed flow rate, respectively.

6.5 Anti-inflammatory activity

Anti-inflammatory potential was evaluated through inhibition of proteins and results are presented though response

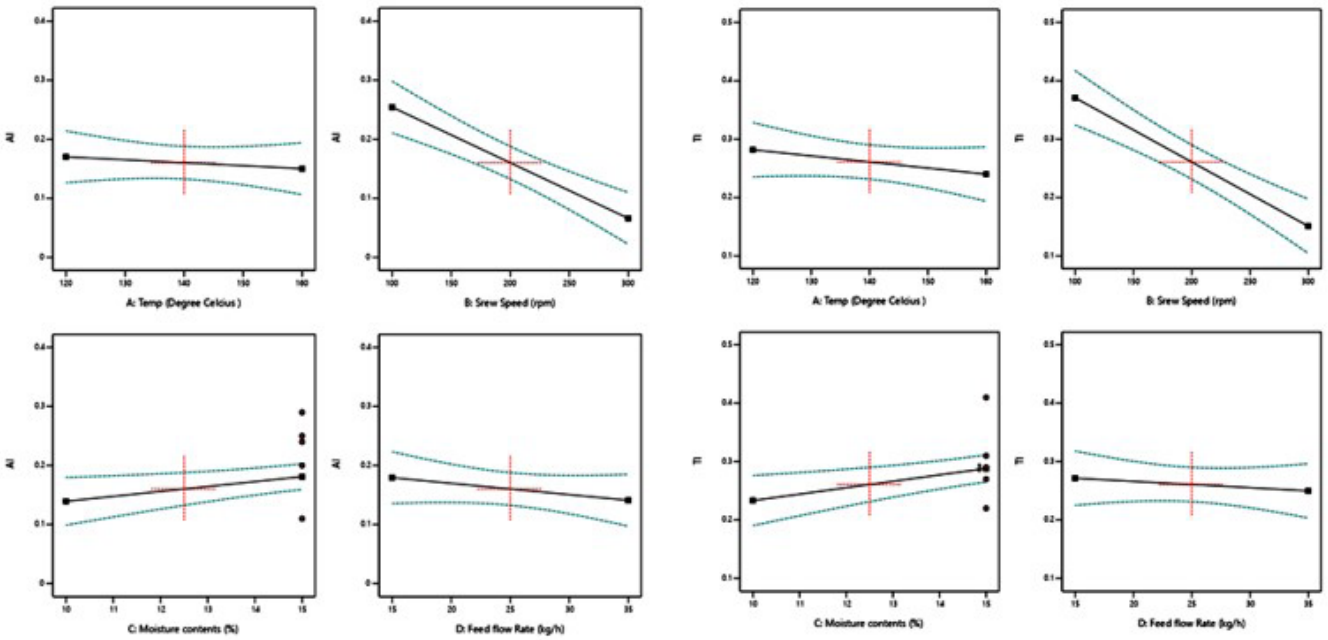

Figure 2. Interactive effect of independent variables on atherogenicity index (AI) and thrombogenicity index (TI) of products.

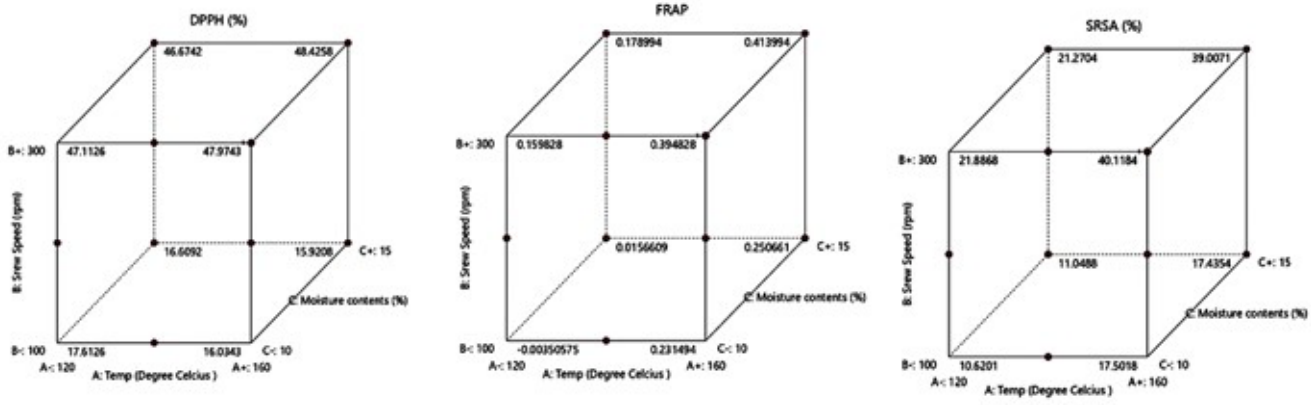

Figure 3. Response surface graph for antioxidant activity (DPPH, FRAP and ARSA).

surface graph as in Figure 4. The result showed that change in temperature and screw speed significantly changed the IP and ACE. The highest IP (38.12 ± 2.89%) and ACE (86.01 ± 5.22%) activity were observed at 160 °C, 15% moisture, 300 rpm and 25% feed flow rate while the lowest IP (7.32 ± 2.66) and ACE (5.22 ± 2.55) activity was observed in product processed at 120 °C, 15% moisture, 100 rpm and 25% feed flow rate, respectively.

7 Discussion

The production of functional foods with bioactive peptides has been proving to be very effect for healthy production system. Its tailored proximate composition along with biological functions. Thus the production of bioactive peptide depends on processing extrusion conditions, proteins contents and its sources and formulation. Temperature and screw speed are the most influential parameter affecting the biochemical changes through changing the structural formation of nutrients (Day & Swanson, 2013). It can be justified that extrusion results in unfolding, denaturation and realignment of protein resulting in significant changes in physicochemical and functional properties (Alam et al., 2016). It was observed a less physiochemical changes in extrudates at 100 °C and increased remarkable with increasing temperature to 130 °C and these properties were decrease on further increase temperature of 160 °C suggesting that extrusion processing significantly affect the proximal composition (Chen et al., 2018). It was reported that Short time heat treatment time results have significant effect on atherogenic and thrombogenic indices of food and feed material (Puhakka et al., 2016). The antioxidant and anti-inflammatory are demonstrated by these product which can be attributed to increase in bioavailability of bioactive peptides

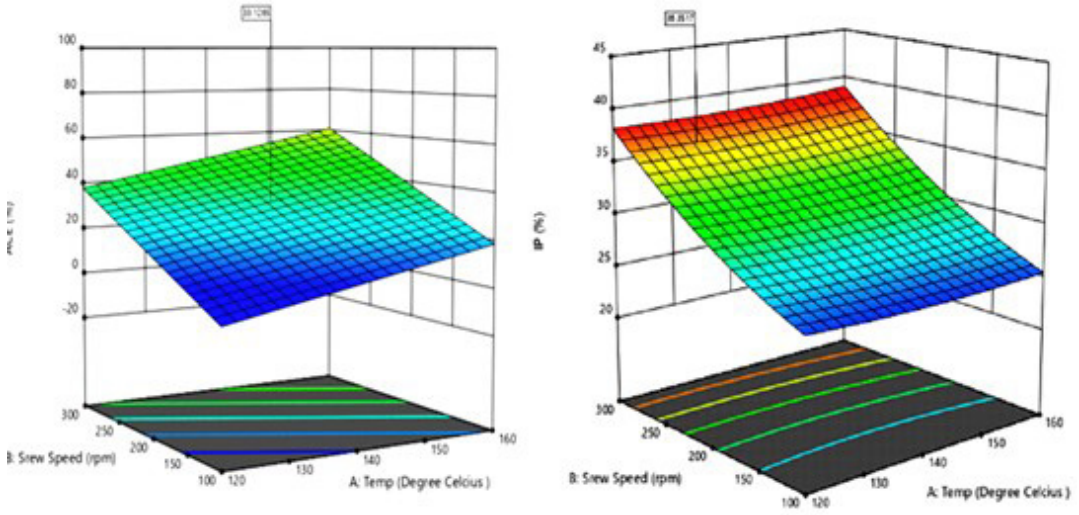

Figure 4. Response surface graph for anti-inflammatory activity. ACE = Angiotensin converting enzyme, IP = Inhibition of proteins.

production and phenolic contents as it was reported temperature-dependent effect (130 °C ≥ 120 °C ≥ 110 °C) which is strongly associated with quantity and type of proteins present in the food material and breakdown to bioactive peptides during extrusion (Oomah et al., 2005; Rico et al., 2021). The extrusion increases the anti-oxidant properties can attribute to proteins or aromatic compounds (Chukwumah et al., 2013). It was observed that low moisture (14%) contents and medium temperature (140 °C) result in higher antioxidant activity. The antioxidant markers such FRAP, DPPH, SRSA were response factors that correlated well with the temperature of extrusion and concentration seems to have an important effect on their activities (Delgado-Licon et al., 2009). It has been observed that extrusion processing produces the bioactive peptides that have strong anti-inflammatory effect. The bioactive peptides produced during extrusion process inhibit the inflammation generated by lipopolysaccharides and macrophages (Montoya-Rodríguez et al., 2014).

8 Conclusion

Multigrain cereal products have been gaining focusing due to gastrointestinal friends and biofunctions. This five cereals products showed better proximal changes and thus increasing the antioxidant and anti-inflammatory properties. The temperature was sole processing factor improve the functional properties and number of peptides spectra in products. This study will help and derive industry to use optimized conditions for production of bioactive products. However still further studies are need to characterized the peptides and its use as nutraceutical.

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