Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.22720

Characterization of bioactive fatty acids and oxidative stability of microwave vacuum dried fish powder supplemented extruded product

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Abstract

This study was aimed to determine the retention of bioactive fatty acids and oxidative stability of fish powder (FP) supplemented extruded products. The *Labeo rohita* fish meat was minced, microwave vacuum dried, and extruded. Product formulations containing FP (0-25%) and semolina (75-100%) were optimized using response surface methodology. The maximum bioactive fatty acids retention was 94.84% in the final product. The highest values of polyunsaturated fatty acids (PUFAs) were 87.97% and 85.66% at 25 °C for 30 and 60 days. Maximum TBARS values were 0.42 and 0.44 mg MDA/kg at 25 °C for 0 and 30 days, respectively. The highest carbonyl contents were 6.02, 7.67, 9.37 nmol/mg protein for 0, 30, and 60 days of storage at 25 °C, respectively. The moderate barrel exit temperature (125 °C) and feed moisture contents (20%) and high screw speed (150 rpm), FP (25%) exhibit the highest retention of PUFAs in a product.

Keywords: fish powder; microwave vacuum drier; thermal extrusion; oxidative stability.

Practical Application: Fish powder can be applied for the production of value-added foods and food fortification has supported the food industry to investigate various approaches for producing fish powder from multiple raw materials.

1 Introduction

Fish has always been used as the richest source of essential PUFAs, including n-3 and n-6 which have beneficial effects on human health. PUFAs have the potential to address depression and other mental disorders (Ab Latif Wani & Ara, 2015). A docosahexaenoic fatty acid is also found abundantly in fish and essential nutrients for eye development, nervous system, and brain development of the fetus (Silva et al., 2015; Fu et al., 2017). The n-6 such as arachidonic acid and n-3 like eicosapentaenoic acid are have been proven precursors of lipid mediator signaling molecules, named "eicosanoids," that play important roles in the regulation of inflammation (Patterson et al., 2012). The cross-sectional studies conducted on human beings have shown that the patients having a higher ratio of n-6 to n-3 demonstrated unpleasant moods while and lower has a significant effect on good mood by probably reducing the vulnerability to depression and cardiovascular diseases (CVDs) (Berger et al., 2017). *Labeo rohita* (rohu) is one of the prime species of fish cultivated in Asia. It has high commercial and nutritional values due to the high amount of PUFAs and white meat (Memon et al., 2011). Rohu belongs to the carp family and is abundantly found in freshwater lakes and rivers of Pakistan, Bangladesh, India, and Thailand. It is considered one of the most popular, crucial, and economic freshwater fish.

Drying is one of the ancient methods of preserving fish meat. The need for developing dried fish powder (FP) and the formation of functional and ready-to-eat foods is growing day by day (Shaviklo et al., 2013). The quality and safety characteristics of dried fish directly depend upon the source of raw material and process applied for drying purposes (Shaviklo et al., 2011, 2012). Novel processing is further helping to prepare FP which is helpful for the storage of fish for a longer period. The microwave vacuum drying (MVD) method is a combination of both microwave and vacuum drying as it has more uniform heat transferring potential and a more energy-saving process for all kinds of foodstuffs (Cui et al., 2008). While extrusion is a multi-functional and multi-purposes thermal and mechanical cooking process widely used in food industries to exhibit higher nutrition value containing essential amino acids and essential fatty acids along with pharmaceutical applications (Li et al., 2007). The geometry of extruders, food mix composition, and processing conditions play a vital role in physiochemical and nutritional changes in food material (Zhang et al., 2014; Shahzad et al., 2020). Research studies have reported that thermal extrusion causes complete inactivation of enzymes in food unlike that of high-temperature short-time processing methods. Most of the anti-nutritional substances are destroyed in extrusion processing. Proteins and

Received 19 July, 2020
Accepted 27 Aug., 2020

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starches processed through extrusion are more digestible and absorbable in the body than un-extruded products. It increases the bioavailability of amino acids and inhibits the activity of trypsin along with other growth-inhibiting substances (Singh et al., 2007; Ahmad et al., 2019).

The lipid oxidation is reduced even at high temperature and makes more absorbable in the human body. Lower temperature, residence time, and optimized mechanical shear give end product of higher quality retaining maximum omega fatty acids. Low lipid levels (less than 5%) aids in extrusion processing and greater product stability and expansion (Kouba & Mourot, 2011). During extrusion, most of the hydrolytic lipases are destroyed thus preventing catabolism of triglycerides into free fatty acids and glycerol. Short retention time inhibits lipid oxidation so maximum lipids get retained in the final product. Millard reaction products resulting in extrusion also act as antioxidants thus preventing lipid oxidation during extrusion (Kouba & Mourot, 2011). In this context, the basic purpose of this research was to examine the retention and oxidative stability of essential unsaturated fatty acids of FP supplemented extruded macaroni with high PUFAs contents.

2 Materials and methods

2.1 Procurement and preparation of sample

Rohu fish (weight of 1500 ± 150 g) was used as raw and experimental material for this research. Fish was purchased from local markets, Faisalabad-Pakistan. Head, fins, tail, and viscera of fish were removed following deboning and mincing then subjected to microwave vacuum drying in a microwave vacuum dryer.

2.2 Microwave Vacuum Drying (MVD)

A lab-scale microwave vacuum dryer (WZD2S, Nanking Sanle, China) was used for drying fish. It had a capacity of drying fish ranging from 8 to 12 kg of fish under different temperatures and pressure. It was optimized to the power of 1300W and operated at 2450 MHz frequency, 90 Pascal pressure for 4.5 hours at 50 °C. The dried minced powder was preserved in polyethylene bags.

2.3 Grinding of dried mince

After completion of the drying process, the dried mince fish was ground to powder form using a spinning blade grinder (Duronic CG250- 250W motor, UK).

2.4 Thermal extrusion optimization

The twin-screw extruder was used to extrude the products. It had a screw diameter of 36 mm with a length to diameter ratio of 24:1. It had a temperature control zone for thermal cooking with a temperature measuring probe and also had a screw speed controller as shown in the supplementary file (Wang et al., 2017). Optimization was performed using Box Behnken Design, RSM. Optimization conditions for BET were ranging from 100-150 °C, SS from 100-150 rpm, FFR from 30-90 kg/hr, FMC from 10-30% and FP from 0-25% (Imran & Anjum, 2014). Semolina (75-100%) was also used for making the final product. For the convenience of experimental design, coding was used which is presented in Table 1.

2.5 Preparation of sample for fatty acids analysis

Total lipids of FP and final product were estimated according to AOAC Method No. 923.07 (Ogbobe & Akano, 1993). Fatty acid methyl ester (FAME) of samples were prepared according to the method prescribed by (Carvalho & Malcata, 2005).

2.6 Gas chromatographic analysis

The FAME sample of 1.0 µL was injected with Helium (1 mL/min) as a carrier gas onto the column that was programmed for operating conditions such as column oven temperature 160 °C at 0 minutes with subsequent increase of 3 °C/min until 180 °C. The column oven temperature was increased from 180 °C to 220 °C at 1 °C/min and was held for 7.5 min at 220 °C. The split ratio was 50% with injector temperature of 240 °C and detector 250 °C. The peak areas and total fatty acids composition were calculated for each sample by retention time using Varian Chem Station software.

2.7 Retention of fatty acids

The retention of fatty acids was calculated according to the following Equation 1:

$$\text{Fatty acids retention \%} = \frac{\text{fatty acids present in product after extrusion}}{\text{fatty acids present in raw material}} X\ 100 \quad (1)$$

2.8 Thiobarbituric acid reactive substances (TBARS) analysis

Product sample (5 g) was taken and homogenized with 11% trichloroacetic acid (TCA) for 1 min at 11000 rpm (Homogenizer: IKA, Wilmington, USA) and subsequently placed in the ice bath for 1.0 min and then homogenized for another 1.0 min.

Table 1. Codes and actual levels of independent variables for optimization of response factors as determined by Box-Behnken design.

Independent variables	Units	Coded levels		
		−1	0	+1
Barrel Exit Temperature (BET)	°C	100	125	150
Feed Flow Rate (FFR)	kg/hr	30	60	90
Screw Speed (SS)	rpm	50	100	150
Feed Moisture Content (FMC)	%	10	20	30
Fish Powder (FP)	%	0	12.5	25

The homogenate was separated through Whatman No.1 and then added 1.0 mL of 20 mM thiobarbituric acid solution to 1.0 mL of filtrate following incubation for 20 hr in dark conditions at 25 °C. The absorbance value was determined at 532 nm through the UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The results were calculated as mg of MDA/kg.

2.9 Carbonyl contents analysis

The carbonyl contents were determined to calculate the oxidation of protein during extrusion and storage time. A sample of 3.0 g was homogenized with 0.15 M KCl (7.4 pH) at 7100 rpm for 90 seconds (Homogenizer: IKA, Wilmington, USA). The precipitation of protein present in the homogenate was done using 10% TCA and centrifuging at 5000 g, 4 °C for 5 minutes (ST 16R, Thermo Scientific, Wilmington, DE). The supernatant is discarded and then 10 mM 2, 4-dinitrophenylhydrazine (DNPH) in 2.0 N HCl was added in the precipitate and incubated at 25 °C for 1 hr in the dark with gentle vortexing after every 15 minutes. The substrate reacted with DNPH was then precipitated using TCA (10%) following centrifugation at 11000 g, 4 °C for 10 minutes. The precipitate was then washed three times using ethanol/ethyl acetate (1:1, v/v) solution and solubilized with guanidine hydrochloride (6M) in 20 mM sodium phosphate buffer having pH 6.5. Centrifuge again at 11000 g, 25 °C for 10 minutes to remove insoluble particles. Absorbance was estimated at 370 nm through the UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Carbonyl contents were estimated through the absorbance coefficient for protein hydrazones. The obtained results were expressed as nmol of carbonyl/mg of protein.

2.10 Sensory evaluation

A trained panel of judges evaluated the product for sensory evaluation using a 9-point hedonic scale (Civille & Carr, 2015). Color, flavor, and overall acceptability were evaluated after product development and the same after a storage period of 60 days interval.

2.11 Statistical analysis

The optimization of microwave and extrusion conditions was performed through RSM using Design Expert 11 software of statistics. The experiment analyses were conducted through the method of Montgomery (1991). All experiments were conducted in triplicates and average values were considered as mean values. The significance of values was calculated statistically through mean using Analysis of variance (ANOVA) at the probability of 0.05 through MATLAB® (Ver. 7.9.0) software (Mathworks, Inc., Natick, USA).

3 Results and discussion

3.1 Results

The powder obtained from fish under microwave vacuum drying contained 9.64 ± 32% total lipids that non-significantly changed during the drying process. The PUFAs that were determined and measured in this study were C18: 2 n-6, C18: 3 n-3, C20:3 n-7, C24:4 n-6, C25:5 n-3, and C22:6 n-3, while the MUFAs were C16:1 n-7, C18:1 n-9, and C20:1 n-9. The SFAs were C14:0, C15:0, C16:0, C17:0, C18:0, C19:0, and C20:0. The PUFAs, MUFAs, and SFAs were 14.30 ± 1.72, 27.60 ± 1.32 and 49.86 ± 2.37% in FP obtained from the drying process. The PUFA retention after different extrusion conditions is presented in Table 2. The highest PUFAs contents (607 ± 8 mg) were found in product with 25% FP extruded under BET (125 °C), SS (150 rpm), FFR (60 kg/hr), FMC (20%) conditions and minimum PUFAs (320 ± 2 mg) were present in a product without FP extruded under BET (125 °C), SS (50 rpm), FFR (60 kg/hr), FMC (20%) as shown in Table 2. The maximum PUFAs retention was 94.84% under optimizing conditions of BET (125 °C), SS (150 rpm), FFR (60 kg/hr), FMC (20%) and FP (25%) and in final products, while the minimum PUFAs retention (80%) was observed in a product at BET (125 °C), SS (50 rpm), FFR (60 kg/hr), FMC (20%) and FP (0%). The supplementary file shows how a response variable (PUFAs) relates to predictor variables. It shows that the response is connected to produce contour lines of constant responses.

The effect of thermal processing on MUFAs and SFAs are shown in Table 3. The results show that the maximum MUFAs retention was 96.48% under optimizing conditions of BET (125 °C), SS (150 rpm), FFR (60 kg/hr), FMC (20%) and FP (25%) in final products while minimum MUFAs retention (66%) was observed at 125 °C BET, 50 rpm SS, 60 kg/hr FFR, 20% FMC and 0% FP. Maximum SFAs contents (1.55 ± 0.007%) were observed in a final product that was extruded under conditions of BET (150 °C), SS (100 rpm), FFR (60 kg/hr), FMC (20%) and FP (25%). The minimum SFAs contents were found to be 0.214% under 100 °C BET, 100 rpm SS, 60 kg/hr FFR, 20% FMC and 0% FP. BET was the most independent parameter affecting the retention of PUFAs. A trend in PUFAs decrease was observed with increasing temperature and decreasing moisture content while an increasing trend in PUFAs was observed with increasing SS and FP as shown in Figure 1.

Highest values of PUFAs retention (87.97% and 85.66%) at a storage temperature of 25 °C for 30 and 60 days were observed in a product extruded under processing conditions of BET (125 °C), SS (150rpm), FFR (60 kg/hr), FMC (20%) and FP (25%). While the minimum PUFAs values (85.80% and 83.87%) were observed at 25 °C storage temperature for 30 and 60 days in product processed at BET (125 °C), SS (150 rpm), FFR (90 kg/hr) and FMC (20%) and FP (12.5%) as shown in Table 2. The minimum value of MUFAs (55 ± 1 and 56 ± 1 mg) at 30 and 60 days storage were found at 125 °C BET, 50 rpm SS, 60 kg/hr FFR, 20% FMC and 0% FP and minimum SFAs (214 ± 2 and 216 ± 1 mg) at 30 and 60 days storage were present at 125 °C BET, 100 rpm SS, 60 kg/hr FFR, 30% FMC and 0% FP as shown in Table 3.

Oxidative stability of the product was measured by determining the TBARS and carbonyl contents are presented in Table 4. The highest TBARS values at processing conditions of BET (125 °C), SS (150 rpm), FFR (90 kg/hr) and FMC (20%) and FP (12.5%) was 0.42 and 0.44 mg MDA/kg at 25 °C storage temperature for 0 and 30 days respectively while 0.55 mg MDA/kg at 60 days storage were in product extruded at BET (125 °C), SS (100 rpm), FFR (30%), FMC (20%) and FP (25%). The highest carbonyl contents of 6.02, 7.67, 9.37 nmol/mg protein

Table 2. Effect of extrusion processing conditions on retention of polyunsaturated fatty acids at different storage intervals.

Extrusion processing run	Independent variables					Dependent variable		
						PUFAs (mg/100 g)		
	Barrel Exit Temp. (BET)	Screw Speed (SS)	Feed Flow Rate (FFR)	Feed Moisture Content (FMC)	Fish Powder (FP)	0 Day	30 Days	60 Days
1	150 (1)	150 (1)	60 (0)	20 (0)	12.5 (0)	444 ± 1[cd]	391 ± 3[fg]	**385 ± 5[fg]**
2	125 (0)	150 (1)	60 (0)	20 (0)	25 (1)	607 ± 8[a]	534 ± 6[b]	**520 ± 2[b]**
3 (C_1)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	460 ± 6[c]	410 ± 3[ef]	**401 ± 3[f]**
4	150 (1)	100 (0)	60 (0)	30 (1)	12.5 (0)	408 ± 9[ef]	360 ± 2[h]	**345 ± 4[hi]**
5	100 (-1)	100 (0)	60 (0)	20 (0)	0 (-1)	380 ± 7[g]	340 ± 1[i]	**329 ± 1[j]**
6	100 (-1)	150 (1)	60 (0)	20 (0)	12.5 (0)	512 ± 9[b]	452 ± 2[cd]	**440 ± 4[d]**
7	125 (0)	150 (1)	30 (-1)	20 (0)	12.5 (0)	490 ± 8[bc]	433 ± 5[de]	**424 ± 2[e]**
8	125 (0)	50 (-1)	60 (0)	10 (-1)	12.5 (0)	409 ± 4[ef]	352 ± 3[h]	**346 ± 4[hi]**
9	150 (1)	100 (0)	60 (0)	20 (0)	0 (-1)	353 ± 7[h]	308 ± 6[lm]	**303 ± 1[m]**
10	125 (0)	100 (0)	60 (0)	30 (1)	25 (1)	539 ± 1[b]	471 ± 3[c]	**461 ± 2[c]**
11	125 (0)	100 (0)	90 (1)	10 (-1)	12.5 (0)	447 ± 6[cd]	386 ± 2[fg]	**379 ± 5[g]**
12 (C_2)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	455 ± 3[cd]	401 ± 2[f]	**391 ± 3[fg]**
13	125 (0)	150 (1)	60 (0)	30 (1)	12.5 (0)	508 ± 5[b]	445 ± 5[cd]	**436 ± 2[d]**
14	125 (0)	100 (0)	60 (0)	10 (-1)	25 (1)	505 ± 9[b]	445 ± 2[cd]	**436 ± 5[d]**
15	125 (0)	100 (0)	30 (-1)	20 (0)	0 (-1)	360 ± 3[h]	319 ± 1[k]	**311 ± 2[ll]**
16	100 (-1)	100 (0)	60 (0)	10 (-1)	12.5 (0)	411 ± 5[ef]	360 ± 3[h]	**354 ± 2[h]**
17	100 (-1)	100 (0)	30 (-1)	20 (0)	12.5 (0)	427 ± 5[de]	371 ± 2[gh]	**366 ± 3[gh]**
18	150 (1)	100 (0)	90 (1)	20 (0)	12.5 (0)	394 ± 1[fg]	344 ± 2[hi]	**338 ± 5[i]**
19	125 (0)	50 (-1)	60 (0)	20 (0)	0 (-1)	320 ± 2[k]	285 ± 5[n]	**277 ± 4[o]**
20	125 (0)	100 (0)	60 (0)	10 (-1)	0 (-1)	363 ± 1[gh]	324 ± 1[jk]	**316 ± 2[kl]**
21	125 (0)	100 (0)	90 (1)	20 (0)	25 (1)	527 ± 3[b]	462 ± 3[c]	**451 ± 5[cd]**
22	125 (0)	150 (1)	60 (0)	20 (0)	0 (-1)	382 ± 5[g]	338 ± 2[i]	**334 ± 1[ij]**
23	125 (0)	100 (0)	90 (1)	30 (1)	12.5 (0)	465 ± 2[c]	419 ± 4[e]	**397 ± 2[f]**
24 (C_3)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	459 ± 7[c]	405 ± 2[f]	**390 ± 4[fg]**
25	100 (-1)	100 (0)	90 (1)	20 (0)	12.5 (0)	433 ± 2[de]	387 ± 3[fg]	**375 ± 2[gh]**
26 (C_4)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	452 ± 5[cd]	405 ± 2[f]	**392 ± 4[fg]**
27	125 (0)	50 (-1)	90 (1)	20 (0)	12.5 (0)	411 ± 1[ef]	369 ± 3[gh]	**357 ± 6[h]**
28	125 (0)	150 (1)	60 (0)	10 (-1)	12.5 (0)	466 ± 2[c]	409 ± 2[ef]	**401 ± 4[f]**
29	125 (0)	100 (0)	30 (-1)	20 (0)	25 (1)	514 ± 7[b]	459 ± 2[c]	**438 ± 2[d]**
30	125 (0)	100 (0)	60 (0)	30 (1)	0 (-1)	390 ± 2[fg]	343 ± 1[hi]	**340 ± 1[i]**
31 (C_5)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	455 ± 3[cd]	405 ± 5[f]	**394 ± 3[fg]**
32	100 (-1)	100 (0)	60 (0)	30 (1)	12.5 (0)	449 ± 2[cd]	391 ± 3[fg]	**387 ± 5[fg]**
33	150 (1)	100 (0)	60 (0)	20 (0)	25 (1)	470 ± 1[c]	414 ± 3[ef]	**405 ± 7[f]**
34	125 (0)	50 (-1)	60 (0)	20 (0)	25 (1)	497 ± 4[bc]	436 ± 6[d]	**422 ± 4[e]**
35	100(-1)	50 (-1)	60 (0)	20 (0)	12.5 (0)	418 ± 2[e]	369 ± 2[gh]	**357 ± 1[h]**
36	150 (1)	100 (0)	30 (-1)	20 (0)	12.5 (0)	392 ± 1[fg]	344 ± 1[hi]	**340 ± 2[i]**
37	150 (1)	100 (0)	60 (0)	10 (-1)	12.5 (0)	403 ± 5[f]	358 ± 4[h]	**348 ± 3[hi]**
38	125 (0)	100 (0)	90 (1)	20 (0)	0 (-1)	348 ± 2[hi]	311 ± 3[ll]	**301 ± 2[m]**
39 (C_6)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	447 ± 6[cd]	399 ± 2[f]	**388 ± 5[fg]**
40	100 (-1)	100 (0)	60 (0)	20 (0)	25 (1)	540 ± 3[b]	475 ± 2[c]	**466 ± 5[c]**
41	125 (0)	150 (1)	90 (1)	20 (0)	12.5 (0)	470 ± 4[c]	426 ± 1[de]	**401 ± 1[f]**
42	150 (1)	50 (-1)	60 (0)	20 (0)	12.5 (0)	388 ± 3[fg]	338 ± 5[i]	**327 ± 3[j]**
43	125 (0)	50 (-1)	30 (-1)	20 (0)	12.5 (0)	422 ± 1[e]	368 ± 2[gh]	**366 ± 3[gh]**
44	125 (0)	100 (0)	30 (-1)	10 (-1)	12.5 (0)	429 ± 5[de]	373 ± 3[gh]	**363 ± 1[gh]**
45	125 (0)	100 (0)	30 (-1)	30 (1)	12.5 (0)	466 ± 2[c]	409 ± 6[ef]	**401 ± 5[f]**
46	**125 (0)**	**50 (-1)**	**60 (0)**	**30 (1)**	**12.5 (0)**	**419 ± 6[e]**	**364 ± 3[gh]**	357 ± 2[h]

C_1, C_2, C_3, C_4, C_5, C_6 are the centre points where extrusion processing was applied. Different superscripts letters indicate the significance difference ($p \leq 0.05$) within the rows and columns. BET = Barrel Exit Temp; SS = Screw Speed; FFR = Feed Flow Rate; FMC = Feed Moisture Content; FP = Fish Powder; PUFAs = polyunsaturated fatty acids.

Table 3. Effect of extrusion processing conditions on retention of SFAs and MUFAs at different storage intervals.

Extrusion processing run	Independent variables					Dependent variables					
	Barrel Exit Temp. (BET)	Screw Speed (SS)	Feed Flow Rate (FFR)	Feed Moisture Content (FMC)	Fish Powder Content (FP)	SFAs (mg/100 g product)			MUFAs (mg/100 g product)		
						0 Day	30 Days	60 Days	0 Day	30 Days	60 Days
1	150 (1)	150 (1)	60 (0)	20 (0)	12.5 (0)	827 ± 2^{kl}	836 ± 3^{k}	846 ± 5^{jk}	375 ± 1^{H}	333 ± 3^{J}	350 ± 2^{I}
2	125 (0)	150 (1)	60 (0)	20 (0)	25 (1)	1379 ± 7^{g}	1388 ± 9^{g}	1411 ± 2^{fg}	714 ± 3^{A}	640 ± 2^{C}	672 ± 6^{AB}
3 (C_1)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	805 ± 4^{m}	816 ± 4^{lm}	839 ± 1^{jk}	386 ± 2^{GH}	342 ± 4^{IJ}	350 ± 3^{I}
4	150 (1)	100 (0)	60 (0)	30 (1)	12.5 (0)	860 ± 6^{j}	880 ± 3^{i}	889 ± 3^{hi}	353 ± 2^{I}	311 ± 1^{K}	333 ± 2^{J}
5	100 (-1)	100 (0)	60 (0)	20 (0)	0 (-1)	214 ± 1^{s}	217 ± 5^{rs}	219 ± 1^{rs}	91 ± 1^{O}	79 ± 1^{OP}	89 ± 1^{O}
6	100 (-1)	150 (1)	60 (0)	20 (0)	12.5 (0)	777 ± 3^{no}	784 ± 1^{n}	793 ± 5^{mn}	437 ± 1^{FG}	387 ± 5^{GH}	410 ± 5^{G}
7	125 (0)	150 (1)	30 (-1)	20 (0)	12.5 (0)	783 ± 5^{n}	795 ± 3^{mn}	805 ± 4^{m}	405 ± 3^{G}	357 ± 3^{I}	378 ± 2^{H}
8	125 (0)	50 (-1)	60 (0)	10 (-1)	12.5 (0)	866 ± 2^{ij}	872 ± 6^{ij}	889 ± 3^{hi}	349 ± 2^{I}	306 ± 2^{KL}	323 ± 3^{JK}
9	150 (1)	100 (0)	60 (0)	20 (0)	0 (-1)	268 ± 2^{q}	275 ± 1^{pq}	277 ± 1^{pq}	83 ± 1^{O}	68 ± 1^{P}	75 ± 2^{OP}
10	125 (0)	100 (0)	60 (0)	30 (1)	25 (1)	1469 ± 5^{ef}	1489 ± 8^{de}	1514 ± 7^{d}	677 ± 5^{AB}	603 ± 6^{DE}	631 ± 4^{CD}
11	125 (0)	100 (0)	90 (1)	10 (-1)	12.5 (0)	820 ± 6^{l}	844 ± 2^{jk}	847 ± 3^{jk}	374 ± 2^{H}	333 ± 3^{J}	352 ± 2^{I}
12 (C_2)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	816 ± 1^{lm}	826 ± 4^{kl}	842 ± 5^{jk}	371 ± 3^{H}	330 ± 2^{J}	349 ± 1^{I}
13	125 (0)	150 (1)	60 (0)	30 (1)	12.5 (0)	777 ± 3^{no}	785 ± 5^{n}	787 ± 4^{n}	410 ± 1^{G}	369 ± 1^{H}	387 ± 3^{GH}
14	125 (0)	100 (0)	60 (0)	10 (-1)	25 (1)	1523 ± 9^{cd}	1554 ± 9^{bc}	1569 ± 1^{b}	652 ± 5^{BC}	585 ± 2^{E}	614 ± 5^{D}
15	125 (0)	100 (0)	30 (-1)	20 (0)	0 (-1)	238 ± 7^{r}	234 ± 2^{r}	236 ± 2^{r}	81 ± 1^{O}	72 ± 2^{OP}	75 ± 2^{OP}
16	100 (-1)	100 (0)	60 (0)	10 (-1)	12.5 (0)	844 ± 4^{jk}	856 ± 1^{j}	859 ± 5^{j}	355 ± 4^{I}	310 ± 5^{K}	337 ± 1^{J}
17	100 (-1)	100 (0)	30 (-1)	20 (0)	12.5 (0)	827 ± 2^{kl}	836 ± 4^{k}	847 ± 6^{jk}	364 ± 2^{HI}	320 ± 3^{JK}	342 ± 1^{IJ}
18	150 (1)	100 (0)	90 (1)	20 (0)	12.5 (0)	871 ± 5^{ij}	884 ± 2^{hi}	896 ± 3^{h}	348 ± 3^{I}	306 ± 4^{KL}	323 ± 3^{JK}
19	125 (0)	50 (-1)	60 (0)	20 (0)	0 (-1)	277 ± 3^{pq}	275 ± 5^{pq}	278 ± 1^{pq}	66 ± 1^{P}	54 ± 1^{Q}	56 ± 3^{Q}
20	125 (0)	100 (0)	60 (0)	10 (-1)	0 (-1)	219 ± 1^{rs}	214 ± 2^{s}	216 ± 1^{s}	92 ± 1^{O}	78 ± 2^{OP}	85 ± 5^{O}
21	125 (0)	100 (0)	90 (1)	20 (0)	25 (1)	1492 ± 8^{de}	1523 ± 7^{cd}	1534 ± 9^{c}	666 ± 6^{B}	590 ± 5^{E}	627 ± 7^{CD}
22	125 (0)	150 (1)	60 (0)	20 (0)	0 (-1)	218 ± 2^{rs}	214 ± 2^{s}	219 ± 1^{rs}	105 ± 1^{N}	89 ± 2^{O}	95 ± 3^{O}
23	125 (0)	100 (0)	90 (1)	30 (1)	12.5 (0)	849 ± 5^{jk}	856 ± 2^{j}	868 ± 5^{ij}	375 ± 3^{H}	333 ± 2^{J}	346 ± 2^{IJ}
24 (C_3)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	815 ± 4^{lm}	826 ± 4^{kl}	840 ± 3^{jk}	384 ± 2^{GH}	340 ± 2^{IJ}	359 ± 3^{HI}
25	100 (-1)	100 (0)	90 (1)	20 (0)	12.5 (0)	807 ± 8^{m}	816 ± 7^{lm}	828 ± 2^{kl}	379 ± 2^{H}	330 ± 3^{J}	349 ± 4^{I}
26 (C_4)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	816 ± 5^{lm}	826 ± 5^{kl}	841 ± 3^{jk}	378 ± 1^{H}	333 ± 1^{J}	349 ± 2^{I}
27	125 (0)	50 (-1)	90 (1)	20 (0)	12.5 (0)	879 ± 2^{i}	887 ± 2^{hi}	892 ± 2^{hi}	366 ± 1^{HI}	324 ± 2^{JK}	340 ± 1^{IJ}
28	125 (0)	150 (1)	60 (0)	10 (-1)	12.5 (0)	843 ± 6^{jk}	858 ± 1^{j}	865 ± 5^{ij}	383 ± 3^{GH}	342 ± 3^{IJ}	359 ± 3^{HI}
29	125 (0)	100 (0)	30 (-1)	20 (0)	25 (1)	1480 ± 9^{e}	1513 ± 7^{d}	1526 ± 8^{cd}	657 ± 5^{BC}	585 ± 5^{E}	614 ± 2^{D}
30	125 (0)	100 (0)	60 (0)	30 (1)	0 (-1)	219 ± 1^{rs}	214 ± 3^{s}	216 ± 1^{s}	111 ± 2^{N}	98 ± 2^{O}	105 ± 2^{N}
31 (C_5)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	812 ± 3^{lm}	816 ± 2^{lm}	843 ± 3^{jk}	375 ± 4^{H}	332 ± 3^{J}	350 ± 4^{I}
32	100 (-1)	100 (0)	60 (0)	30 (1)	12.5 (0)	787 ± 2^{n}	805 ± 4^{m}	813 ± 2^{lm}	387 ± 3^{GH}	340 ± 5^{IJ}	359 ± 5^{HI}
33	150 (1)	100 (0)	60 (0)	20 (0)	25 (1)	1555 ± 7^{bc}	1581 ± 3^{ab}	1598 ± 9^{a}	626 ± 2^{CD}	558 ± 3^{F}	589 ± 5^{E}
34	125 (0)	50 (-1)	60 (0)	20 (0)	25 (1)	1548 ± 8^{bc}	1570 ± 3^{b}	1586 ± 6^{ab}	613 ± 1^{D}	552 ± 7^{F}	576 ± 6^{EF}
35	100(-1)	50 (-1)	60 (0)	20 (0)	12.5 (0)	869 ± 2^{ij}	872 ± 5^{ij}	889 ± 2^{hi}	344 ± 2^{IJ}	306 ± 2^{KL}	323 ± 2^{JK}
36	150 (1)	100 (0)	30 (-1)	20 (0)	12.5 (0)	870 ± 5^{ij}	874 ± 1^{ij}	896 ± 5^{h}	355 ± 1^{I}	315 ± 1^{K}	335 ± 2^{J}
37	150 (1)	100 (0)	60 (0)	10 (-1)	12.5 (0)	854 ± 1^{j}	867 ± 3^{ij}	880 ± 4^{i}	366 ± 4^{HI}	324 ± 2^{JK}	342 ± 3^{IJ}
38	125 (0)	100 (0)	90 (1)	20 (0)	0 (-1)	227 ± 3^{r}	224 ± 2^{r}	226 ± 2^{r}	73 ± 1^{OP}	63 ± 1^{P}	66 ± 1^{P}
39 (C_6)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	813 ± 6^{lm}	820 ± 5^{l}	847 ± 2^{jk}	386 ± 2^{GH}	340 ± 1^{IJ}	362 ± 1^{HI}
40	100 (-1)	100 (0)	60 (0)	20 (0)	25 (1)	1420 ± 4^{fg}	1444 ± 5^{f}	1462 ± 6^{ef}	679 ± 2^{AB}	603 ± 3^{DE}	631 ± 3^{CD}
41	125 (0)	150 (1)	90 (1)	20 (0)	12.5 (0)	688 ± 6^{p}	693 ± 2^{op}	705 ± 2^{o}	306 ± 1^{KL}	273 ± 2^{M}	285 ± 2^{L}
42	150 (1)	50 (-1)	60 (0)	20 (0)	12.5 (0)	885 ± 2^{hi}	897 ± 1	906 ± 6^{gh}	322 ± 4^{JK}	288 ± 2^{L}	304 ± 5^{KL}
43	125 (0)	50 (-1)	30 (-1)	20 (0)	12.5 (0)	844 ± 5^{jk}	856 ± 3^{j}	865 ± 3^{ij}	362 ± 4^{HI}	324 ± 3^{JK}	342 ± 3^{IJ}
44	125 (0)	100 (0)	30 (-1)	10 (-1)	12.5 (0)	849 ± 2^{jk}	858 ± 2^{j}	865 ± 3^{ij}	390 ± 3^{GH}	348 ± 4^{I}	365 ± 1^{HI}
45	125 (0)	100 (0)	30 (-1)	30 (1)	12.5 (0)	801 ± 2^{m}	819 ± 5^{l}	821 ± 1^{l}	404 ± 1^{G}	361 ± 1^{HI}	378 ± 3^{H}
46	125 (0)	50 (-1)	60 (0)	30 (1)	12.5 (0)	866 ± 5^{ij}	877 ± 2^{i}	885 ± 4^{hi}	357 ± 2^{I}	315 ± 2^{K}	337 ± 2^{J}

C_1, C_2, C_3, C_4, C_5, C_6 are the centre points where extrusion processing was applied. Different superscripts letters $^{(a-s)}$ indicate the significance difference ($p \leq 0.05$) within the rows and columns within SFAs. Different superscripts letters $^{(A-Q)}$ indicate the significance difference ($p \leq 0.05$) within the rows and columns within MUFAs. BET = Barrel Exit Temp; SS = Screw Speed; FFR = Feed Flow Rate; FMC = Feed Moisture Content; FP = Fish Powder; SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids.

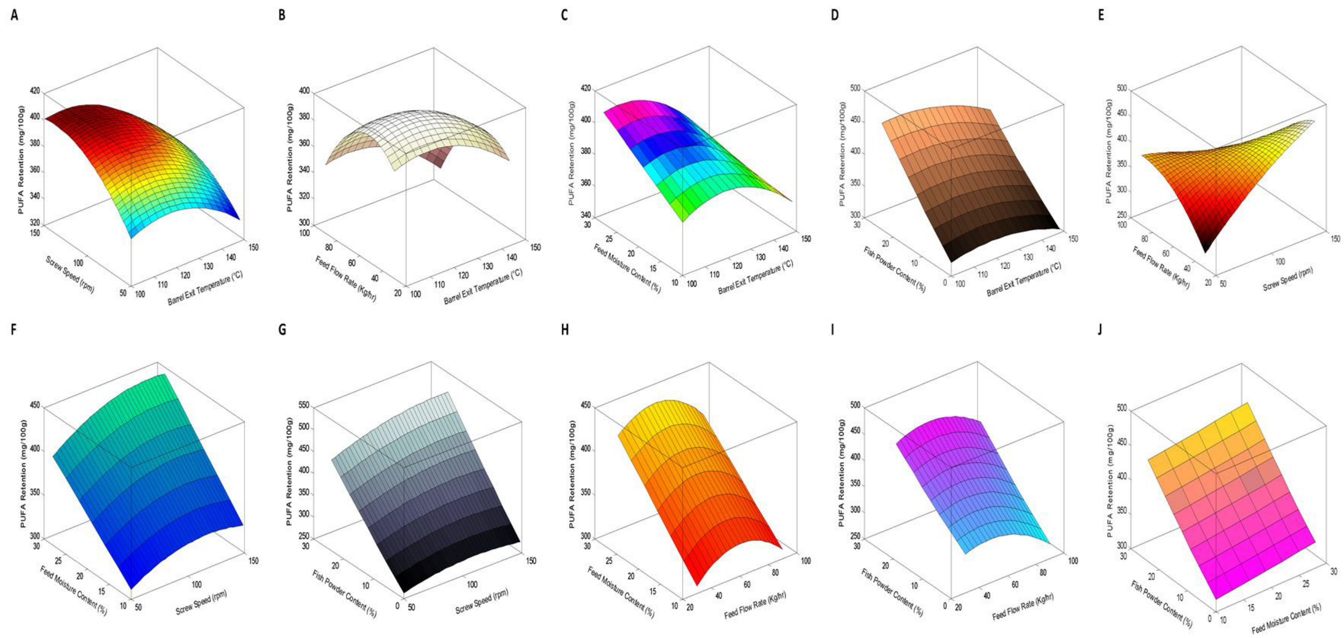

Figure 1. Mutual interaction effects of BET (100-150 °C), SS (50-150 rpm), FFR (30-90 kg/hour), FMC (10-30%) and FP (0-25%) on PUFAs retention during thermal extrusion in functional macaroni production.

were observed in product extruded under BET (125 °C), SS (100 rpm), FFR (60 kg/hr), FMC (30%) and FP (25%) for 0, 30 and 60 days of storage at 25 °C. The sensory evaluation score for products is shown in Table 5. The results for color, flavor and overall acceptability have shown that the product extruded at 125 °C BET, 150 rpm SS, 60 kg/hr FFR, 20% FMC and 25% FP have the gain highest score (7.30 ± 0.50 and 6.59 ± 0.65), (7.04 ± 0.56, 6.54 ± 0.32) for color and flavor at 0 and 60 days storage, respectively. The product extruded at 125 °C BET, 150 rpm SS, 60 kg/hr FFR, 30% FMC and 12.5% FP have the best overall acceptability score.

3.2 Discussion

Fish and fish products are consumed as the major source of PUFAs, however, in low-income countries, due to high postharvest losses, this vital source of micronutrients and essential fatty acids are rarely available and affordable to the communities. In fishery sector, the post-harvest losses increases from 10 to 12 million tons per year also have a low shelf life (14-16 days) under ice storage condition (Nowsad et al., 2015). In Pakistan, most of the fish are consumed by roasting and frying technology that has few hours of storage that further leads to CVDs (Gadiraju et al., 2015). So this study was designed to use FP to develop fish powder supplemented macaroni with high PUFAs and better oxidative stability. Thus PUFAs optimized fished powder was obtained through MVD. In MVD the PUFAs non-significantly changed and remained unaffected from their original composition and contents. In MVD, due to a higher vacuum degree, the boiling temperature of the water is relatively lowered which results in easily drying of fish mince and could improve the quality of the finished product (Hearn et al., 1987). The addition of FP not only increases the PUFAs and MUFAs but also the protein contents of the final product in functional macaroni. The PUFAs and MUFAs increased from 0.4 to 0.64% and 0.1 to 0.74%, respectively, providing a significant contribution to the recommended intakes of unsaturated fatty acids. Thus, extrusion processing plays an important role in the retention of PUFAs and MUFAs and their oxidative stability. It was reported that twin-screw extrusion with a high temperature of 180 °C and a high SS of 300 rpm did not significantly change the polyenoic fatty acids (Zadernowski et al., 1997). The BET, SS along FP addition have a highly significant effect on the retention of polyenoic fatty acids and particular PUFAs while FMC affects slight significance whereas the FFR does not significantly influence them. It can also be substantiated that the mutual influence of BET and FMC was slightly significant because the increase in BET at high FMC negatively affects the retention of PUFAs and MUFAs.

It was observed that SFAs significantly increased during the extrusion process. The higher temperature results in the conversion of double bonds from Cis to Trans fatty acids (Camire, 2000). These changes further occur during storage conditions leading to a change in the composition of fatty acids and oxidation of the product. It was observed that the better retention of TBARS values and carbonyl contents were detected in products with a high quantity of PUFAs and MUFAs. Changes in composition were rapid in the first 30 days as compared to the next 30 days of storage at 25 °C. Björck & Asp (1983) also found that with the increase in an extrusion temperature decrease in fat content occurred and this statement is comparable to current findings. This might be due to the expulsion of fumes at higher temperatures from fats. So, the change in parameters like BET, SS, and FMC also affect the number of fats on different levels. BET and FMC are both the variables that mainly affect the composition retention and storage stability of PUFA's. Fitzpatrick et al. (2004) stated

Table 4. Effect of thermal extrusion processing conditions on TRARS and carbonyl contents of product at 25 °C for different storage intervals.

Extrusion processing run	Independent variables					TBARS (mg MDA/kg)			Carbonyl contents (nmol/mg protein)		
	Barrel Exit Temp. (BET)	Screw Speed (SS)	Feed Flow Rate (FFR)	Feed Moisture Content (FMC)	Fish Powder Content (FP)	Storage time (days) 0	30	60	Storage time (days) 0	30	60
1	150 (1)	150 (1)	60 (0)	20 (0)	12.5 (0)	0.27 ± 0.01^{de}	0.29 ± 0.02^{de}	0.34 ± 0.02^{d}	3.99 ± 0.10^{R}	5.64 ± 0.05^{L}	7.27 ± 0.19^{EF}
2	125 (0)	150 (1)	60 (0)	20 (0)	25 (1)	0.42 ± 0.04^{c}	0.44 ± 0.03^{bc}	0.51 ± 0.07^{ab}	5.91 ± 0.15^{IJ}	7.56 ± 0.08^{D}	9.27 ± 0.07^{B}
3 (C_1)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	0.26 ± 0.02^{de}	0.31 ± 0.02^{d}	0.33 ± 0.02^{d}	3.93 ± 0.08^{R}	5.54 ± 0.12^{LM}	7.10 ± 0.13^{FG}
4	150 (1)	100 (0)	60 (0)	30 (1)	12.5 (0)	0.24 ± 0.01^{e}	0.26 ± 0.01^{de}	0.30 ± 0.04^{d}	4.08 ± 0.09^{QR}	5.67 ± 0.13^{KL}	7.32 ± 0.11^{EF}
5	100 (-1)	100 (0)	60 (0)	20 (0)	0 (-1)	0.10 ± 0.01^{fg}	0.11 ± 0.01^{f}	0.13 ± 0.01^{f}	2.89 ± 0.07^{V}	4.06 ± 0.17^{QR}	5.19 ± 0.12^{NO}
± 6	100 (-1)	150 (1)	60 (0)	20 (0)	12.5 (0)	0.29 ± 0.02^{de}	0.32 ± 0.02^{d}	0.37 ± 0.05^{cd}	3.87 ± 0.08^{RS}	5.46 ± 0.14^{M}	7.10 ± 0.15^{FG}
7	125 (0)	150 (1)	30 (-1)	20 (0)	12.5 (0)	0.28 ± 0.02^{de}	0.31 ± 0.04^{d}	0.35 ± 0.07^{d}	4.12 ± 0.11^{Q}	5.73 ± 0.16^{K}	7.37 ± 0.13^{E}
08	125 (0)	50 (-1)	60 (0)	10 (-1)	12.5 (0)	0.23 ± 0.03^{e}	0.25 ± 0.01^{e}	0.27 ± 0.01^{de}	4.21 ± 0.11^{PQ}	5.80 ± 0.11^{JK}	7.47 ± 0.16^{D}
9	150 (1)	100 (0)	60 (0)	20 (0)	0 (-1)	0.09 ± 0.01^{fg}	0.10 ± 0.01^{fg}	0.13 ± 0.01^{f}	3.11 ± 0.10^{U}	4.47 ± 0.13^{O}	5.81 ± 0.12^{JK}
10	125 (0)	100 (0)	60 (0)	30 (1)	25 (1)	0.38 ± 0.04^{cd}	0.41 ± 0.04^{c}	0.47 ± 0.06^{b}	6.02 ± 0.13^{I}	7.67 ± 0.14^{CD}	9.37 ± 0.15^{A}
11	125 (0)	100 (0)	90 (1)	10 (-1)	12.5 (0)	0.24 ± 0.01^{e}	0.27 ± 0.02^{de}	0.32 ± 0.03^{d}	3.84 ± 0.09^{S}	5.43 ± 0.17^{MN}	7.09 ± 0.12^{FG}
12 (C_2)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	0.27 ± 0.05^{de}	0.30 ± 0.03^{d}	0.33 ± 0.02^{d}	3.90 ± 0.12^{RS}	5.51 ± 0.12^{LM}	7.11 ± 0.16^{FG}
13	125 (0)	150 (1)	60 (0)	30 (1)	12.5 (0)	0.29 ± 0.02^{de}	0.31 ± 0.01^{d}	0.35 ± 0.05^{d}	3.63 ± 0.14^{T}	5.23 ± 0.14^{N}	6.89 ± 0.12^{H}
14	125 (0)	100 (0)	60 (0)	10 (-1)	25 (1)	0.40 ± 0.06^{c}	0.43 ± 0.04^{bc}	0.54 ± 0.03^{a}	5.87 ± 0.13^{J}	7.53 ± 0.10^{D}	9.23 ± 0.15^{BC}
15	125 (0)	100 (0)	30 (-1)	20 (0)	0 (-1)	0.10 ± 0.01^{fg}	0.12 ± 0.01^{f}	0.13 ± 0.01^{f}	3.02 ± 0.10^{UV}	4.20 ± 0.09^{Q}	5.39 ± 0.11^{MN}
16	100 (-1)	100 (0)	60 (0)	10 (-1)	12.5 (0)	0.28 ± 0.03^{de}	0.30 ± 0.01^{d}	0.33 ± 0.02^{d}	3.77 ± 0.09ST	5.37 ± 0.08^{MN}	7.03 ± 0.09^{G}
017	100 (-1)	100 (0)	30 (-1)	20 (0)	12.5 (0)	0.27 ± 0.01^{de}	0.30 ± 0.02^{d}	0.34 ± 0.04^{d}	3.88 ± 0.16^{RS}	5.47 ± 0.16^{M}	7.11 ± 0.15^{FG}
18	150 (1)	100 (0)	90 (1)	20 (0)	12.5 (0)	0.24 ± 0.02^{e}	0.26 ± 0.01^{de}	0.30 ± 0.02^{d}	4.27 ± 0.12^{P}	5.86 ± 0.12^{J}	7.54 ± 0.18^{D}
19	125 (0)	50 (-1)	60 (0)	20 (0)	0 (-1)	0.06 ± 0.01^{fg}	0.07 ± 0.01^{fg}	0.09 ± 0.01^{fg}	3.11 ± 0.08^{U}	4.29 ± 0.11^{P}	5.49 ± 0.15^{LM}
20	125 (0)	100 (0)	60 (0)	10 (-1)	0 (-1)	0.07 ± 0.01^{fg}	0.09 ± 0.02^{fg}	0.10 ± 0.01^{fg}	2.94 ± 0.10^{V}	4.07 ± 0.13^{QR}	5.28 ± 0.12^{N}
21	125 (0)	100 (0)	90 (1)	20 (0)	25 (1)	0.35 ± 0.03^{d}	0.39 ± 0.07^{cd}	0.53 ± 0.08^{ab}	5.78 ± 0.11^{JK}	7.42 ± 0.18^{DE}	9.14 ± 0.14^{C}
0.22	125 (0)	150 (1)	60 (0)	20 (0)	0 (-1)	0.11 ± 0.01^{f}	0.13 ± 0.01^{f}	0.15 ± 0.01^{f}	2.98 ± 0.12^{V}	4.17 ± 0.12^{Q}	5.37 ± 0.13^{MN}
23	125 (0)	100 (0)	90 (1)	30 (1)	12.5 (0)	0.23 ± 0.01^{e}	0.25 ± 0.02^{e}	0.28 ± 0.01^{de}	3.78 ± 0.13ST	5.37 ± 0.11^{MN}	7.04 ± 0.12^{G}
24 (C_3)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	0.27 ± 0.03^{de}	0.30 ± 0.02^{d}	0.33 ± 0.03^{d}	3.92 ± 0.11^{R}	5.51 ± 0.15^{LM}	7.09 ± 0.21^{FG}
25	100 (-1)	100 (0)	90 (1)	20 (0)	12.5 (0)	0.24 ± 0.02^{e}	0.27 ± 0.01^{de}	0.31 ± 0.02^{d}	3.96 ± 0.12^{R}	5.58 ± 0.14^{L}	7.23 ± 0.16^{F}
26 (C_4)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	0.26 ± 0.03^{de}	0.31 ± 0.03^{d}	0.34 ± 0.04^{d}	3.91 ± 0.09^{R}	5.51 ± 0.11^{LM}	7.11 ± 0.13^{FG}
27	125 (0)	50 (-1)	90 (1)	20 (0)	12.5 (0)	0.21 ± 0.01^{ef}	0.23 ± 0.01^{e}	0.27 ± 0.02^{de}	4.10 ± 0.10^{QR}	5.71 ± 0.19^{KL}	7.36 ± 0.17^{E}
28	125 (0)	150 (1)	60 (0)	10 (-1)	12.5 (0)	0.28 ± 0.02^{de}	0.32 ± 0.02^{d}	0.36 ± 0.03^{cd}	3.84 ± 0.13^{S}	5.45 ± 0.14^{M}	7.10 ± 0.17^{FG}
29	125 (0)	100 (0)	30 (-1)	20 (0)	25 (1)	0.38 ± 0.03^{cd}	0.43 ± 0.05^{bc}	0.55 ± 0.05^{a}	5.76 ± 0.17^{K}	7.42 ± 0.15^{DE}	9.12 ± 0.19^{C}
30	125 (0)	100 (0)	60 (0)	30 (1)	0 (-1)	0.06 ± 0.01^{fg}	0.07 ± 0.01^{fg}	0.10 ± 0.01^{fg}	2.86 ± 0.11^{V}	4.04 ± 0.12^{QR}	5.25 ± 0.13^{N}
31 (C_5)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	0.26 ± 0.03^{de}	0.30 ± 0.03^{d}	0.34 ± 0.05^{d}	3.91 ± 0.14^{R}	5.51 ± 0.10^{LM}	7.11 ± 0.09^{FG}
32	100 (-1)	100 (0)	60 (0)	30 (1)	12.5 (0)	0.26 ± 0.02^{de}	0.29 ± 0.02^{de}	0.33 ± 0.02^{d}	3.79 ± 0.12ST	5.39 ± 0.12^{MN}	7.05 ± 0.16^{G}
33	150 (1)	100 (0)	60 (0)	20 (0)	25 (1)	0.32 ± 0.03^{d}	0.39 ± 0.05^{cd}	0.48 ± 0.04^{b}	5.97 ± 0.18^{I}	7.63 ± 0.11^{CD}	9.33 ± 0.23^{AB}
34	125 (0)	50 (-1)	60 (0)	20 (0)	25 (1)	0.31 ± 0.05^{d}	0.37 ± 0.03^{cd}	0.46 ± 0.04^{b}	5.86 ± 0.13^{J}	7.52 ± 0.16^{D}	9.21 ± 0.22^{BC}
35	100 (-1)	50 (-1)	60 (0)	20 (0)	12.5 (0)	0.25 ± 0.01^{e}	0.29 ± 0.02^{de}	0.34 ± 0.02^{d}	4.16 ± 0.17^{Q}	5.75 ± 0.15^{K}	7.41 ± 0.11^{DE}
36	150 (1)	100 (0)	30 (-1)	20 (0)	12.5 (0)	0.26 ± 0.02^{de}	0.29 ± 0.01^{de}	0.35 ± 0.04^{d}	4.22 ± 0.09^{PQ}	5.82 ± 0.14^{J}	7.48 ± 0.09^{D}
37	150 (1)	100 (0)	60 (0)	10 (-1)	12.5 (0)	0.25 ± 0.02^{e}	0.28 ± 0.01^{de}	0.33 ± 0.02^{d}	4.30 ± 0.10^{P}	5.92 ± 0.18^{IJ}	7.60 ± 0.11^{CD}
38	125 (0)	100 (0)	90 (1)	20 (0)	0 (-1)	0.09 ± 0.01^{fg}	0.10 ± 0.01^{fg}	0.12 ± 0.01^{f}	3.06 ± 0.11^{UV}	4.26 ± 0.09^{PQ}	5.81 ± 0.12^{JK}
39 (C_6)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	0.27 ± 0.01^{de}	0.31 ± 0.02^{d}	0.34 ± 0.03^{d}	3.89 ± 0.10^{RS}	5.50 ± 0.17^{LM}	7.10 ± 0.18^{FG}
40	100 (-1)	100 (0)	60 (0)	20 (0)	25 (1)	0.37 ± 0.02^{cd}	0.42 ± 0.05^{c}	0.54 ± 0.04^{a}	5.92 ± 0.18^{IJ}	7.58 ± 0.09^{CD}	9.28 ± 0.22^{B}
41	125 (0)	150 (1)	90 (1)	20 (0)	12.5 (0)	0.28 ± 0.03^{de}	0.30 ± 0.01^{d}	0.33 ± 0.02^{d}	3.73 ± 0.16ST	5.34 ± 0.13^{N}	6.99 ± 0.12^{GH}
42	150 (1)	50 (-1)	60 (0)	20 (0)	12.5 (0)	0.22 ± 0.01^{ef}	0.25 ± 0.02^{e}	0.29 ± 0.05^{de}	4.41 ± 0.11^{O}	6.01 ± 0.12^{I}	7.67 ± 0.13^{CD}
43	125 (0)	50 (-1)	30 (-1)	20 (0)	12.5 (0)	0.24 ± 0.02^{e}	0.27 ± 0.01^{de}	0.30 ± 0.02^{d}	4.17 ± 0.14^{Q}	5.78 ± 0.14^{JK}	7.43 ± 0.17^{DE}
44	125 (0)	100 (0)	30 (-1)	10 (-1)	12.5 (0)	0.26 ± 0.01^{de}	0.29 ± 0.03^{de}	0.33 ± 0.04^{d}	4.02 ± 0.17^{R}	5.63 ± 0.13^{L}	7.27 ± 0.10^{EF}
45	125 (0)	100 (0)	30 (-1)	30 (1)	12.5 (0)	0.25 ± 0.02^{e}	0.28 ± 0.01^{de}	0.32 ± 0.02^{d}	3.82 ± 0.10^{S}	5.43 ± 0.19^{MN}	7.08 ± 0.19^{FG}
46	125 (0)	50 (-1)	60 (0)	30 (1)	12.5 (0)	0.22 ± 0.01^{ef}	0.24 ± 0.02^{e}	0.28 ± 0.01^{de}	4.04 ± 0.11^{QR}	5.63 ± 0.17^{L}	7.29 ± 0.20^{EF}

C_1, C_2, C_3, C_4, C_5, C_6 are the center points where extrusion processing was applied. Values with same superscript letters ($^{a-fg}$) show the homogenous group within the rows and columns for TBARS values ($p \leq 0.05$). Values with same superscript letters ($^{A-V}$) show the homogenous group within the rows and columns carbonyl contents ($p \leq 0.05$). BET = Barrel Exit Temp; SS = Screw Speed; FFR = Feed Flow Rate; FMC = Feed Moisture Content; FP = Fish Powder; TBARS = Thiobarbituric acid reactive substance; MDA= malondialdehyde.

Table 5. Effect of thermal extrusion processing conditions on the sensory attributes of product at 25 °C during storage intervals.

Extrusion processing run	Independent variables					Sensory attributes					
	Barrel Exit Temp. (BET)	Screw Speed (SS)	Feed Flow Rate (FFR)	Feed Moisture Content (FMC)	Fish Powder Content (FP)	Color		Flavor		Overall acceptability	
						0	60	0	60	0	60
1	150 (1)	150 (1)	60 (0)	20 (0)	12.5 (0)	6.92 ± 0.43^{bc}	6.22 ± 0.39^{fg}	6.33 ± 0.37^{de}	5.73 ± 0.45^{gh}	6.80 ± 0.40^{b}	6.21 ± 0.65^{de}
2	125 (0)	150 (1)	60 (0)	20 (0)	25 (1)	7.30 ± 0.50^{a}	6.59 ± 0.65^{d}	7.04 ± 0.56^{a}	6.54 ± 0.32^{c}	6.44 ± 0.81^{cd}	5.91 ± 0.45^{f}
3 (C_1)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	6.20 ± 0.37^{fg}	5.60 ± 0.48^{i}	6.28 ± 0.45^{de}	5.79 ± 0.60^{gh}	6.84 ± 0.65^{ab}	6.25 ± 0.36^{de}
4	150 (1)	100 (0)	60 (0)	30 (1)	12.5 (0)	6.30 ± 0.39^{ef}	5.66 ± 0.55^{i}	6.25 ± 0.33^{e}	5.71 ± 0.56^{gh}	6.77 ± 0.45^{b}	6.22 ± 0.74^{de}
5	100 (-1)	100 (0)	60 (0)	20 (0)	0 (-1)	5.33 ± 0.31^{jk}	4.91 ± 0.27^{lm}	5.82 ± 025^{g}	5.45 ± 0.35^{i}	6.44 ± 0.33^{cd}	6.07 ± 0.39^{ef}
6	100 (-1)	150 (1)	60 (0)	20 (0)	12.5 (0)	6.73 ± 0.45^{cd}	6.17 ± 0.43^{fg}	6.29 ± 0.47^{de}	5.83 ± 0.55^{g}	6.82 ± 0.70^{ab}	6.37 ± 0.71^{d}
7	125 (0)	150 (1)	30 (-1)	20 (0)	12.5 (0)	6.39 ± 0.43^{ef}	5.88 ± 0.54^{h}	6.46 ± 0.55^{cd}	6.05 ± 0.45^{f}	6.93 ± 0.74^{a}	6.51 ± 0.47^{cd}
8	125 (0)	50 (-1)	60 (0)	10 (-1)	12.5 (0)	6.12 ± 0.38^{fg}	5.41 ± 0.60^{j}	6.21 ± 0.38^{e}	5.62 ± 0.60^{h}	6.72 ± 0.58^{bc}	6.13 ± 0.52^{e}
9	150 (1)	100 (0)	60 (0)	20 (0)	0 (-1)	5.21 ± 0.55^{k}	4.72 ± 0.42^{m}	5.94 ± 0.40^{fg}	5.51 ± 0.45^{hi}	6.43 ± 0.56^{cd}	6.02 ± 0.26^{ef}
10	125 (0)	100 (0)	60 (0)	30 (1)	25 (1)	7.10 ± 0.55^{b}	6.43 ± 0.71^{ef}	7.02 ± 0.62^{a}	6.53 ± 0.80^{c}	6.36 ± 0.66^{d}	5.87 ± 0.45^{fg}
11	125 (0)	100 (0)	90 (1)	10 (-1)	12.5 (0)	6.10 ± 0.38^{fg}	5.47 ± 0.48^{j}	6.32 ± 0.39^{de}	5.79 ± 0.32^{gh}	6.79 ± 0.65^{b}	6.27 ± 0.80^{de}
12 (C_2)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	6.18 ± 0.88^{fg}	5.59 ± 0.41^{ij}	6.34 ± 0.46^{de}	5.87 ± 0.34^{fg}	6.84 ± 0.49^{ab}	6.37 ± 0.96^{d}
13	125 (0)	150 (1)	60 (0)	30 (1)	12.5 (0)	6.88 ± 0.42^{cd}	6.43 ± 0.60^{ef}	6.48 ± 0.50^{cd}	6.13 ± 0.48^{ef}	6.99 ± 0.55^{a}	6.66 ± 0.91^{bc}
14	125 (0)	100 (0)	60 (0)	10 (-1)	25 (1)	6.90 ± 0.53^{c}	6.12 ± 0.55^{fg}	6.83 ± 0.51^{b}	6.24 ± 0.88^{e}	6.29 ± 0.75^{de}	5.71 ± 0.62^{gh}
15	125 (0)	100 (0)	30 (-1)	20 (0)	0 (-1)	5.40 ± 0.96^{j}	4.98 ± 0.33^{l}	5.91 ± 0.96^{fg}	5.53 ± 0.37^{hi}	6.59 ± 0.58^{c}	6.22 ± 0.35^{de}
16	100 (-1)	100 (0)	60 (0)	10 (-1)	12.5 (0)	6.41 ± 0.38^{ef}	5.84 ± 0.37^{h}	6.18 ± 0.36^{ef}	5.72 ± 0.56^{gh}	6.66 ± 0.65^{bc}	6.24 ± 0.72^{de}
17	100 (-1)	100 (0)	30 (-1)	20 (0)	12.5 (0)	6.50 ± 0.91^{e}	5.92 ± 0.57^{gh}	6.23 ± 0.48^{e}	5.83 ± 0.47^{g}	6.70 ± 0.46^{bc}	6.34 ± 0.38^{d}
18	150 (1)	100 (0)	90 (1)	20 (0)	12.5 (0)	6.20 ± 0.39^{fg}	5.53 ± 0.48^{ij}	6.22 ± 0.88^{e}	5.65 ± 0.88^{h}	6.69 ± 0.70^{bc}	6.09 ± 0.27^{ef}
19	125 (0)	50 (-1)	60 (0)	20 (0)	0 (-1)	5.21 ± 0.40^{k}	4.72 ± 0.33^{m}	5.85 ± 0.28^{g}	5.43 ± 0.52^{i}	6.52 ± 0.51^{cd}	6.11 ± 0.74^{ef}
20	125 (0)	100 (0)	60 (0)	10 (-1)	0 (-1)	5.32 ± 0.88^{jk}	4.93 ± 0.27^{l}	5.89 ± 0.88^{fg}	5.54 ± 0.91^{hi}	6.57 ± 0.42^{c}	6.21 ± 0.48^{de}
21	125 (0)	100 (0)	90 (1)	20 (0)	25 (1)	6.98 ± 0.56^{bc}	6.23 ± 0.71^{fg}	6.94 ± 0.67^{ab}	6.39 ± 0.78^{d}	6.36 ± 0.71^{d}	5.78 ± 0.56^{g}
22	125 (0)	150 (1)	60 (0)	20 (0)	0 (-1)	5.42 ± 0.41^{j}	5.01 ± 0.50^{l}	6.06 ± 0.29^{ef}	5.67 ± 0.96^{gh}	6.69 ± 0.49^{bc}	6.29 ± 0.52^{de}
23	125 (0)	100 (0)	90 (1)	30 (1)	12.5 (0)	6.30 ± 0.44^{ef}	5.84 ± 0.39^{h}	6.43 ± 0.36^{d}	6.07 ± 0.60^{ef}	6.89 ± 0.59^{ab}	6.51 ± 0.37^{cd}
24 (C_3)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	6.27 ± 0.39^{f}	5.80 ± 0.56^{hi}	6.31 ± 0.47^{de}	5.96 ± 0.54^{fg}	6.84 ± 0.72^{ab}	6.45 ± 0.84^{cd}
25	100 (-1)	100 (0)	90 (1)	20 (0)	12.5 (0)	6.52 ± 0.96^{de}	6.05 ± 0.37^{g}	6.16 ± 0.88^{ef}	5.80 ± 0.36^{gh}	6.68 ± 0.57^{bc}	6.30 ± 0.36^{de}
26 (C_4)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	6.21 ± 0.34^{fg}	5.54 ± 0.96^{ij}	6.35 ± 0.96^{de}	5.78 ± 0.54^{gh}	6.75 ± 0.62^{b}	6.23 ± 0.69^{de}
27	125 (0)	50 (-1)	90 (1)	20 (0)	12.5 (0)	6.03 ± 0.39^{gh}	5.37 ± 0.36^{jk}	6.24 ± 0.33^{e}	5.69 ± 0.55^{gh}	6.73 ± 0.58^{bc}	6.24 ± 0.58^{de}
28	125 (0)	150 (1)	60 (0)	10 (-1)	12.5 (0)	6.37 ± 0.43^{ef}	5.89 ± 0.55^{h}	6.41 ± 0.39^{d}	6.02 ± 0.51^{f}	6.91 ± 0.65^{ab}	6.51 ± 0.85^{cd}
29	125 (0)	100 (0)	30 (-1)	20 (0)	25 (1)	6.90 ± 0.96^{c}	6.14 ± 0.66^{fg}	6.89 ± 0.66^{ab}	6.33 ± 0.69^{de}	6.33 ± 0.55^{d}	5.78 ± 0.37^{g}
30	125 (0)	100 (0)	60 (0)	30 (1)	0 (-1)	5.47 ± 0.43^{j}	5.09 ± 0.41^{kl}	6.04 ± 0.34^{f}	5.67 ± 0.49^{gh}	6.64 ± 0.60^{bc}	6.27 ± 0.64^{de}
31 (C_5)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	6.33 ± 0.41^{ef}	5.80 ± 0.36^{hi}	6.29 ± 0.47^{de}	5.87 ± 0.96^{fg}	6.83 ± 0.51^{ab}	6.41 ± 0.38^{cd}
32	100 (-1)	100 (0)	60 (0)	30 (1)	12.5 (0)	6.62 ± 0.39^{d}	6.11 ± 0.47^{fg}	6.23 ± 0.37^{e}	5.82 ± 0.54^{g}	6.74 ± 0.88^{bc}	6.33 ± 0.90^{d}
33	150 (1)	100 (0)	60 (0)	20 (0)	25 (1)	6.75 ± 0.63^{cd}	6.08 ± 0.59^{g}	6.79 ± 0.61^{b}	6.31 ± 0.75^{de}	6.21 ± 0.96^{de}	5.72 ± 0.73^{gh}
34	125 (0)	50 (-1)	60 (0)	20 (0)	25 (1)	6.73 ± 0.54^{cd}	5.91 ± 0.65^{gh}	6.87 ± 0.57^{ab}	6.24 ± 0.80^{e}	6.24 ± 0.79^{de}	5.74 ± 0.18^{g}
35	100 (-1)	50 (-1)	60 (0)	20 (0)	12.5 (0)	6.30 ± 0.96^{ef}	5.75 ± 0.96^{hi}	6.12 ± 0.33^{ef}	5.67 ± 0.37^{gh}	6.61 ± 0.70^{bc}	6.16 ± 0.88^{e}
36	150 (1)	100 (0)	30 (-1)	20 (0)	12.5 (0)	6.22 ± 0.46^{fg}	5.56 ± 0.43^{ij}	6.20 ± 0.45^{ef}	5.64 ± 0.39^{h}	6.72 ± 0.47^{bc}	6.17 ± 0.58^{e}
37	150 (1)	100 (0)	60 (0)	10 (-1)	12.5 (0)	6.11 ± 0.40^{fg}	5.49 ± 0.39^{ij}	6.14 ± 0.50^{ef}	5.61 ± 0.51^{h}	6.64 ± 0.63^{bc}	6.18 ± 0.61^{e}
38	125 (0)	100 (0)	90 (1)	20 (0)	0 (-1)	5.33 ± 0.36^{jk}	4.99 ± 0.47^{l}	5.82 ± 0.37^{g}	5.53 ± 0.36^{hi}	6.61 ± 0.54^{bc}	6.32 ± 0.49^{de}
39 (C_6)	125 (0)	100 (0)	60 (0)	20 (0)	12.5 (0)	6.07 ± 0.38^{g}	5.46 ± 0.51^{j}	6.35 ± 0.91^{de}	5.85 ± 0.50^{g}	6.87 ± 0.69^{ab}	6.32 ± 0.83^{de}
40	100 (-1)	100 (0)	60 (0)	20 (0)	25 (1)	6.81 ± 0.88^{cd}	6.11 ± 0.91^{fg}	6.82 ± 0.67^{b}	6.31 ± 0.67^{de}	6.21 ± 0.60^{de}	5.73 ± 0.46^{g}
41	125 (0)	150 (1)	90 (1)	20 (0)	12.5 (0)	6.47 ± 0.45^{e}	5.96 ± 0.50^{gh}	6.46 ± 0.51^{cd}	6.05 ± 0.44^{f}	6.94 ± 0.67^{a}	6.50 ± 0.74^{cd}
42	150 (1)	50 (-1)	60 (0)	20 (0)	12.5 (0)	6.42 ± 0.37^{ef}	5.81 ± 0.54^{hi}	6.11 ± 0.35^{ef}	5.59 ± 0.88^{hi}	6.59 ± 0.61^{c}	6.06 ± 0.39^{ef}
43	125 (0)	50 (-1)	30 (-1)	20 (0)	12.5 (0)	6.02 ± 0.91^{gh}	5.45 ± 0.38^{j}	6.27 ± 0.44^{de}	5.81 ± 0.49^{g}	6.72 ± 0.57^{bc}	6.23 ± 0.62^{de}
44	125 (0)	100 (0)	30 (-1)	10 (-1)	12.5 (0)	6.14 ± 0.88^{fg}	5.62 ± 0.44^{i}	6.23 ± 0.32^{e}	5.82 ± 0.51^{g}	6.79 ± 0.52^{b}	6.36 ± 0.38^{d}
45	125 (0)	100 (0)	30 (-1)	30 (1)	12.5 (0)	6.35 ± 0.44^{ef}	5.90 ± 0.52^{gh}	6.36 ± 0.48^{de}	6.02 ± 0.55^{f}	6.92 ± 0.71^{a}	6.57 ± 0.88^{c}
46	125 (0)	50 (-1)	60 (0)	30 (1)	12.5 (0)	6.13 ± 0.37^{fg}	5.54 ± 0.54^{ij}	6.31 ± 0.88^{de}	5.83 ± 0.60^{g}	6.80 ± 0.66^{b}	6.31 ± 0.91^{de}

C_1, C_2, C_3, C_4, C_5, C_6 are the center points where extrusion processing was applied. Different superscripts letters indicate the significance ($p \leq 0.05$) within for color, flavor and overall acceptability. BET = Barrel Exit Temp; SS = Screw Speed; FFR = Feed Flow Rate; FMC = Feed Moisture Content; FP = Fish Powder.

that storage conditions may influence the levels of fat and the same results are noticed in this study. With the change in BET, SS and FMC also change the levels of fat. The high temperature not only inactivates the lipase enzymes but also facilities the binding of lipids with carbohydrates contents that ultimately affect the oxidative stability of lipid in extruded products (Thachil et al., 2014).

4 Conclusion

The moderate BET (125 °C) with high SS (150 rpm), moderate FMC (20%), and high FP (25%) facilitate the highest retention of PUFAs and MUFAs in end products. The Highest PUFAs retention for short time storage takes place in product extruded at moderate BET with moderate SS high FFR and moderate FP. Moderate conditions have been recommended as the best option for good quality products with maximum retention of long-chain PUFAs and MUFAs and storage stability. Moreover, results from this work will aid in the formulation of healthier food products supplemented with fish dried powder and may address a critical industrial demand in terms of formulation options. Further studies can be conducted to determine the maximal shelf life of products supplemented with fish PUFAs along with protein for value addition and treatment of nutritional disorders through their absorption, metabolism, and distribution pattern into biological tissues.

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