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Effect of Extrusion Processing Variables on Finger Millet Flours with Respect to Their Functional Properties Using Response Surface Methodology

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Authors' contributions

This work was carried out in collaboration between all authors. This work has been done by author SN as part of M. Tech thesis work under the guidance of author SGR and she is also responsible for preparation of manuscript. Author EV assisted in statistical analysis and provided technical guidance in designing experiments. All authors read and approved the final manuscript.

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ABSTRACT

Finger millet is fiber rich nutritious food which can be used by diabetic patients and also by healthy persons to maintain body weight owing to their inherent good amount of resistant starch and dietary fiber. Various degrees of structural transformations in the starches can be obtained through wide range of degree of cooking possible through extrusion processing. Response surface methodology was used to study the effect of amylose content (19.42%, 24.19%, 25.74%), moisture (21, 23 and 25%), screw speed (250, 300, 350 rpm) and barrel temperature (160,180 200°C) and their interactive effect on the extrudate properties. Percentage amylose in the finger millet flours was found to have significant effect on the bulk density, sectional expansion index and resistant starch of

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the extrudates. Expansion of extrudates was negatively correlated to amylose (p=0.022). Barrel temperature was found to be most prominent for modification of RS in finger millet extrudates. Higher amylose content lead to formation of more resistant starch in extrudates of finger millet. The effect of screw speed and barrel temperature was mostly seen on the water absorption index and water solubility index. Quadratic regression model ($R^2 > 0.68$) was found appropriate to model the physico-chemical parameters of the exrudates as function of extruder and raw material properties.

Keywords: Extrusion; finger millet; resistant starch; response surface methodology.

1. INTRODUCTION

In recent years, millets have gained importance because of their nutritional strength in terms of dietary and functional fiber, and also due to reportedly significant lower glycemic index resulting with consumption of millet based diet [1]. The functionality of starch in millets is comparable to other cereals. The higher proportion of non-starchy polysaccharides, dietary fiber and low glycemic index render millets as ideal ingredients in many food formulations meant for specific target groups. Dietary starch is good source of energy though its nutritive value is linked with its digestibility in small intestine. The nutritive value of starch is dependent on the content of resistant starch (RS). Resistant starch is defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy humans [2,3]. At present, RS may be classified into four groups (RS1-RS4) based on their hysical and chemical characteristics. RS1 corresponds to physically inaccessible starches trapped in a cellular matrix, as in legume seeds; RS2 comprises native uncooked granules of starch, such as raw potato or banana starches, whose crystallinity makes them less susceptible to hydrolysis; RS3 consists of retrograded starches, which may be formed in cooked foods that are kept room temperature and RS4 represents chemically modified starches.The presence of RS has shown to exert positive effect on a human body, it stimulates the beneficial micro flora, reduces colonic pH, post prandial blood level of glucose and blood level of cholesterol [4]. Considering their health benefits, products containig high levels of RS might well qualify as fnctional foods [5].

One way of manufacturing functional food is thermomechanical process called as Extrusion [6]. The technology gives us the cost effective, time saving and versatile method of manufacturing of functional foods. The extrusion process consists of converting a non-cohesive granular material (grits and flour), composed of biopolymers (starch, proteins) into a re-structured solid in one operation. This thermomechanical cooking involves the conversion of solid material in a viscoelastic fluid or “melt”. That is, the transport mechanism through the extruder changes along the screw from solid flow to fluid flow. As a consequence of the pressure built up during fluid flow, high shear stresses are developed, which cause structural transformations in the material.

A number of factors can affect the rate of starch hydrolysis, such as amylose ⁄ amylopectin ratio, the type and arrangement of the crystal structures, the average molecular weights of the components, food particle size, amylose-lipid complexes, existence of other materials in the food matrix (sugar, protein, etc.) and enzyme inhibitors [7,8,9]. RS content has been found to positively correlate with amylose content in cereal crops. Author [10] have reported an increase in the RS fraction during extrusion processing of mango starch depending on the inherent level of amylose fraction. Processing raw food materials in most cases destroys RS1 and RS2, but it can produce RS3. Reason being RS formed during processing is associated with amylose retrogradation [11]. Depending on the conditions, processing of cereal grains may cause an increase or a decrease in the RS content. Most of the studies on RS formation have been done on pure starch systems and few on flours of rice, wheat, barley and peas but millet remain untouched despite of their known importance.

Present work was undertaken with an objective to study the effect of extrusion parameters such as moisture content, variation in amylose, temperature and screw speed on resistant starch formation.

2. MATERIALS AND METHODS

2.1 Sample Preparation

To elucidate the effect of amylose content on their extrudates quality especially with respect to resistant starch content for fingermillet, PR 202 as low amylose, GPU 48 as medium amylose variant and HR 911 as higher amylose variety were chosen for conducting extrusion studies. Extrusion cooking of finger millet was performed using a heavy-duty twin screw extruder (DSE 25, Brabender, Germany). The barrel was enrobed with cold/ tap water circulation to maintain the temperature. The extruder barrel was fitted with 5.0 mm die nozzle. After initial laboratory trials, extrudates were prepared keeping constant feed rate of 50 r.p.m. The barrel temperatures were varied from 160 to 200°C in the last heating zone.

2.2 Amylose Determination

The iodine is absorbed within the helical coils of amylose to produce a blue colored complex. 100mg of powdered sample was weighed, to this sample 1ml of ethanol and 10 ml of 1N NaOH was added. This sample was kept overnight and volume made up to 100 ml. Approximately 2.5 ml of extract was taken in conical flask and 20 ml water and 2-3 drops phenolphthalein indicator was added. To this 0.1N HCl was added drop by drop until pink color just disappears. 1 ml of iodine reagent was added and volume was made up to 50 ml in volumetric flask. Absorbance was measured at 590 nm. Amylose was calculated by using standard curve prepared from amylose against blank (1 ml of iodine reagent in 50 ml water) [12]. Total starch was determined according to Anthrone method spectrophotometrically by measuring absorbance at 630 nm. Amylose content was determined by iodometric titration method [13] by measuring absorbance at 590 nm using pure amylose (Merck, India) as standard.

2.3 Density

The density (ρ_e) of extrudate was determined by rapeseed displacement method. A measuring cylinder of capacity 250 ml was used. Rapeseed was filled into the measuring cylinder up to volume (V). The container was then emptied and to this a weighed amount of product, M_1 (g) was placed inside the cylinder. The remaining space was filled with the same amount of rapeseed which was earlier emptied from the cylinder. The rapeseed now fills up to the mark, V_1 (mL). Thus, the volume of the extrudate is the difference between V and V_1, say V_e (mL). The density of the extrudate was therefore defined as:

$$\rho_e(kg/m^3) = \frac{M_1}{V_e} \times 10^{-3} \tag{1}$$

2.4 Sectional Expansion Index (SEI)

SEI was determined by dividing the cross sectional area (mean of five measurements) of the extrudates by the cross-sectional area of the die nozzle [14]. The die had a diameter of 5 mm.

Water absorption index (WAI) and Water solubility index (WSI) was determined by the method of Anderson [15]. The ground extrudate was suspended in water at room temperature (30°C) in tared test tubes, gently stirred intermitently for 30 mins and then centrifuged at 3000 g for 15 min. (Sigma, U.K.). The supernatant was decanted into an evaporating dish of known weight. The WSI is the weight of dry solids in the supernatant expressed as a percentage of the original weight of sample. The WAI is the weight of gel obtained after removal of the supernatant per unit weight of original dry solids. WAI and WSI were calculated using the formulae:

$$WSI(\%) = \frac{Total\ soluble\ solids}{weight\ of\ dry\ sample} \times 100 \tag{2}$$

$$WAI(\%) = \frac{Weight\ of\ swollen\ gel}{weight\ of\ dry\ sample - total\ soluble\ solids} \tag{3}$$

2.5 Resistant Starch

Resistant starch was determined using the resistant starch assay kit (Megazyme, Ireland). Amyloglucosidase and pancreatic amylase were used to digest the finely ground extrudates for 16 h at 37°C, non-resistant starch is solubilized and hydrolysed to D-glucose. The reaction is terminated by ethanol, and resistant starch centrifuged and washed repeatedly to remove glucose. This is followed by hydrolysis of resistant starch by KOH (2M) and its hydrolysis by amyloglucosidase. D-glucose is then measured using glucose oxidase-peroxidase enzymes by measuring absorbance at 510 nm (Varian Cary 50).

2.6 Statistical Analysis

Response surface methodology was used to evaluate the simultaneous effect of moisture (%), barrel temperature (°C), amylose content (%) and screw speed (min^{-1}) on the extrudate properties and resistant starch from fingermillet varieties PR 202 as low amylose, GPU 48 as medium amylose variant and HR 911 as high amylose variety. The experimental plan was based on three level four factor Box Behnken design (MINI TAB version 11). Face centered cubic design was selected and the value of alpha was kept at 1.00. The design included three replicates at the center points. All experiments were done in random order in triplicates and the average values were used for the regression analysis. The effect of extrusion process variables on the extrudate properties and their resistant starch was then analyzed by regression analysis through MINI TAB version 11 software. The quadratic polynominal regression equation including the effects of the linear, the quadratic and the interaction of the four variables, amylose content (X_1), moisture content (X_2), barrel temperature (X_3) and screw speed (X_4) on the response value (Y) was given as

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_{ii}^2 + \sum\sum \beta_{ij} X_i X_j + e_i \tag{4}$$

where Y is the response variable; β_o is the constant coefficient (intercept); β_i is the linear coefficient (main effect); β_{ii} is the quadratic coefficient; β_{ij} is the two factors interaction coefficient and e_i is the random error. The numerical optimization was carried out with the help of Design expert version 7.1.2 to visualize the effect of interaction between the factors.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties of the Flours

The starch content of the finger millet cultivars ranged from 67 to 84% and the amylose content was between 19.43 and 25.5% Prior to conducting experiments, the screw speed, barrel temperature, die head, feed rate were standardized to obtain extrudates acceptable in terms of crunchiness and crispness studied by texture profile analysis.

3.2 Bulk Density

The bulk density of the extrudates varied from 88.56 kg/ m^3 to 126.04 kg/ m^3. Amylose content of the flours affected the bulk density of the extrudates most significantly (Table 1). These findings are in concurrence to most researchers [16,17,18,19]. Moisture content of the finger millet flours had a decreasing effect on the bulk density. This may be attributed to higher moisture content in the feed coming out of the extruder die head, resulting in higher pressure differential leading to higher expansion and thus lesser bulk density. Barrel temperature had a negative correlation to bulk density at temperatures higher than 180°C. It may be assumed that at temperatures below 180°C, starch in the flours did not reach its melting point and therefore its structural transformation might not have taken place. Yu [20] have also reported direct relation between bulk density and moisture content. Second order polynomial equation was fit to the experimental data of bulk density, which gave high value of $R^2 > 92\%$. Quadratic effects of amylose and barrel temperature are significant. While interaction between moisture level and barrel temperature have significant effect on bulk density of the extrudates ($p=0.023$). Analysis of variance (Table 2) shows level of significance for the effect of linear, quadratic and interaction for bulk density.

3.3 Sectional Expansion Index (SEI)

SEI varied in the extrudates from 1.4 to 2.18. The effect of amylose content, moisture, barrel temperature and screw speed on SEI is presented in Table 1. The most significant effect observed was of amylose content (p=0.000). SEI decreased with increase in amylose content. This may be explained due to fact that higher amylose content leads to higher viscosity during cooling of the extrudates i.e, once the feed material comes out of the extruder die head. Higher viscosity during this phase impedes increase in pores due to water evaporation. The effect of barrel temperature and screw speed though negative was not significant. This is consistent with published studies that low moisture favours lateral and transverse expansion because of high melt viscosity, but melt elasticity reduces with low moisture to adversely affect expansion [21].

Table 2 indicates the level of significance of the effect for linear, quadratic and interaction terms on SEI.

3.4 Water Absorption Index (WAI) and Water Solubility Index (WSI)

WAI of finger millet extrudates ranged from 6.95 to 12.158%. As expected the most prominent effect on WAI was of screw speed and barrel temperature. The interactive effect of amylose and moisture on WAI is also significant (Table 3). High screw speed or increase in temperature led to higher mechanical energy being transmitted to the feed ingredients leading to greater structural transformation or gelatinization and therefore higher WSI.

Though R^2 for the regression model is low, these results indicate the major tools to modify WAI and WSI during extrusion processing. Table 4 indicates the level of significance of the effect for linear, quadratic and interaction terms on WSI. WSI gives an indication of the degree of starch conversion and molecular degradation possibly due to melting and degradation of amylopectin crystals into dextrins and soluble polysaccharides [22,23]. WSI was not found to be significantly affected by the four variables tested (Table 3). WSI varied from 7.5 to 35.5 %. This shows increase in WSI with temperature and screw speed. High screw speed or increase in temperature lead to higher mechanical energy being transmitted to the feed ingredients leading to greater structural transformation or gelatinization and therefore higher WSI. Table 4 indicates the level of significance of the effect for linear, quadratic and interaction terms on WSI.

Table 1. Regression coefficients of bulk density and section expansion index of the finger millet extrudates

Terms	Bulk density		Expansion ratio	
	Coefficient	p-value	Coefficient	p-value
Constant (a)	104.98	0.000	2.09	0.000
X_1 (β_1)	3.83	0.022	-0.27	0.000
X_2 (β_2)	-1.17	0.436	-0.01	0.858
X_3 (β_3)	2.24	0.148	-0.01	0.869
X_4 (β_4)	1.85	0.225	0.02	0.598
X_1X_1 (β_{11})	6.59	0.010	-0.03	0.000
X_2X_2 (β_{22})	6.03	0.017	-0.01	0.864
X_3X_3 (β_{33})	-16.32	0.000	-0.03	0.620
X_4X_4 (β_{44})	3.77	0.108	0.09	0.134
X_1X_2 (β_{12})	0.20	0.938	0.03	0.714
X_1X_3 (β_{13})	-0.88	0.732	-0.51	0.468
X_1X_4 (β_{14})	-3.14	0.235	0.09	0.193
X_2X_3 (β_{23})	6.54	0.023	-0.02	0.747
X_2X_4 (β_{24})	-4.28	0.114	-0.02	0.799
X_3X_4 (β_{34})	3.12	0.238	0.00	0.998
R^2	92.1%		88%	
S	5.019		0.1359	

Where, X_1 is amylose (19.42%, 24.19%, 25.74%); X_2 is Moisture (2%, 3% and 4%), X_3 is barrel temperature (160, 180 and 200°C) and X_4 is screw speed (250, 300 and 350 rpm)

Table 2. ANOVA of bulk density and section expansion index of the finger millet extrudates

Source	Bulk density			SEI		
	DF	F	P	DF	F	P
Linear	4	2.91	0.067	4	11.85	0.000
Square	4	28.6	0.000	4	9.53	0.001
Interaction	6	2.16	0.121	6	0.46	0.822
Total	26			26		

Table 3. Regression coefficients of WAI and WSI of the finger millet extrudates

Terms	WAI		WSI	
	Coefficient	p-value	Coefficient	p-value
Constant (a)	9.618	0.000	18.32	0.000
X_1 (β_1)	0.07	0.859	0.31	0.858
X_2 (β_2)	0.09	0.830	-0.70	0.690
X_3 (β_3)	0.61	0.162	-2.35	0.196
X_4 (β_4)	0.48	0.268	-0.71	0.687
X_1X_1 (β_{11})	0.21	0.743	3.06	0.257
X_2X_2 (β_{22})	0.46	0.473	3.15	0.245
X_3X_3 (β_{33})	-0.38	0.546	3.43	0.208
X_4X_4 (β_{44})	0.77	0.232	3.72	0.174
X_1X_2 (β_{12})	0.47	0.520	0.50	0.869
X_1X_3 (β_{13})	-2.24	0.080	-5.00	0.118
X_1X_4 (β_{14})	0.12	0.868	-1.06	0.727
X_2X_3 (β_{23})	-1.13	0.137	-1.02	0.736
X_2X_4 (β_{24})	1.18	0.120	-2.00	0.514
X_3X_4 (β_{34})	1.01	0.178	10.44	0.004
R^2	67.3		64.3	
S	1.416		5.942	

where, X_1 is amylose (19.42%, 24.19%, 25.74%); X_2 is Moisture (2%, 3% and 4%), X_3 is barrel temperature (160, 180 and 200°C) and X_4 is screw speed (250, 300 and 350 rpm)

Table 4. ANOVA of WAI and WSI of the finger millet extrudates

Source	WAI			WSI		
	DF	F	P	DF	F	P
Linear	4	0.91	0.487	4	0.56	0.696
Square	4	0.8	0.546	4	0.86	0.517
Interaction	6	2.97	0.051	6	2.65	0.071
Total	26			26		

Table 5. Regression coefficients for resistant starch in finger millet extrudates

Terms	Resistant starch	
	Coefficient	p-value
Constant (a)	-595.3	0.046
X_1 (β_1)	38.4	0.191
X_2 (β_2)	38.5	0.218
X_3 (β_3)	4.8	0.039
X_4 (β_4)	0.8	0.265
X_1X_1 (β_{11})	0.2	0.932
X_2X_2 (β_{22})	1.4	0.514
X_3X_3 (β_{33})	0.0	0.084
X_4X_4 (β_{44})	0.0	0.699
X_1X_2 (β_{12})	-5.1	0.059
X_1X_3 (β_{13})	-0.1	0.464
X_1X_4 (β_{14})	0.0	0.725
X_2X_3 (β_{23})	-0.2	0.192
X_2X_4 (β_{24})	-0.1	0.208
X_3X_4 (β_{34})	0.0	0.389
R^2	64.3	
S	4.943	

Where, X_1 is amylose (19.42%, 24.19%, 25.74%); X_2 is Moisture (2%, 3% and 4%), X_3 is barrel temperature (160, 180 and 200°C) and X_4 is screw speed (250, 300 and 350 rpm)

3.5 Resistant Starch

Though researcher [24] reported very low content of resistant starch (0.01%), processing can lead to either increase in digestibility owing to gelatinization or decrease due to subsequent retrogradation during cooling. Fig. 1 indicate the role of temperature as most prominent for modification of RS in finger millet extrudates. Increasing temperature increase the amount of RS. Amylose content and moisture content had a negative interactive effect for formation of RS in extrudates (Table 6). This is in concurrence to the findings of several researchers [25,26] who have shown strong correlation of amylose with the RS content (R^2 values of 0.98 – 0.99).

Effect of different extrusion variables on resistant starch can be seen in fig 1, where the moisture content of flours has significant effect on formation resistant starch and screw speed has little effect on formation. It is beacuase of action of moisture as plasticizer for retrogradation. Thus with increase in flour moisture content resistant starch content of extruded products incresed.

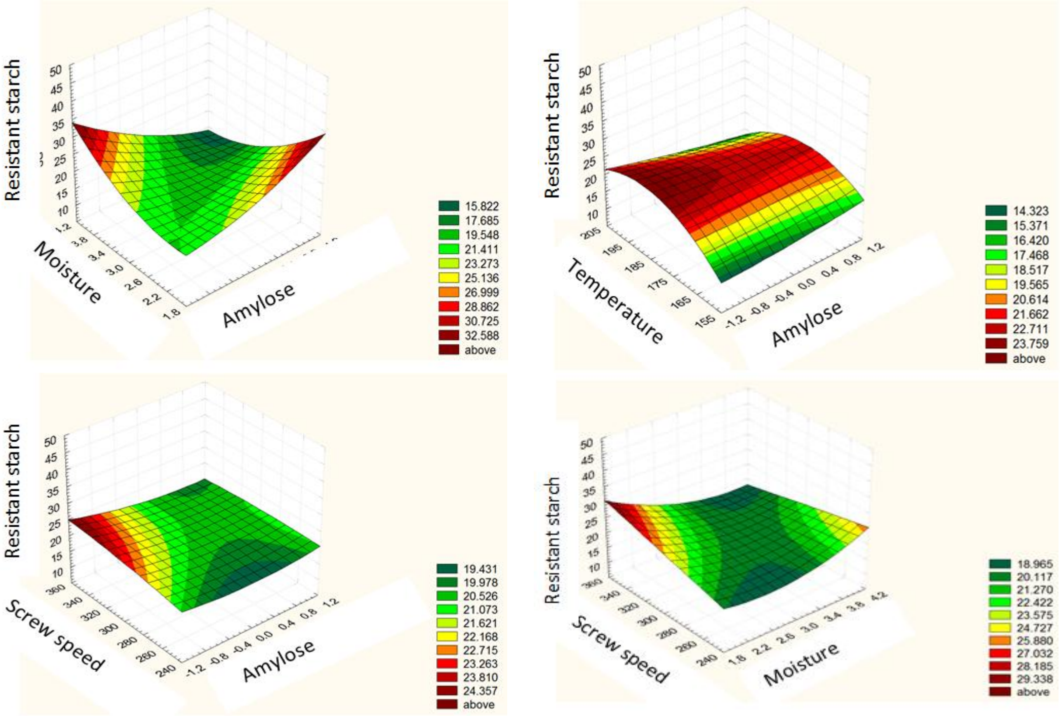

Fig. 1. RSM of effect of extrusion processing variables and amylose content on the resistant starch content of the extrudates

This is in agreement with the kim [27]. Low level of resistant starch at low temeprature suggests, that at this temperature, although crystallites forming the structure of RS in the native flour is torn apart, no new compensating or additional crystallites are formed between amylose chains since the extrusion temperature is probably below the threshold for starch fragmentation. But decrease in resistant starch at higher temperature suggests that melting of crystallites that form the structure [11].

Screw speed has little effect on RS, however it reduces the resistant starch with increase in shearing action of screw. It's because of degradation of amylose into molecules smaller degree of polymerization (<26) that could not be incorporated in crystalline structure and results in decrease in RS [28,29]. Amylose content is generally an important factor that increases RS production during extrusion cooking. At high moisture content, RS may be formed and increased at high amylose level, probably by a retrogradation tendency with the formation of strong intermolecular hydrogen bonds in the amylose fraction. Strong correlation between the amylose and RS can be found in this study.

Table 6. ANOVA of WAI and WSI of the finger millet extrudates

Source	DF	F	P
Linear	4	1.94	0.168
Square	4	1.42	0.287
Interaction	6	1.59	0.234
Total	26		

4. CONCLUSION

A physical property of extruded products depends greatly on the extrusion parameters. Moisture content, barrel temperature and amylose content had the significant effect on bulk density, WAI, WSI. The amylose content had the profound effect on the formation of resistant starch. Thus we can infer that the higher amylose variety is more suitable to produce foods meant for dietetic purposes. Higher degree of processing through increase in moisture and temperature cause increase in RS higher amylose varieties but the screw speed had little effect on RS formation. However further studies on more varieties can consolidate these findings on a sound footing.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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