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Research Paper

Explore the possibility of conventional and non-conventional flours for the development of gluten-free multigrain pasta

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ABSTRACT

The aim of this study was to evaluate mixture of gluten free conventional (rice and maize) and non-conventional flour (waterchestnut, sorghum, pearl millet, pigeon pea) and flaxseed for the development of multigrain gluten-free pasta. To achieve better association between different blends twin-screw extruder (feed moisture 30 & 34% and barrel temperature 90 & 105°C) and 2% gaur gum was used for pasta preparation. Pasta prepared using 50% rice (blend I) exhibited the highest degree of starch gelatinization. The majority of samples exhibited acceptable water absorption (112-124%) and volume expansion (0.90 -1.83mg/l). The gruel solid loss ranged from 6.68 to 13.35%, owing to the meager structuring capacity of the non-gluten flours. However, higher barrel temperatures inversely affected the gruel solid loss. Luminosity, redness, and yellowness were higher for samples containing 50% rice (blend I). Pasting and textural properties are enhanced with feed moisture and barrel temperature. Higher feed moisture possessed dominating effect on the bioactive composition of pasta. Barrel temperature depicted a higher correlation (r=0.798) for protein digestibility whereas feed moisture (r=0.854) for starch digestibility. Highest in vitro starch digestibility (36.01g/100g) and protein digestibility (85.54 g/100g) was obtained for pasta with 50% water chestnut (blend II). Highest sensory score pasta was obtained for the pasta produced using rice (50%), pearl millet (15%), pigeon pea (15%), maize and sorghum (10% each) and extruded at feed moisture (30%) and barrel temperature (90ºC) scored highest and was well accepted.

Key words: Bioactive composition, cooking quality, digestibility, gluten free, multigrain

Pasta products, to a great extent devoured everywhere throughout the world are customarily made from durum wheat semolina, known to be the best crude material for pasta production (Nilusha *et al.*, 2019). However, the growing promoting quality of the healthy convenience foods like pasta has led researchers to enhance pasta quality in terms of nutrition and ease of preparation. Augmentations of conventional and non-conventional raw material for pasta preparation represent an intriguing source of proteins, fibres, vitamins and minerals

Customer demands for functional and nourished foods. Presently a-days sustenance is not expended to fulfill hunger but rather additionally to counteract nourishment related sicknesses and improve physical and mental prosperity of customers (Betoret *et al*., 2011). Accumulated data on the human health benefits of minor food intake dependent on cereals and pseudocereals has resulted in an intensified emphasis on the phytochemical content of various cereal grains. The researchers are therefore focused on investigating ways to boost the overall nutritional balance of foods rich in carbohydrates. Subsequently, the shifted towards the concept of utilizing multigrains came into existence.

Another important concern of today's market is the concept of gluten free products. With the prevalence of fact that some people with a specific genetic nature suffer from celiac disease (or non-tropical sprue) upon consumption of food containing gluten like wheat, rye, oats or barley. In particular, celiac disease is a chronic small intestine malabsorption condition triggered by gluten exposure in a genetically predisposed person characterized by a strong immune response to wheat gliadin fraction and rye prolamins (secalins), barley (hordeins), and probably oats (avidins).

In this regard, functional foods that include multigrain and are gluten-free provide an excellent opportunity to increase product quality. Early functional foods were characterized by fortification with vitamins and/or minerals. The f1ood industry has explored ways of enhancing the overall nutritional balance of foods rich in carbohydrates and has focused on increasing their dietary fiber (DF) content at the cost of carbohydrates that are readily digestible. Subsequently, the focus has shifted towards the concept of utilizing multigrain's. for the production of convenience foods .

The main objective of this research was to explore the different levels of important gluten-free flours for the preparation of pasta and to study effect of extrusion variables on quality characteristics and digestibility of pasta.

MATERIALS AND METHODS

Proximate composition of raw materials

Raw Material: Commercial varieties of rice, waterchestnut, maize, sorghum, pigeon pea and flaxseed was used for the study. Pearl millet was procured from Director Seeds, Punjab Agricultural University, Ludhiana, India. Cyclotec mill was used to produce flour from rice, waterchestnut, maize, sorghum, pearl millet and pigeon pea sifted to pass through a 60 mesh sieve (BIS) which corresponds to the particle size of 250µm.

Proximate analysis: The moisture content, ash, protein, fat, and dietary fibre were analysed according to methods by American Association of Cereal Chemists. Crude fibre was estimated using Fibertec (Foss Company, Hillerod, Denmark).

Extrusion: On a co-rotating intermeshing twin screw extruder model BCH, extrusion experiments were carried out on (Clextral, Firminy, France). The diameter of the barrel was 2.5 mm and its length to diameter ratio (L/D) was 16:1. Throughout the experiment, the temperatures of the first, second and third zones were maintained at 30, 40 and 50 ° C, respectively, while the temperature in the fourth zone ranged from 70°C to 110°C. The extruder was powered by a motor of 8.5 kW, with variable speeds from 0 to 682 rpm. The extruder was fitted with a torque indicator, which displays the percentage of torque that the drive motor draws in relation to the current.

Experimental design: Rice, waterchestnut, maize, sorghum, pearl millet, pigeon pea and flaxseed were blended in variable proportions to obtain multigrain blends. Different combinations used for the study are as under:

Blends	Composition (%)							
	Rice	Water chestnut	Pearl Millet	Pigeon Pea	Maize	Sorghum	Flaxseed (%)	Guar Gum (%)
Combination(CI)	50	0	15	15	10	10	2	2
Combination(CII)	0	50	15	15	10	10	2	2
Combination (CIII)	25	25	15	15	10	10	2	2

Degree of starch gelatinization (DSG): Degree of gelatinization of prepared pasta was determined using colorimetric method (Cai and Diosady, 1993)

Cooking characteristics: The pasta cooking quality was determined by minimum cooking time, water absorption, expansion of volume and solid loss of gruel as per method of American Association of Cereal Chemists.

Amylose leaching: The content of amylose in cooking water was analysed according to the method defined by Matsuo *et al.,* (1992).

Pasting properties: Using the Rapid Visco Analyzer (RVA) model starch Master (Newport Scientific, Warrie Wood, Australia), the pasting properties of processed pasta were determined using the method developed by Rafiq *et al.,* (2016).

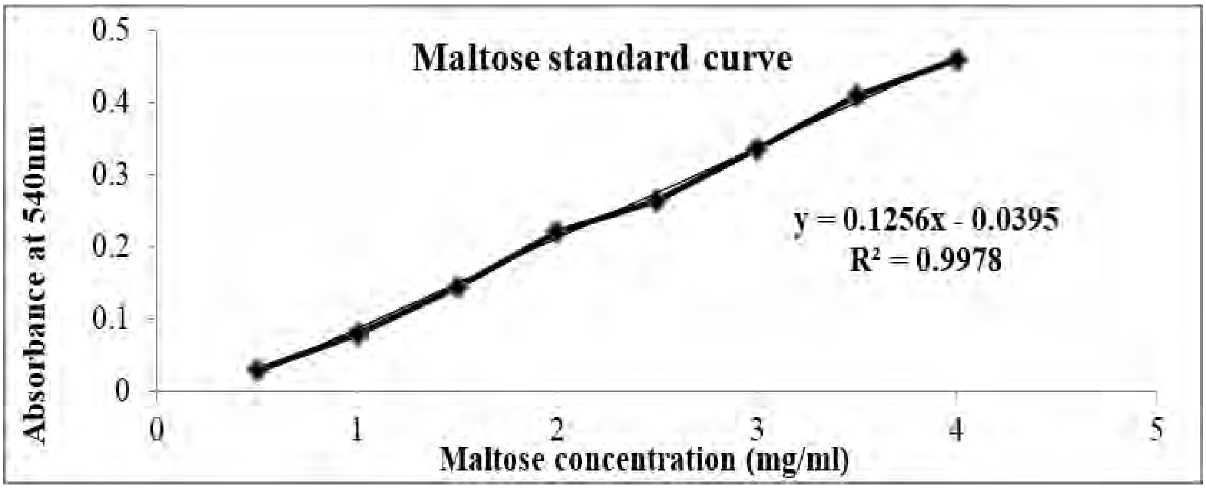
Color: The color of dried pasta samples were measured using a color meter, CR-300 (Minolta Camera Co. Ltd., Japan). Results are expressed in the CIE L*a*b* (CIE, 1986). The value of hue angle (h*) and chroma (C) for each sample was calculated using the following equations.

$$h^* = arctan\left(\frac{b^*}{a^*}\right)$$

$$C = \sqrt{a^2 + b^2}$$

Texture profile analysis: Textural characteristics of the cooked pasta were determined by using modified method prepared by Rafiq et al., (2016).

***In vitro* starch digestibility:** *In vitro* starch digestibility of pasta was determined according to the method of Onyango *et al,* (2004a).



Standard curve of maltose at 540nm

***In vitro* protein digestibility:** *In vitro* protein digestibility of prepared pasta was determined by following Akeson and Stahmann method (1964).

Bioactive compositions: Bioactive compositions were determined by standard protocol. Total phenolic contents was estimated using Folin-Ciocalteu reagent and were expressed as mg gallic acid equivalent (GAE)/100 g dry weight, antioxidant activity by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging inhibition. The total flavonoid content was measured using the Aluminium chloride colorimetric method.

Sensory quality: All product samples were coded and evaluated for degree of liking or disliking on a 9-point hedonic scale, using descriptive categories.

RESULTS AND DISCUSSION

Proximate analysis of raw materials: The nutritional composition of cereals flour (semolina, milled rice, maize, sorghum, pearl millet), legume (pigeon pea), waterchestnut and flaxseed was expressed on 14 % moisture basis. Significant ($p<0.05$) difference in all the nutritional parameters of raw material was observed. Among cereals highest crude protein content was observed in pearl millet (9.93%) while lowest in maize (5.47%). The fat content of cereal flours ranged between 0.73-5.22%, being maximum in pearl millet and minimum in milled rice (0.73%). The crude fibre content of cereals also varied significantly. Maximum crude fibre content was observed in the pearl millet flour (2.69%) and minimum in rice (0.94%).The proximate composition of whole grain cereals studied was in agreement with the studies of other researchers who used these whole grains as raw material.

Pigeon pea was found to contain 20.5% crude protein, 2.78% fat, 3.7% crude fiber, 3.63% ash and carbohydrate 55.39% whereas waterchestnut contained 4.2% crude protein, 1.02% fat, 0.78% crude fiber, 0.65% ash and carbohydrate 79.35%. Similar results were reported by Sacchetti et al., (2004) for water chestnut and Oshodi et al., (1993) for pigeon pea. The proximate composition of flaxseed revealed 19% crude protein, 33.63% crude fat, 4.18% crude fiber, 3.05% ash and 26.14% carbohydrate.

Degree of starch gelatinization of multigrain pasta: Marchant and Blanshard, (1978) based on non-equilibrium thermodynamics, postulated that three constituent processes for starch gelatinization are: (1) water diffusion into the starch granules, (2) a helix-coil transition facilitated by hydration that is a melting process, and (3) swelling as a result of crystallite disintegration (melting).Degree of gelatinization of multigrain pasta by amylose- iodine complexing varied significantly with blend composition (Fig.1). Pasta containing 50% rice i.e blend CI, the degree of starch gelatinization varied between 67.18 to 94.61% with respect to processing variables. However, the higher feed moisture and temperature favoured starch gelatinization. An increase in gelatinization by 29% was observed by raising feed moisture from 30 to 34%, keeping temperature constant (105°C). Pasta prepared from blend CII (waterchestnut@50%) and blend CIII (rice:waterchestnut in 1:1 ratio) starch gelatinization ranged in between 55.35 – 72.89% and 51.01 – 65.41%, respectively.

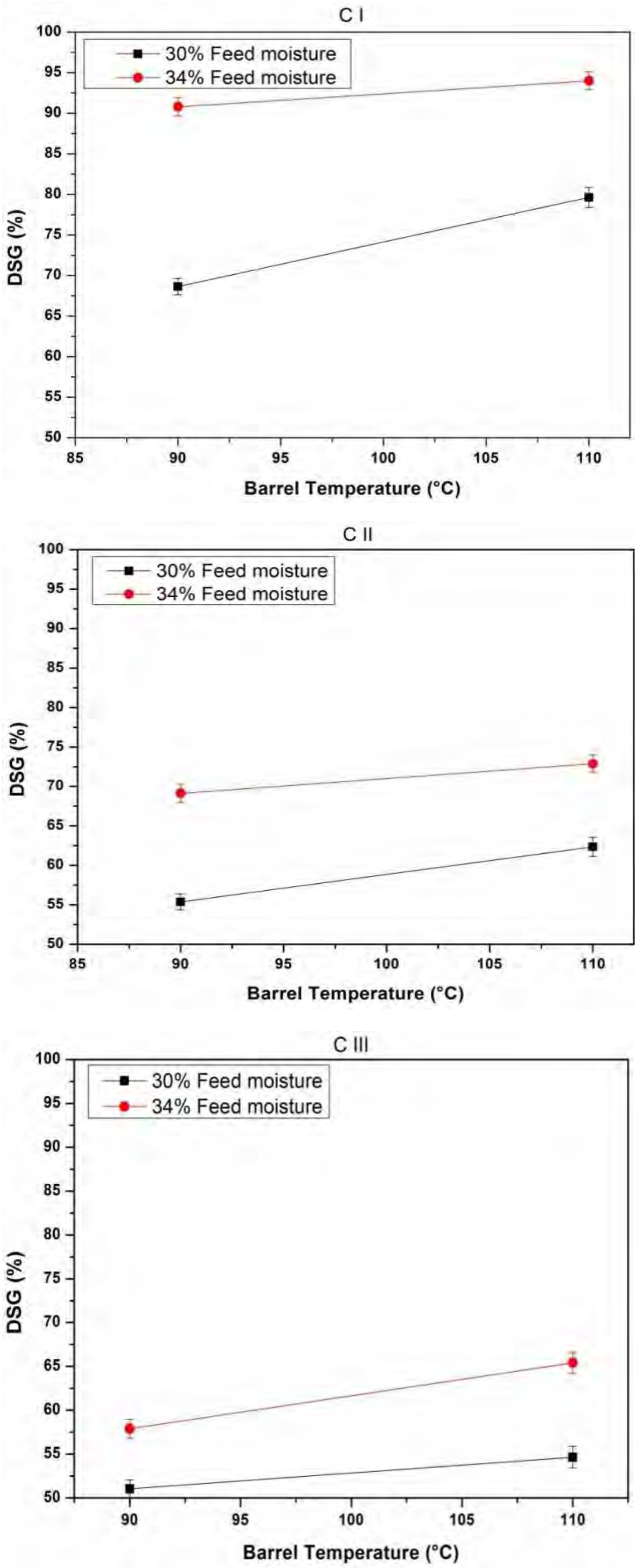
Amylose content, distribution of amylopectin branch chains, morphology, lipid complex amylose chains, and protein content are recorded to influence the transition temperatures of gelatinization. It was worthy to note that an increase in feed moisture by 4%, enhanced degree of starch gelatinization by 24% in pasta produced from blend CII and by 16.45% in pasta from blend CIII at constant temperature (105°C). Moreover, the positive effect of barrel temperature was also observed but the effect of high feed moisture was more prominent.

The presence of protein and fibre rich ingredients (pearl millet and legume@15% each) in blend mixture competes for water and hinders complete gelatinization of starch. Tudorica et al., (2002) suggested that changes in the microstructure of cereal based products by adding some soluble fibre rich compounds could lead to limitation of water availability for starch gelatinization due to the hydration of soluble nonstarch polysaccharides. Irrespective of multigrain blend composition, the positive effect of gelatinization with both barrel temperature and feed moisture was noticed.

Fig.1 revealed that irrespective of blend composition, higher barrel temperature (105°C) and feed moisture (34%) had significant ($p<0.05$) positive effect on the degree of starch gelatinization of pasta. Amongst all the samples, the highest degree of starch gelatinization (94.61%) was observed in pasta prepared from blend CI (rice@50%) and processed at 105°C barrel temperature and 34% moisture in the feed.

While the lowest degree of starch gelatinization (51.01%) was observed in pasta prepared from blend CIII (rice: waterchestnut in 1:1 ratio) and processed at 90°C barrel temperature and 30% feed moisture. This can be attributed to swelling of the amorphous phase which occurs when starch is heated in excess water contributes to the disruption of the crystallite regions by tearing molecules from the crystallites.

If the temperature increase is applied continuously, eventually the whole population of granules will be gelatinized. Researchers observed that dextrinization predominates at moisture contents below 20% whereas gelatinization predominates at moisture content above 20%.

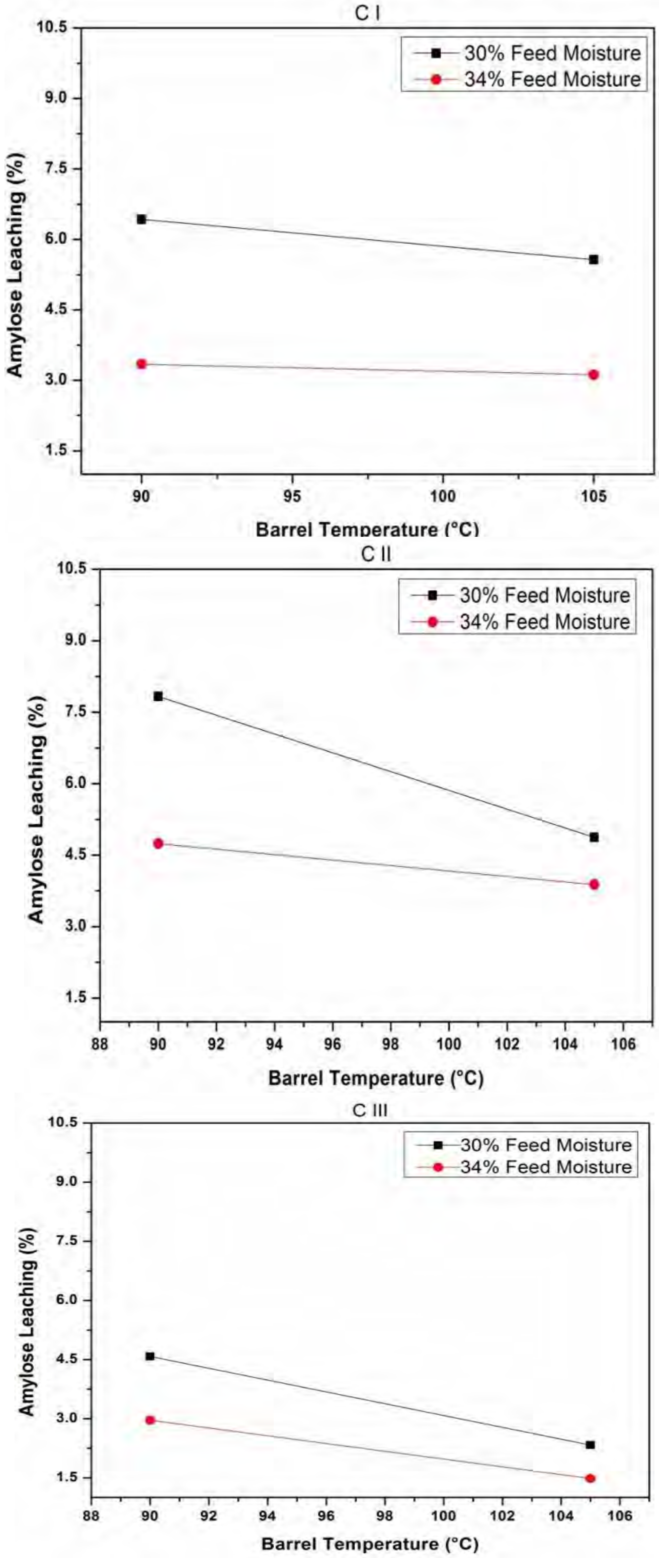


(a)

Cooking quality of multigrain pasta: The data pertaining to cooking quality of multigrain pasta processed at different extrusion conditions is presented in Table 1.

Minimum cooking time: Statistically, significant ($p \leq 0.05$) difference was observed in minimum cooking time of pasta. Minimum cooking time of pasta prepared from blend CI (rice@50%) ranged in between 9.00 to 10.45 min with respect to processing variables. Whereas multigrain pasta prepared from waterchestnut 50% and rice:waterchestnut in 1:1 ratio depicted minimum cooking time of 9.40 to 11.52 min and 10.10 to 11.13 min respectively (Table 1). With respect to processing variables, overall a positive effect of feed moisture on minimum cooking time was observed. The existence of consistency of the protein-starch network seems to minimize the water diffusion through the pasta matrix, thereby increases the time that the water requires to enter granules. Chillo et al., (2008) found that the lack of a gluten network in the amaranth flour based spaghetti affected the optimal cooking time.

Water absorption ratio: Water absorption ratio of pasta also varied significantly ($p<0.05$) at different processing conditions (Table 1). The highest mean water absorption ratio (124.15%) was observed in rice-waterchestnut (1:1) based pasta followed by pasta prepared from waterchestnut@50% (122.01%). The results of water absorption ratio of multigrain pasta revealed that water absorption increased by the extrusion processing. Extrusion temperature as well as feed moisture resulted in increased water absorption. Water absorption ratio of rice: waterchestnut (1:1 ratio) based pasta increased from 117.02 to 124.15% by increasing the barrel temperature from 90 to 105°C at 34% feed moisture. Parallel trend in increase in water absorption ratio of pasta prepared from other multigrain blends were observed. This signifies a positive relation of feed moisture and barrel temperature with water absorption. In white teff extrudates, Kebede et al., (2010) recorded a consistent increase in water absorption with an increase in barrel temperature. As the temperature rose, the chains of amylose and amylopectin formed an expandable matrix that translated into a greater capacity for water retention.



(b)

Fig 1: Effect of feed moisture and barrel temperature on (a) Degree of starch gelatinization of gluten-free multigrain pasta (B) Amylose leaching during cooking of gluten-free multigrain pasta where C I (Rice, pearl millet, pigeon pea, maize, sorghum, flaxseed, guar gum), **C II** (Water chestnut, pearl millet, pigeon pea, maize, sorghum, flaxseed, guar gum), **C III** (Rice, water chestnut, pearl millet, pigeon pea, maize, sorghum, flaxseed, guar gum).

Volume expansion: Volume expansion of multigrain pasta (Table 1) ranged in between 1.48 to 1.70 ml/g (blend C I), 1.42 to 1.60 ml/g (blend CII) and 1.09 to 1.90 ml/g (blend CIII). Higher volume expansion was observed for pasta containing rice as one of ingredient (blend CI and CIII). However, it was observed that increasing trend in volume expansion was combined outcome of barrel temperature and feed moisture. Incorporation of pearl millet and legume @15% each in pasta enhanced the overall volume expansion. Vijaykumar et al., (2010) stated that with the increase in the level of millet flour blend, the elongation index of cooked and uncooked noodles increased significantly ($p < 0.01$).

Gruel solid loss: The gruel solid loss of pasta from blend CI varied in between 6.05-10 ml/g % whereas for pasta from blend CII and blend CIII ranged in between 7.63-10.61 ml/g and 8.35-11.20 ml/g respectively (Table 1). The processing variables significantly ($p<0.05$) affected the gruel solid loss of prepared pasta. Enormous decrease in gruel solid loss was observed at higher extrusion temperature and high moisture content in feed. Decrease in gruel solid loss by 16% in pasta prepared from blend containing 50% rice (blend CI), 17% in pasta containing 50% waterchestnut (blend CII) and 23% in pasta from rice: waterchestnut (1:1) was observed by increasing barrel temperature from 90 to 105°C at 34% feed moisture. The presence of higher amounts of starch in both rice and waterchestnut and the absence of a well-structured protein reticulum can hinder the excessive swelling of the starch granules, resulting in organic matter being dispersed in the cooking water. Steadman et al., (2001) recorded that there was more gruel solid loss of fiber rich pasta such as buckwheat pasta, as part of it leaches out of the pasta during cooking. Due to the low structuring ability of the non-gluten flours, all the gluten-free samples displayed high levels of matter loss.

Amylose leaching of multigrain pasta: The principle starch component in the cooking water is amylose, thus amylose leaching during cooking can be used for prediction of cooking loss. The leached amylose in cooked water was determined by colometric method. Pasta prepared from various blends at different extrusion conditions varied significantly ($p<0.05$) in amylose leaching during cooking. Fig.1 illustrated that amylose leaching of pasta containing rice 50% ranged in between 3.12-6.43 percent.

It was noticed that by raising feed moisture from 30 to 34% at constant extrusion temperature (90°C) the decrease in amylose leaching was 48%. However, for waterchestnut 50% (blend CII) and rice: waterchestnut at 1:1 ratio (blend CIII) based pasta amylose leaching varied in between 3.88-7.83% and 1.48-4.58%, respectively. Martinez et al., (2009) recorded that 2-5 g/100 g of amylose leach is derived from commercial spaghetti in cooking water. In addition, Cleary and Brennan (2006) indicated that partial or full substitution with fibre content for durum wheat semolina can lead to negative changes in the quality of pasta, including increased cooking loss. At constant extrusion temperature (90°C), an increase in feed moisture by 4% resulted in decrease in amylose leaching (39%) for pasta prepared from blend CI (rice 50%) whereas a decrease of 35% in pasta prepared from blend CIII was observed. The decreased amylose leaching of pasta at higher extrusion conditions (feed moisture and barrel temperature) could be attributed to increasing binding at elevated moisture and temperature.

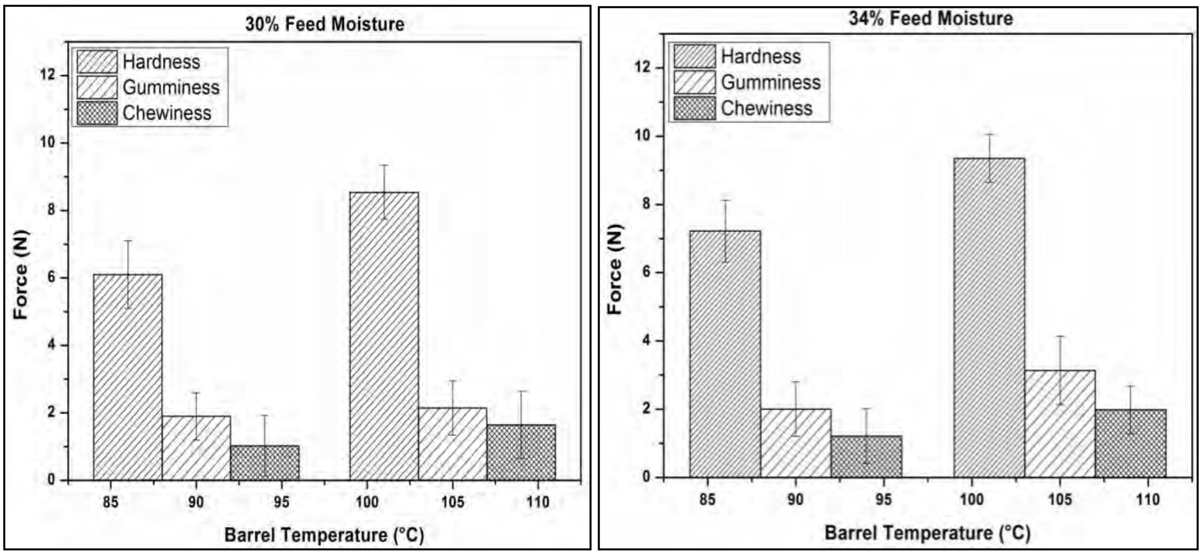
Pasting properties of multigrain pasta: Table 2 presents the RVA pasting properties of multigrain pasta. During heating and cooling in excess of water, major differences were observed in sample behavior. The type of starch (granule size, amylose/amylopectin ratio and amylopectin chain-length distribution) is responsible for the pasting profile. The consistency of the cooking and the textural characteristics of the product are influenced by the amount of water that pasta can retain in its structure. The pasting temperature of the samples was not detected, indicating that the multigrain pasta was fully cooked during extrusion processing. Peak viscosity of multigrain pasta from blend CI (rice@50) ranged in between 134-226 cP. With increasing extrusion temperature from 90 to 105°C, a decrease in peak viscosity was assessed. Multigrain pasta prepared from blend CII depicted peak viscosity in between 168-248 cP and for pasta prepared from blend CIII peak viscosity varied from 176-294 cP. Overall, a significant decreasing trend in peak viscosity of multigrain pasta was observed with increased feed moisture and barrel temperature.

A high percentage of ungelatinized starch was correlated with higher peak viscosity levels, while a lower percentage of peak viscosity suggested a percentage of gelatinized starch due to varying degrees of depolymerization and molecular entanglement resulting from processing conditions. A similar finding was previously reported (McPherson et al, 2000). However, Biliaderis (2009) noted that the crystallinity of cereals subjected to heat-moisture treatment is lost. Disruption of starch granules at high temperatures and mechanical shear stress results in amylose leaching out during the holding time of the viscosity test. A breakdown in viscosity is commonly associated with this time. The holding viscosity of multigrain pasta varied from 118-184cP for blend CI, 119-169cP for blend CII and 175-231cP for blend CIII, the lower values of holding viscosity were observed for combination CI and CII. During certain unit operations, the ability of starches to withstand processing conditions (like heating at high temperature and shear stress) and its behavior after cooling is an important factor for stability of products. Temperature reduction is typically associated with re-association between starch molecules, with the formation of a gel structure resulting in the formation of amylose in particular, thereby increasing the viscosity to the final viscosity. Waterchestnut containing multigrain pasta (CII@50% and CIII@25% waterchestnut) depicted higher final viscosity which ranged in between 323-989 cP and 416-810 cP respectively. In case multigrain pasta from rice@50% (CI blend) lower values of final viscosity (404-655cP) were obtained. This can be attributed to more thermal degradation of rice based multigrain pasta, which is also evident from higher degree of gelatinization for rice based blend CI. Altogether, feed moisture and barrel temperature facilitated the lowering of final viscosity. Analogous trend were observed for breakdown and setback viscosity of multigrain pasta. Usually low setback values indicates low rate of starch retrogradation and syneresis. In addition, the decrease observed in the final viscosity and setback (difference between the minimum viscosity during heating and the final viscosity after cooling) experienced by extreme extrusion suggested an extension of the impact on the amylose chains which, due to their fragmentation during extrusion, could lose the ability to retrograde during cooling. This effect agrees with previous results of Doublier *et al.,* (1986).

Color profile of multigrain pasta: Color is a very important quality parameter of food products since it influences consumer acceptability. The L* a* b* tristimulus values were measured to characterize the color. The color values (L*, a* and b*) including hue angle and chroma of multigrain pasta are presented in Table 3. Statistically, a non-significant ($p>0.05$) difference in color of cooked pasta from all blends was noticed. However, the same samples viewed differently in terms of color values in dried form. Multigrain pasta prepared from blend CI (50% rice) at 34% feed moisture and 105°C barrel temperature exhibited higher luminosity L* (49.83), redness a* (1.70) and yellowness *b** (12.93). As presumed with the rising feed moisture, greatest positive gain in luminosity as well as a* and b* values were observed.

Multigrain pasta from waterchestnut based blend-CII displayed luminosity values ranged from 43.97-49.07 whereas redness varied from 0.27-1.57 and yellowness from 8.13- 11.57. L* value of pasta increased by 10% on raising barrel temperature 90 to 105°C at 30 % feed moisture. Scientists observed that addition of other grains in multigrain blend caused a* parameter to shift towards small positive whereas addition of protein rich ingredients elevates b* value. Overall, significant ($p<0.05$) increase in the L* a* b* of the pasta was noticed as a function of both extrusion temperature and feed moisture. However, the color value of pasta prepared from rice: waterchestnut at 1:1 ratio (blend CIII) ranged in between 44.83-48, a* between 0.90-1.63 and b* value between 8.50-12.07. A large difference in the hue angle and chroma between different treatments was found, regardless of the pasta composition. The hue angle and chroma values of multigrain pasta prepared from three blends (CI, CII and CIII) varied from 82 to 88.13° and 8 to 13.05 with respect to processing conditions and blend composition. Higher chroma values were observed for pasta containing rice (50%-blend CI), followed by pasta containing rice: waterchestnut at 1:1 ratio. These results are validated by the verdicts of Fratianni et al., (2005).

Texture profile of multigrain pasta: Fig. 2 demonstrated the textural properties of the cooked pasta to the minimum cooking time in terms of hardness/firmness, gumminess, chewiness, adhesiveness and resilience. There were significant differences ($p<0.05$) among textural characteristics of multigrain pasta. The hardness values of the multigrain pasta were (6.1–9.35 N) for blend CI, (5.41 – 8.22N) for blend CII and (7.21- 10.21N) for blend CIII. Firmness/hardness represents a dominant element dictating the texture of cooked pasta.



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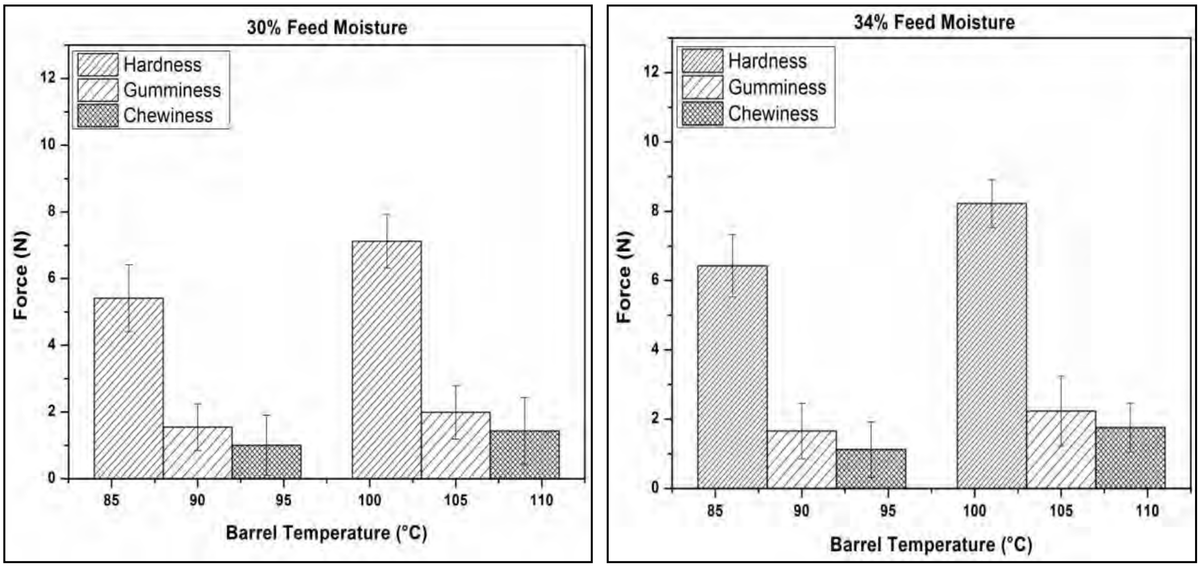

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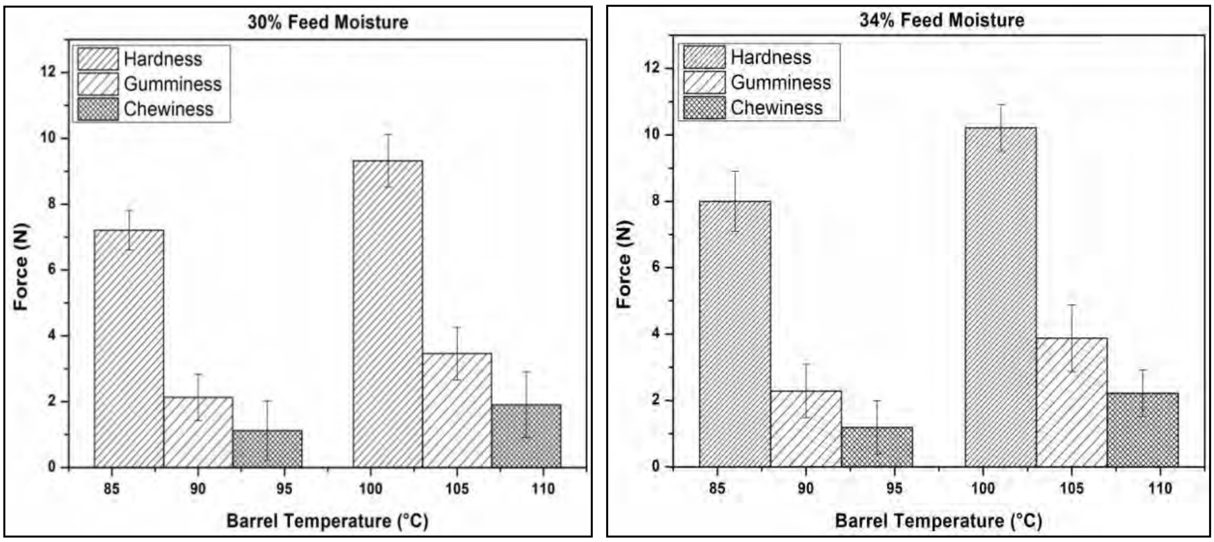

(c)

Fig 2: Effect of feed moisture and barrel temperature on textural characteristics of gluten-free multigrain pasta prepared from

(a) C I (Rice, pearl millet, pigeon pea, maize, sorghum, flaxseed, guar gum)
(b) C II (Waterchestnut, pearl millet, pigeon pea, maize, sorghum, flaxseed, guar gum
(c) C III (Rice, waterchestnut pearl millet, pigeon pea, maize, sorghum, flaxseed, guar gum)

The significant difference in firmness/hardness is due to different composition of blends as well as processing conditions. Marti et al., *(*2013) proposed that the characteristics of texture are strictly related to the raw material used and the conditions of processing applied during pasta production. Our studies observed that higher firmness values were depicted for rice based multigrain pasta (CI and CIII) which can be associated to better structural compatibility of rice with other ingredients in blend. Altogether higher extrusion processing conditions relatively have dominating effect on firmness. However, effect of barrel temperature was more dominant with highest correlation coefficient of r=0.932 for pasta containing rice: waterchestnut at 1:1 ratio (CIII). The adhesiveness value (-0.02 to -0.03g-s) for the multigrain pasta was not statistically ($p>0.05$) significant and thus product was characterized by low stickiness. Gumminess and chewiness of pasta was directly and positively related to feed moisture which could be attributed to enhanced hydrophobic interaction between protein and starch which in turn improved pasta texture. Both gumminess and chewiness were higher for pasta produced at high feed moisture and barrel temperature compared to pasta produced at low feed moisture and low barrel temperature. For pasta produced at a higher barrel temperature, greater resilience has been observed. Wojtowicz, (2012) also published similar findings during a review on the effect of process conditions on the selected texture properties of pre-cooked buckwheat pasta.

In vitro digestibility of multigrain pasta

***In vitro* starch digestibility:** Raw starch is resistant to enzyme hydrolysis (Jenkins et al., 1986) and digestion before gelatinization occurs. The rise in carbohydrate digestibility may be attributed to the pre-regelatinization of starch due to extrusion cooking assisted by the loss of granular rigidity. Also, mechanical disruption of starch during extrusion cooking facilitated the digestion and absorption processes. *In vitro* starch digestibility can be determined from the reducing sugar release during the enzymatic hydrolysis of foods. Such values can yield information regarding the potential glycemic impact of a food from determining the rate and extent of starch degradation (Vermeulen and Turnbull, 2000). Swelling and dispersion of starch molecules during gelatinization makes them accessible to digestive enzymes. Thus degree of gelatinization has direct impact on starch digestibility. The extrusion processing of multigrain blends enhanced starch digestibility. The *in vitro* starch digestibility of multigrain pasta varied between 25.10 to 31.10 g/100g for blend CI (Table 4). At constant temperature (105°C) increase in feed moisture (30 to 34%) enhanced starch digestibility by 9%.

For waterchestnut based pasta (CII), the *in vitro* starch digestibility ranged in between 27.54-36.01 g/100g and for multigrain pasta from blend CIII digestibility varied from 26 – 33.12 g/100g. It was noticed that waterchestnut@50% (CII) and 25% (CIII) containing pasta depicted higher starch digestibility. Inclusively all around, the starch digestibility was enhanced with rising barrel temperature and feed moisture, which could be attributed to increased gelatinization. Highest *in vitro* starch digestibility 36.10 g/100g was obtained for pasta containing waterchestnut (50%) and processed at high feed moisture (34%) and barrel temperature (105°C).

In vitro starch digestibility depicted high positive correlation with barrel temperature viz r = 0.838 for blend CI, r = 0.826 for blend CII and r = 0.854 for blend CIII. Tudorica et al., (2002) suggested that changes in the microstructure of cereal based products by adding some soluble fibre rich compounds could lead to reduction in the digestibility of pasta by hinder enzyme to reach substrate.

***In vitro* protein digestibility:** *In vitro* digestibility of the protein is an important aspect defining the nutritional quality of a protein along with its amino acid composition and bioavailability. In extruder, due to simultaneous action of high pressure, shear and high temperature, structure and digestibility of proteins and starch are highly modified in extruded material (Anton et al., 2009). The data pertaining for protein digestibility of multigrain pasta prepared from various blends (CI, CII and CIII) is displayed in Table 4. The *in vitro* protein digestibility of multigrain pasta ranged in between 61.69 – 78.95 g/100g for blend CI, 76.85-85.54 g/100g for blend CII and 72.64-82.10 g/100g for CIII (Table 4).

Multigrain pasta containing waterchestnut (50%) depicted relatively higher protein digestibility followed by pasta with rice:waterchestnut in 1:1 ratio. Digestibility of protein enhanced by 11% for blend CI, 7.6% for blend CII and 11.07% for blend CIII when barrel temperature was raised. Friedman and Masters, (1980) proposed that severity of processing conditions may alter the protein digestibility by denaturation of the protein and protein cross-linking, racemization, degradation and formation of complexes with sugar. Highest *in vitro* protein digestibility 85.54 g/100g was obtained for pasta containing waterchestnut (50%) and processed at high feed moisture (34%) and barrel temperature (105°C). Similar effect of extrusion variables were observed by Rafiq et al., (2017) during preparation of cereal-legume pasta. *In vitro* protein digestibility depicted high positive correlation with barrel temperature viz r = 0.782 for blend CI, r = 0.760 for blend CII and r = 0.798 for blend CIII.

Bioactive composition of multigrain pasta

Total phenolic content: The total phenolic content (TPC) for multigrain pasta ranged in between 2.02 – 2.72 mg GAE/100 g for blend CI, 1.53-2.29 mg GAE/100g for blend CII and 1.62-2.92 mg GAE/100g for blend CIII (Table 4). Several reports have shown that sorghum and millet are inexpensive and nutritionally comparable or even superior to major cereals (Duodu et al., 2003). Overall, multigrain pasta containing both rice and waterchestnut (blend CIII) exhibited highest phenolic content (2.92 mg GAE/100g) at 34% feed moisture and 90°C barrel temperature. These results are in agreement with Delgado-Licon et al., (2009) who observed similar effects during extrusion of bean-corn mixture. Although, total phenolic content retention/production of all multigrain pasta was more at higher feed moisture, which can be attributed to either release of more phenolic compounds during extrusion or less loss of these compounds at high feed moisture. However contradictory effects were observed for barrel temperature as increased extrusion temperature lessens the total phenolic content in the processed pasta. Altan et al., (2009) reported that heating of foods over 80°C may destroy or alter the nature of phenolic compounds, thus reduction in total phenolic content of pasta may be attributed either to the decomposition of phenolic content due to the high extrusion temperature or alteration in molecular structure of phenolic compounds that may lead to reduction in the chemical reactivity of phenolic compounds or decrease their extractability due to certain degree of polymerisation

Antioxidant activity: Processing is usually thought to cause substantial losses of antioxidants in food products. Some authors, however, have observed its positive influence on antioxidant levels. In the present study, the effect of extrusion processing on antioxidant activity of multigrain pasta was evaluated using DPPH assay. Based on hydrogen donating capacity of the target substances in a methanolic media, chromogen radical DPPH scavenging activity of multigrain pasta varied from 56.71 to 72.55% for rice based pasta (CI), 45.69- 62.63% for waterchestnut based pasta (CII) and 39.30-53.98% for rice:waterchestnut (1:1 ratio) based pasta (CIII) (Table 4). The higher antioxidant activity was depicted in pasta containing 50% rice (blend CI) followed by pasta with 50% waterchestnut (blend CII). Addition of sorghum (10%), flaxseed (2%) and pearl millet (15%) in blends contributed to enhance the antioxidant activity of pasta. Antioxidant activity of blend CIII (rice and waterchestnut 1:1) also varied significantly ($p< 0.05$). At high feed moisture (34%), increase in barrel temperature from 90 to 105°C deduced the antioxidant activity of pasta by 5.40% for CI, 16% for CII and 7.15% for CIII. A lower reduction was observed in rice containing blends (CI and CIII). Overall highest antioxidant value was obtained for pasta prepared from CI (rice@50%) and processed at 34% feed moisture and 90°C barrel temperature. Altogether a positive effect of feed moisture on antioxidant activity was observed. A beneficial effect of a higher moisture content of the raw material on the degree of retention of chemical compounds was observed by Korus et al., (2007).

Total Flavonoid content: Total flavonoid content (TFC) varied significantly ($p<0.05$) among various blends. The total flavonoid content of multigrain pasta ranged from 173 to 220 µg Quercetin/mg for blend CI (containing 50% rice). However, when the moisture of the feed increased from 30% to 34%, keeping the temperature constant (90°C), a narrow variation was observed. Significant difference ($p<0.05$) was also observed for pasta produced from blend CII (50% waterchestnut) and ranged in between 186 to 283µg Quercetin/mg (Table 4). Although, when the temperature of extrusion increased from 90 to 105°C keeping the feed moisture constant at 30%, a significant decrease in TFC was observed. Huang et al., (2006) reported similar results for buckwheat and sweet potato, respectively, upon thermal processing. Furthermore, TFC varied significantly among pasta prepared from blend CIII and ranged from 220 to 305µg Quercetin/mg. The increased barrel temperature of feed adversely affected the TFC at constant feed moisture (30%). This may be attributed to the higher moisture content of feed, as the moist heat is more destructive and produced the synergistic effect along with high temperature. Some researchers also showed similar effect of extrusion processing on buckwheat grit cakes.

Sensory quality: Evaluation score with respect to apperance, color, flavor were high for multigrain pasta prepared from combination I (CI) at feed moisture (30%) and barrel temperature (90ºC) scored highest and was well accepted followed by pasta prepared from combination III (CIII) at same moisture and temperature. For multigrain pasta panelists scored varied in between “liked moderately” to liked slightly” for all parameters. Overall sensory score variation is reasonably well agreeable to differences in other characteristics.

Nutritional analysis of product: Multigrain pasta prepared from various raw material possessed variation with respect to their chemical constituents. The composition of multigrain pasta (expressed at 14 % moisture basis) ranged as crude protein content from 4.88 to 16.19%, crude fat content from 0.90 to 2.34%, ash from 0.50 to 1.62%, crude fibre from 0.99 to 3.40% and carbohydrates from 62.83 to 78.13%.

CONCLUSION

Addition of multigrain’s increased the protein, fat, and fiber content of pasta. Gluten-free multigrain pasta prepared from various blends at different extrusion conditions varied significantly ($p<0.05$) during extrusion. Overall a positive effect of feed moisture on minimum cooking time was observed with respect to processing variables. Irrespective of blend composition an increasing trend in water absorption ratio as well as volume expansion with increased extrusion processing was observed. A slight higher gruel solid loss and moderate amylose leaching was observed owing to the poor structuring capacity of the non-gluten flours. Higher luminosity, redness and yellowness were observed for samples containing 50% rice (blend I). Pasting and textural properties enhanced with feed moisture and barrel temperature. Higher feed moisture possessed dominating effect on bioactive composition of pasta. Barrel temperature depicted higher correlation ($r=0.798$) for protein digestibility whereas feed moisture ($r=0.854$) for starch digestibility. Highest *invitro* starch and protein digestibility was obtained for pasta with waterchestnut@50% (CII) whereas lower values were obtained for multigrain pasta prepared from rice@50% (CI). Based on sensory score pasta produced using rice (50%), pearl millet (15%), pigeon pea (15%),maize and sorghum (10% each) and extruded at feed moisture (30%) and barrel temperature (90ºC) scored highest and was well accepted. The addition of multigrain’s increased the protein, fat, and fiber content of pasta.

Table 1: Effect of processing variables on cooking quality of gluten-free multigrain pasta

Process Variables			Cooking Characteristics			
Multigrain Blends	**Feed Moisture (%)**	**Barrel Temp (°C)**	**Minimum cooking time (min)**	**Water Absorption (%)**	**Volume Expansion (ml/g)**	**Gruel solid loss (%)**
CI	30	90	10.45±0.11^{a}	112.02±0.75^{d}	1.48±0.03^{d}	10.00±0.06^{a}
		105	9.45±0.12^{c}	118.12±0.90^{b}	1.62±0.06^{a}	8.53±0.011^{d}
	34	90	9.00±0.09^{b}	115.08±0.15^{c}	1.55±0.01^{c}	7.21±0.09^{c}
		105	10.01±0.11^{d}	121.02±0.71^{a}	1.70±0.05^{b}	6.05±0.03^{b}
CII	30	90	11.52±0.10^{d}	113.08±0.65^{a}	1.42±0.02^{d}	10.61±0.016^{a}
		105	10.39±0.12^{c}	119.02±0.75^{c}	1.51±0.03^{b}	7.79±0.026^{d}
	34	90	9.40±0.21^{a}	116.08±1.00^{b}	1.49±0.01^{c}	9.21±0.031^{b}
		105	10.18±0.08^{b}	122.02±1.15^{d}	1.60±0.04^{a}	7.63±0.015^{c}
CIII	30	90	10.24±0.09^{b}	114.18±0.80^{a}	1.09±0.04^{b}	11.20±0.021^{a}
		105	11.00±0.09^{c}	120.28±0.95^{c}	1.83±0.03^{b}	9.57±0.009^{c}
	34	90	10.10±0.02^{a}	117.02±1.00^{b}	1.11±0.01^{c}	10.87±0.015^{b}
		105	11.13±0.10^{d}	124.15±0.90^{d}	1.90±0.05^{a}	8.35±0.010^{d}

Values are mean ± standard deviation

Means with different superscript letter within each column are statistically significant according to Duncan's Multiple Range Test ($p < 0.05$)

Table 2: Effect of process variables on pasting properties of multigrain pasta

Process variables			Pasting Properties				
Multigrain Blends	**Feed Moisture (%)**	**Barrel Temp (°C)**	**Peak Viscosity (cP)**	**Hold Viscosity (cP)**	**Final Viscosity (cP)**	**Breakdown Viscosity (cP)**	**Setback Viscosity (cP)**
CI	30	90	226±2.10[a]	184±2.88[a]	655±2.31[a]	42±1.73[a]	481±3.01[a]
		105	182±2.73[c]	149±23.3[c]	432±3.01[c]	33±1.23[c]	293±1.70[c]
	34	90	197±2.05[b]	158±1.74[b]	540±2.06[b]	39±2.10[b]	390±2.29[b]
		105	134±2.51[d]	118±1.42[d]	404±1.77[d]	16±1.00[d]	296±2.54[c]
CII	30	90	248±3.04[a]	169±1.43[a]	989±1.98[a]	79±2.11[a]	820±1.43[a]
		105	194±2.31[c]	133±2.00[c]	543±2.32[b]	61±3.01[c]	410±1.43[b]
	34	90	212±1.55[b]	137±1.52[b]	411±2.10[c]	75±1.23[b]	274±1.12[c]
		105	168±2.65[d]	119±2.00[d]	323±1.89[d]	49±1.98[d]	204±1.71[d]
CIII	30	90	294±3.21[a]	231±1.23[a]	810±2.32a	63±1.09[a]	579±1.97[a]
		105	225±2.87[c]	175±1.11[c]	632±3.00[b]	50±1.01[c]	457±0.99[b]
	34	90	261±3.42[b]	203±1.01[b]	552±2.11[c]	58±1.21[b]	349±1.21[c]
		105	176±2.01[d]	135±1.87[d]	416±0.91[d]	41±1.22[d]	281±1.01[d]

Values are mean ± standard deviation

Means with different superscript letter within each column are statistically significantly according to Duncan's Multiple Range Test ($p < 0.05$)

Table 3: Effect of process variables on color profile of multigrain pasta

Process Variables			Color Characteristics				
Multigrain Blends	**Feed Moisture (%)**	**Barrel Temp (°C)**	**L***	**a***	**b***	**Hue Angle (°)**	**Chroma**
CI	30	90	42.50±0.20[d]	0.23±0.19[d]	6.83±0.23[d]	88.05±0.22[a]	8.00±0.10[d]
		105	49.00±0.30[b]	0.97±0.18[b]	11.50±0.20[b]	85.20±0.28[c]	11.54±0.10[b]
	34	90	46.10±1.11[c]	0.77±0.09[c]	10.50±0.21[c]	85.83±0.01[b]	10.53±0.08[c]
		105	49.83±0.22[a]	1.70±0.13[a]	12.93±0.11[a]	82.51±0.08[d]	13.05±0.02[a]
CII	30	90	43.97±0.09[d]	0.63±0.01[c]	8.13±0.09[d]	85.54±0.13[b]	8.16±0.14[d]
		105	49.07±1.55[a]	1.57±0.03[a]	11.57±1.32[a]	82.29±0.14[d]	11.67±0.11[a]
	34	90	44.30±0.89[c]	0.27±0.16[d]	8.33±0.21[c]	88.13±0.13[a]	8.24±0.01[c]
		105	47.00±0.12[b]	0.89±0.09[b]	9.40±0.13[b]	84.57±0.10[c]	9.44±0.10[b]
CIII	30	90	44.83±0.21[d]	0.90±0.13[c]	8.60±1.12[c]	84.03±1.01[a]	8.65±0.76[c]
		105	46.53±1.65[b]	0.90±0.10[c]	8.50±1.32[d]	83.98±0.99[b]	8.55±0.32[d]
	34	90	45.90±1.03[c]	1.47±0.14[b]	10.43±1.11[b]	82.00±1.02[d]	10.54±0.13[b]
		105	48.00±1.11[a]	1.63±0.21[a]	12.07±1.09[a]	82.32±1.09[c]	12.18±0.76[a]

Values are mean ± standard deviation

Means with different superscript letter within each column are statistically significantly according to Duncan's Multiple Range Test ($p < 0.05$)

Table 4: Effect of processing variables on bioactive compounds of multigrain pasta and on *in vitro* digestibility of multigrain pasta

Process variables			Bioactive Components			*In vitro* Digestibility	
Multigrain Blends	Feed Moisture (%)	Barrel Temp (°C)	Total Phenolic Content (mg GAE/100 g)	Antioxidant Activity (% RSA)	Total Flavanoid Content (µg Quercetin/mg)	*In vitro* Starch Digestibility (g/100g)	*In vitro* Protein Digestibility (g/100g)
CI	30	90	2.22±0.01[c]	56.71±2.81[c]	213.00±4.57[b]	25.10±0.98[d]	61.69±1.32[d]
		105	2.02±0.11[d]	65.69±3.01[b]	173.95±3.33[d]	28.32±0.78[b]	72.46±1.02[b]
	34	90	2.72±0.03[a]	72.55±2.81[a]	220.83±3.24[a]	27.00±0.11[c]	70.43±1.21[c]
		105	2.57±0.04[b]	68.63±2.57[ab]	201.76±4.42[c]	31.10±0.21[a]	78.95±1.19[a]
CII	30	90	1.98±0.08[c]	51.20±2.50[b]	270.82±4.37[b]	27.54±0.12[d]	76.85±1.08[d]
		105	1.53±0.09[d]	45.69±2.75[c]	220.82±2.75[c]	31.12±0.32[b]	80.06±1.24[b]
	34	90	2.29±0.10[a]	62.63±2.52[a]	283.32±2.52[a]	29.00±1.03[c]	79.00±1.43[c]
		105	2.02±0.03[b]	52.75±2.71[b]	186.57±2.83[d]	36.01±1.21[a]	85.54±1.02[a]
CIII	30	90	2.43±0.05[d]	49.11±2.96[a]	305.20±1.84[a]	26.00±1.13[d]	72.64±1.25[d]
		105	1.61±0.07[c]	39.30±2.81[b]	220.82±3.33[d]	30.01±1.21[b]	75.65±1.18[b]
	34	90	2.92±0.01[a]	53.98±3.05[a]	295.82±2.12[b]	28.23±1.12[c]	73.01±1.20[c]
		105	2.62±0.04[b]	50.12±2.75[a]	273.95±3.11[c]	33.12±1.09[a]	82.10±1.21[a]

Values are mean ± standard deviation

Means with different superscript letter within each column are statistically significantly according to Duncan's Multiple Range Test ($p < 0.05$)

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