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Research Paper

RESISTANT STARCH CONTENTS AND THE IN VITRO STARCH, PROTEIN AND MINERAL DIGESTIBILITY OF MILLET AND PULSE BASED PASTA PRODUCTS

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Samples from millet and pulse based pasta products were analyzed for their contents of Resistant Starch (RS), digestible starch and total starch, as well as the *in vitro* starch digestibility. The RS analysis was based on α-amylase and amyloglucosidase hydrolysis followed by colorimetric assay of the glucose released. The results showed that the RS sources were found in the millet and pulse based pasta group (21.21±0.90 to 22.43±0.47%) and control group (21.12±0.71%). Extrusion cooking condition significantly ($p<0.05$) increased the protein digestibility by 90.64% in control pasta group, and 93.75 to 95.47% in experimental pasta group; whereas, there was a reduction in *in vitro* starch digestibility by 90.0 to 94.5% in experimental group and 90.0% in control group. The highest concentrations of mineral elements were found in millet and pulse based pasta products. Pasta products analysed were subjected to *in vitro* enzymatic digestion, simulating the digestive process occurring in the human alimentary tract. The supernatants thus obtained were analyzed for their content of the previously determined mineral components; the percentage of minerals released from the products was calculated.

Keywords: Millet and pulse based pasta products, Resistantstarch, In vitro digestibility

INTRODUCTION

Millets have been reported to be rich sources of dietary fibre which is present in soluble and insoluble forms, and proved to play an important role in the management of metabolic disorders like diabetes mellitus, cardiovascular disease, hyperlipidemia and improved bowel motility which in turn reduces the incidence of colon cancer (Hathan and Prasanna, 2011). The hypoglycemic effect of minor millets due to their high crude fiber, dietary fibre and antioxidants, low carbohydrate content, low digestibility, and presence of β-glucans which are water soluble gums are helpful in repairing glucose metabolism (Itagi *et al.*, 2012). These grains release sugar slowly in the blood and also diminish glucose absorption. The

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hypolipidemic and hypoglycemic effects of minor millets have been attributed to the presence of higher levels of complex carbohydrates, dietary fibre and resistant starch (Pathak and Srivastava, 1998).

Extrusion is one of the most common industrial processes used to make snacks, and it is among the most versatile technological processes for making food products, usually from cereals. Extrusion technology has many advantages including its versatility, high productivity, low cost, and the ability to produce high quality products of unique shapes (Koksel *et al*., 2004). Extrusion-cooking is a versatile and feasible alternative for manufacturing snacks and water reconstitutable foods, and it has been the object of studies in order to enhance the nutritional and functional properties of extrudates for the development ofproducts (Hernandez-Diaz *et al*., 2007). In developed countries, many convenience foods are prepared by extrusion technology, and extruded products such as noodles and pasta products are popularly consumed. Examples of extruded productsinclude spaghetti, macaroni, vermicelli and noodles which are consumed worldwide. Pasta is usually prepared from durum wheat as its main ingredient; butit can also be prepared from hard or soft wheat flour by adding various protein sources. Pasta is considered a slowly digestible starch food, a feature governed by the particular physical characteristics of the product (Granfeldt, 1994).

Bioavailability is be defined as the proportion of the total mineral in a food, meal or diet that is utilized for normal body functions (Fairweather-Tait, 1992). For a nutrient to be absorbed by a living organism, the first and primary condition to be fulfilled is that the nutrient be released from the bonds in which it occurs in a food substance. This release of nutrients is accomplished through enzymatic hydrolysis in the digestive system. *In vitro* bioaccessibility/bioavailability methods are useful to provide knowledge on the possible interactions between nutrients and/or food components, the effects of luminal factors (including pH and enzymes), food preparation and processing practices, nature of the food matrix etc. on either micronutrient absorbability (a component of bioavailability) or on the potential for a nutrient to be absorbed (i.e., bioaccessibility). *In vitro* methods are less expensive, faster, and offer better control on experimental variables than human or animal studies (Sandberg, 2005). These methods do not involve any physiological aspect; therefore they can only determine the total amounts of released mineral components available for absorption. The aim of this study was to estimate the content and the potential bioavailability of starch, protein and minerals from millet and pulse based pasta products, and also to assess the percentage of available mineral components in the process of *in vitro* enzymatic digestion.

MATERIALS AND METHODS

Kodo millet or varagu (CO 3) (*Paspalum scrobiculatum),*little millet or samai (CO 6) (*Panicum sumatrens)* and pearl millet or cumbu (COC 9) (*Pennisetum typhoideum*) were obtained from the Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Whole wheat (*Triticum aestivum*), horse gram and soybean were purchased from the local departmental store.

Sample Preparation

Development of Modified Flour from Millets

To optimize the technology for the development of modified flour to be utilized as the functional food ingredient, the physical modification method was followed.

Physical Modification Technique (Autoclaving-Cooling Cycle)

The physical modification (autoclaving-cooling cycle method) technique was used, following the standard Berry (1986) procedure for the preparation of modified starch(kodo millet, little millet and pearl millet), with slight modification. The kodo millet, little millet and pearl millet grains were cleaned, washed, soaked for 2 hours, ground, and then pressure-cooked at 121 ºC (15 lb/in^2) separatelyfor one hour in an autoclave. The gelatinized starch mixture was cooled to room temperature and it was frozen at 4 ^{o}C for 24 hours which was termed as one cycle. Then, three additional cycles were carried out, followed by cabinet-drying for about 4-6 hours at 40 ^{o}C according to the respective starches, and then ground into fine particles.

Preparation of Horse Gram Flour

Horse gram was roasted at 120 ^{o}C for 10 minutes until it changed to light brown color and developed roasted flavor. The roasted horse gram was ground into flour.

Preparation of Soybean Flour

Soybean was cleaned well, sprinkled with one per cent moisture and kept for one hour. It was roasted in a preheated hot sand bath (120 ^{o}C) for 10 minutes and sieved using a metal sieve. The soybean was milled in a mini dhal mill, the hull was winnowed and ground into flour.

Preparation of Pasta from Millet and Pulse

The various treatments of whole wheat flour with combinations of modified millet flour and pulse flour was carried out in various proportions to formulate low glycemic pastaproducts. The process for development of pastaproducts with cereal, millet and pulse is given in Table 1. Hundred grams of functional flour blend was added tohot water (70 ºC) and mixed well. Then, it was steamed in an idly steamer for 15 minutes. The steamed functional flour blend was fed into the barrel of extruder; after moistening with hot water (70 ºC), the blend was mixed thoroughly in the extruder by the shaft. This mass was kneaded for 15 minutes to ensure thorough distribution of moisture. Extrusion was done after fixing the appropriate die, and the extruded products were steamed for 20 minutes using idly steamer. The steamed extruded products were then cooled and dried in a cabinet drier for four hours at 60 °C.

Nutrient Analysis

Starch content of the sample was estimated using Anthrone reagent described by Sadasivam

Table 1: Proportions to Formulate Pasta Products

Treatments	Whole Wheat Flour (g)	Kodo Millet Flour (g)	Little Millet Flour (g)	Pearl Millet Flour (g)	Horse Gram Flour (g)	Soybean Flour (g)	Egg White (g)	Guar Gum (g)	Water (ml)	Salt (g)
T_1 (Control)	90	-	-	-	-	-	10	2	50	2
T_2	50	15	-	15	10	-	10	2	75	2
T_3	50	15	-	15	-	10	10	2	75	2
T_4	50	-	15	15	10	-	10	2	75	2
T_5	50	-	15	15	-	10	10	2	75	2

and Manickam (2008). The resistant starch content of pastaproducts was assessed using the method described by McCleary and Monaghan (2002).

In Vitro Starch Digestibility

In Vitro Starch Digestibility (IVSD) was determined according to the method of Singh *et al.* (1982). About 50 mg of sample was taken in a test tube and mixed with 1 ml of 0.2 M phosphate buffer (pH 6.9). Pancreatic *alpha amylase* (0.5 mL) (Sigma, cat. No. 6880, 20 mg enzyme dissolved in 50 mL of the same buffer) was added to the sample and incubated at 37 ºC for 2 h. Immediately after the incubation period, 2 ml of 3, 5-DNS reagent (prepared by dissolving 200 mg crystalline phenol, 1 g of 3, 5-dinitrosalycylic acid and 50 mg sodium sulphite in 1% NaOH solution) was added. This mixture was heated for 5-15 min in a boiling water bath. Then, 1.0 mL of 40% K-Na-Tartarate solution was added in the test tubes and allowed to cool at the room temperature (25 ºC). There after, a solution was made up to 25 mL with distilled water and filtered prior to the measurement of absorbance at 550 nm. A blank was run simultaneously. A standard curve was prepared using maltose. Values are expressed as mg of maltose released per gram of sample (db). All the experiments were conducted at least in duplicate.

In Vitro Protein Digestibility

The *in vitro* protein digestibility was determined as described by Moulishwar *et al.* (1993). A known weight of the sample containing 100 milligrams of protein was suspended in 15 mL of 0.1 N HCl containing 19.5 mg of pepsin, and incubated at 37 ºC for 3 hours. The suspension was neutralized with 0.5 N NaOH and then 25 ml of phosphate buffer (8.0) containing 6 mg of pancreatin was added, and incubated at 37 ºC for 24 hours. The volume was made upto 100 ml with distilled water, and 50 ml of aliquot was treated with 15 ml of 10% trichloroacetic acid (TCA) and left overnight to precipitate the proteins. The suspensions were centrifuged at 3000 rpm for 20 min. Protein in the supernatant was estimated by micro-kjeldahl method and protein digestibility was calculated from the following equation:

$$In\ Vitro\ Protein\ Digestibility\ (\%) = \frac{Digested\ \ proteins}{Total\ \ proteins} \times 100$$

Total Minerals Determination

Minerals were extracted from the samples by the dry ashing method described by Hart and Fisher (1971). Calcium and magnesium was determined by titration method described by Jackson (1973). Phosphorus was determined by the method described by Piper (1966). The amount of iron, zinc and manganese were determined using Atomic Absorption Spectroscopy. Sodium and potassium were determined by flame photometer according to AOAC (1984).

In Vitro Availability of Minerals

HCl extractability of minerals in foods is an index of their bioavailability from foods (Chompreeda and Fields, 1984). For HCl extractability of minerals, the powdered sample was extracted with 0.03 N hydrochloric acid by shaking the contents at 37 ºC for 3 hrs. The clear extract obtained after filtration with Whatman No. 42 filter paper was oven-dried at 100 ºC and then placed in a muffle furnace at 550 °C for 4 hrs. Thereafter, the samples were cooled; then, about 5 mL of 5 N HCl was added and boiled gently for 10 min; then again after cooling, the samples were diluted to 100 mL with distilled water. Minerals were determined as described above.

$$Mineral\ \ extractability\ \ in\ 0.03\ N\ \ HCl = \frac{In\ Vitro\ \ Mineral\ \ Extractability\ (\%)}{Total\ \ Mineral} \times 100$$

RESULTS AND DISCUSSION

Total Starch and *in Vitro* Starch Digestibility

The *in vitro* starch and protein digestibility of pasta products are given in Table 2. Resistant starch and dietary fibre content was found to be high in experimental pasta when compared to control pasta due to the incorporation of modified millet flour and pulse flour. Among the treatment groups, resistant starch content was higher in T_4 (22.43±0.47) followed by other groups, the values of which ranged from 21.12±0.71 to 21.87±0.67%. Timea (2009) studied the resistant starch content of maize, wheat, rice and tapioca incorporated with extruded samples, and found it to be 11.31±1.71 to 15.51±0.41%. Total starch content of the experimental samples was comparatively lower than that of the control samples. The *in vitro* starch digestibility of millet and pulse blended pasta products ranged from 90.0 to 94.5%. Incorporation of millet and pulse flour imparts low digestible starch content; thus, products with indigestible compounds undergo slow enzymatic hydrolysis of carbohydrates. Hence, due to reduced starch content these experimental samples form a therapeutic diet suitable for lifestyle disorder patients (especially diabetics and obese).

Total Protein and *In Vitro* Protein Digestibility

The total protein content of pasta products (noodles) was found to be maximum in millet and pulse incorporated pasta (18.98 to 20.90 g/100 g) than the control pasta (13.42 g/100 g). Among all these pasta products, soya flour incorporated noodles (T_3 and T_5) were found to have higher protein content than horse gram flour incorporated noodles (T_2 and T_4) and control (T_1). The *in vitro* protein digestibility of millet and pulse blended pasta products ranged from 93.75 to 95.47%. Modification of millets improved its nutritive value by decreasing the tannin content and improving the *in vitro* protein and starch digestibility. The moist heat treatment (pre-cooking) increased the protein digestibility of the blend. Pre-cooking might have caused an early conformational change of the protein chain in simple form, making it more digestible. Heating improves digestibility due to protein denaturation which results in the opening of protein structure. Dahlin and Lorenz (1993) has reported that heating may enhance flour protein digestibility by rendering the protein more susceptible to hydrolysis due to structural changes, destruction of enzyme inhibitors or decrease in lipid-protein and starch-protein complexes. Abdoulaye *et al.* (2012) found that *in vitro* protein digestibility of extruded products ranged from 73.94 to 82.17%

Table 2: In Vitro Starch and Protein Digestibility (%) of Extruded Products

Treatments	Digestible Starch (g/100 g)	Resistant Starch (g/100 g)	Total Starch (g/100 g)	IVSD (%)	Total Protein (g/100 g)	IVPD
T_1	29.64 ± 0.60	21.12 ± 0.71	50.76 ± 1.99	90.0 ± 2.39	13.42 ± 0.54	90.64 ± 3.53
T_2	16.05 ± 0.69	21.87 ± 0.67	37.92 ± 0.44	90.0 ± 2.03	18.98 ± 0.39	93.75 ± 2.96
T_3	15.37 ± 0.22	21.21 ± 0.90	36.58 ± 1.34	94.5 ± 3.92	20.72 ± 0.21	94.38 ± 4.17
T_4	14.85 ± 0.42	22.43 ± 0.47	37.28 ± 0.80	90.5 ± 3.26	19.54 ± 0.47	94.66 ± 3.33
T_5	14.93 ± 0.37	21.77 ± 0.68	36.70 ± 1.38	93.5 ± 4.13	20.90 ± 0.45	95.47 ± 2.76

in millet and soybean blended extruded products. Susanna and Prabhasankar (2013) found that *in vitro* protein digestibility of gluten-free pasta using sorghum flour, soya flour, channa flour and control pasta using durum wheat flour to be 95.18±0.89 and 91.34±0.65% respectively. Pragya and Sarita (2012) investigated that *in vitro* protein digestibility of iron rich namakpare mixes made using finger millet flour and soybean flour ranged from 62.34 to 78.26%. The present study is in line with the above-mentioned study.

Total Minerals

Total minerals, *in vitro* available minerals and bioavailability of minerals are given in Table 3. The total mineral and *in vitro* available mineral content of calcium, phosphorus, iron, magnesium, copper and zinc were higher in experimental pasta than control pasta. Manganese, sodium and potassium were higher in control pasta than other treatment groups. The respective total mineral and *in vitro* available mineral content per 100 g of pasta products were as follows.Calcium ranged

Table 3: Total Mineral, in Vitro Available Mineral (mg/100 g DWB) and Percent Mineral Bioavailability of Noodles

Minerals	T_1	T_2	T_3	T_4	T_5
Calcium					
Total calcium (mg)	51.70 ± 2.29	180.65 ± 3.42	150.76 ± 5.30	180.46 ± 2.44	150.38 ± 4.74
In vitro available calcium (mg)	18.18 ± 0.76	119.64 ± 4.90	100.75 ± 4.51	122.44 ± 4.08	102.36 ± 4.16
Availability (%)	35.16 ± 0.48	66.23 ± 1.79	66.83 ± 1.56	67.85 ± 1.89	68.07 ±1.46
Phosphorus					
Total phosphorus (mg)	310.48 ± 9.60	347.18 ± 9.69	384.59 ± 10.39	353.75 ± 13.07	391.16 ± 12.06
In vitro available phosphorus (mg)	195.47 ± 7.40	242.15 ± 7.44	269.58 ± 6.56	245.74 ± 6.20	278.14 ± 12.03
Availability (%)	62.96 ± 1.87	69.75 ± 1.88	70.10 ± 2.78	69.47 ± 2.96	71.11 ± 2.37
Iron					
Total iron (mg)	5.86 ± 0.19	6.66 ± 0.01	6.75 ± 0.23	7.68 ± 0.22	7.82 ± 0.25
In vitro available iron (mg)	2.56 ± 0.09	3.57 ± 0.12	3.35 ± 0.05	3.92 ± 0.10	3.95 ± 0.16
Availability (%)	43.69 ± 1.42	53.60 ± 1.98	49.63 ± 0.98	51.04 ± 1.19	50.51± 0.46
Magnesium					
Total magnesium (mg)	125.97 ± 5.67	137.45 ± 4.17	135.56 ± 3.67	130.53 ± 5.18	129.26 ± 5.24
In vitro available magnesium (mg)	72.12 ± 2.85	90.12 ± 3.82	93.31 ± 4.04	86.23 ± 1.09	90.20 ± 2.19
Availability (%)	57.25 ± 0.88	65.57 ± 2.78	68.83 ± 1.43	66.06 ± 2.49	69.78 ± 1.07
Manganese					
Total manganese (mg)	4.02 ± 0.17	3.78 ± 0.05	3.87 ± 0.03	3.73 ± 0.15	3.81 ± 0.16
In vitro available manganese (mg)	2.21 ± 0.09	2.18 ± 0.08	2.22 ± 0.07	2.20 ± 0.03	2.27 ± 0.05
Availability (%)	54.97 ± 2.13	57.67 ± 1.25	57.36 ± 2.53	58.98 ± 1.33	59.58 ± 1.23

Table 3 (Cont.)

Sodium					
Total sodium (mg)	41.05 ± 1.68	38.92 ± 1.61	37.48 ± 0.47	39.71 ± 1.00	38.28 ± 1.21
In vitro available sodium (mg)	28.10 ± 0.33	25.10 ± 0.70	24.23 ± 0.72	25.34 ± 0.25	23.40 ± 0.63
Availability (%)	68.45 ± 0.80	64.49 ± 2.15	64.65 ± 1.11	63.81 ± 0.89	61.13 ± 2.20
Potassium					
Total potassium (mg)	290.60 ± 11.68	273.88 ±11.36	241.48 ± 5.00	270.71 ± 9.28	238.31 ± 6.66
In vitro available potassium (mg)	200.21 ± 7.76	190.42 ± 2.41	169.51 ± 6.26	188.62 ± 7.46	160.35 ± 6.79
Availability (%)	68.89 ± 0.49	69.53 ± 1.76	70.20 ± 0.95	69.68 ± 0.82	67.29 ± 1.06
Copper					
Total copper (mg)	2.07 ± 0.08	2.38 ± 0.06	2.35 ± 0.01	2.32 ± 0.06	2.29 ± 0.09
In vitro available copper (mg)	0.49 ± 0.02	0.59 ± 0.02	0.61 ± 0.03	0.59 ± 0.01	0.61 ± 0.02
Availability (%)	23.67 ± 0.43	24.79 ± 0.29	25.96 ± 0.94	25.43 ± 0.76	26.64 ± 1.10
Zinc					
Total zinc (mg)	5.39 ± 0.22	5.86 ± 0.14	5.87 ± 0.16	6.30 ± 0.11	6.39 ± 0.08
In vitro available zinc (mg)	2.63 ± 0.01	2.87 ± 0.04	2.97 ± 0.06	2.93 ± 0.10	3.20 ± 0.05
Availability (%)	48.79 ± 1.45	48.98 ± 1.02	50.61 ± 1.37	46.51 ± 1.38	50.08 ± 0.36

from 51.70 to180.65 mg and 18.18 to 122.44 mg; phosphorus ranged from 310.48 to 391.16 mg and 195.47 to 278.14 mg; iron ranged from 5.86 to 7.82 mg and 2.56 to 3.95 mg; magnesium ranged from 125.97 to 137.45 mg and 72.12 to 93.31 mg; manganese ranged from 3.73 to 4.02 mg and 2.18 to 2.27 mg; sodium ranged from 37.48 to 41.05 mg and 23.40 to 28.10 mg; potassium ranged from 238.31 to 290.60mg and 160.35 to 200.21 mg; copper ranged from 2.07 to 2.38 mg and 0.49 to 0.61 mg and zinc content ranged from 5.39 to 6.39 mg and 2.63 to 3.20 mg. Abdoulaye *et al.* (2012) found that the ash, calcium, phosphorous, iron and magnesium content of extrudates made with germinated millet flour and roasted soybean flour ranged from 3.29 to 3.55%, 133.62 to 159.62, 548.87 to 576.11, 5.99 to 8.99 and 139.40 to 162.94 mg/100 g. Kulkarni *et al.* (2012) reported calcium, phosphorous and iron content of 21, 112 and 2.2 mg/100 g in control (wheat flour) noodles and 160, 186 and 5.61 mg/100 g in noodles supplemented with malted ragi flour.

Bioavailability of Minerals

Bioavailability can be described as that portion of a nutrient that can be used. This means that any potentially available part of a nutrient after gastrointestinal digestion should be attributed to its availability. The *in vitro* available percent of mineral availability was high in experimental pasta than control pasta. Assessment of mineral bioavailability of pasta products showed that the availability of calcium ranged from 35.16 to 68.07%, phosphorus ranged from 62.96 to 71.11%; iron ranged from 43.69 to 53.60%; magnesium ranged from 57.25 to 69.78%; manganese ranged from 54.97 to 59.58%;

sodium ranged from 61.13 to 68.45%; potassium ranged from 67.29 to 70.20%; copper ranged from 23.67 to 26.64% and zinc ranged from 46.51 to 50.61% respectively. Pragya and Sarita (2012) found that the *in vitro* iron bioavailability of iron rich namakpare mixes made using finger millet flour and soybean flour ranged from 28.67 to 42.00%.

CONCLUSION

Total starch, protein and mineral composition, as well as, *in vitro* starch, protein and mineral digestibility of the products showed that the extrudates may be of high benefits to the group of people requiring low digestible carbohydrates for daily survival. Results of the present study showed that food extrusion significantly influenced the chemical and *in vitro* digestibility of the extrudates. The general acceptability, increased *in vitro* protein and mineral digestibility, and reduced *in vitro* carbohydrate digestibility of the extrudates implies that the product is appropriate for diabetics.

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