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Development and Evaluation of Amylase Rich Malted Mixes (ARMMS) to Improve the Nutritional and Health Status of Pre School Children

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Authors' contributions

This work was carried out in collaboration between both the authors. Author VK designed the study, wrote the protocol and supervised the work. Authors VK and KUM carried out all laboratories work and performed the statistical analysis. Author KUM managed the analyses of the study. Author VK wrote the first draft of the manuscript. Author KUM managed the literature searches. Authors VK and KUM edited the manuscript. Both the authors read and approved the final manuscript.

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ABSTRACT

Two types of ready- to- use (RTE) amylase rich malted mixes (ragi or wheat mixed with green gram) were formulated and *laddu, roti, kheer* and *porridge* were suitably prepared and analyzed for overall acceptability. The results of organoleptic evaluation rated between very poor (1) to very good (5) for all the sensory attributes such as appearance, colour, flavor, texture and overall acceptability were measured. The results of physical parameters reveal that malting decreased grain length, width, kernel weight (0.45 to 19.0 g), volume (0.50 –31.2 ml), hardness (1.12 to 5.9 kg/cm^2) and reduced the bulk density. Wheat malted mix had significantly higher ($P<0.05$) fat (2.27 g), carbohydrate (98.0 g) and calorie (396 kcal) content. Significantly higher ($P<0.05$) calcium (440

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mg), thiamine (0.7 mg) and riboflavin (0.9 mg) content was observed in ragi malted mix compared to native and germinated grains. When compared to the native grains and malted mixes germinated green gram had significantly higher protein (33.0 g), fiber (11.5 g), iron (8.0 g) and vitamin C (157.8 mg) content.

Keywords: Organoleptic evaluation; amylase rich malted mixes; physical parameters.

1. INTRODUCTION

Flours of germinated cereals that are rich in the enzyme amylase constitute amylase- rich foods (ARF). Even small quantities of this type of foods liquefy and reduce the bulk of the cereal-based diet [1] .Thus, ARF helps in increasing the energy density of weaning gruels and in reducing its bulk [1]. It will also help in enhancing the digestibility due to conversion of carbohydrates into simple sugars. Amylase rich food is germinated cereal flour, which is extremely rich in the enzyme alpha-amylase. The alpha-amylase cleaves the long carbohydrate chains in the cereal flour into shorter dextrin. It modifies the starch content of the cereals so that they do not thicken and would therefore not require dilutions resulting in enhanced digestibility [2]. This remarkable property makes it possible to offer a low viscosity yet high energy dense preparation. This study was conducted to develop ready- to- use (RTE) amylase- rich malted mixes (ARMMS) using malted wheat *(Triticum aestivum)* Ragi (finger millet, *Eleusinecoracana)* in combination with green gram (mung bean, *Vigna radiate*)and to assess the physico-chemical and organoleptic quality characteristics such as appearance, colour, flavor, texture and overall acceptability of the developed malted mixes. Addition of ARF to existing standard supplementary food, as used under the BINP program is a simple and effective means to increase the intake of food by changing its consistency, thus making it easier for malnourished children to ingest [3]. In developing countries malting and fermentation have been shown to be the most effective and convenient for the improvement of nutritive value and reduction of bulk and viscosity of cereal foods [4,5,3]. There is a growing interest in the fermentation of food products using composite blends of raw, malted and fermented maize with other legumes as a way of improving nutritional quality [6,7]. This technology requires good knowledge of the functional properties of the flour. Increased food intake after the addition of amylase rich flour to supplementary feed for malnutrition children in rural community of Bangladesh [8] was observed. Viscosity reduction using ARF was also better with the fermented gruels than with gruels from unfermented flours. The implications of these results for the formulation of complementary flour blends for infant feeding are very beneficial.

2. METHODOLOGY

Material required for the study such as ragi, wheat, green gram, sugar and milk powder were procured from local market in Hyderabad, India. Two types of ready- to- use ARMMS (ragi or wheat mixed with green gram) were formulated using a standard procedure [1]. Suitable products namely *Laddu, Roti, Kheer* and *Porridge* were prepared with amylase- rich- malted mixes.

The formulated ARMM and products were subjected to five point hedonic scales for organoleptic evaluation [9] for colour, flavor, texture, taste, appearance and overall acceptability by 15 selected trained panel members from staff and students of Post Graduate and Research Centre, Rajendranagar.

Whole ragi, wheat and green gram were subjected to physical parameters using standard procedures [10] for colour, texture, length, width, kernel weight, kernel volume and hardness in native and malted grains. For the assessment of colour, texture, length and width, an average of 10 whole grains was taken.

Proximate composition (moisture, protein, fat, fiber and ash) of whole grains, germinated grains and ARMMs were analyzed using standard [11] methods. Vitamins C was assessed by colorimetric method [12]. The dehydro ascorbic acid was condensed with 2:4 – dinitro phenyl hydrazine and the product treated with sulfuric acid to give a red colour ,the intensity of which was measured .Thiamine was assessed by modified thiochrome method [11]. A model HP 1090 high – performance liquid chromotograph equipped with an HP 1046 flurometric detector was used. The chromatographic column was a 25cm x 4mm i.d. Stainless steel cartridge packed with Sphorsisorb C85 µm. a 10- µl volume of elute was chromotographed, using a mixture of 70% phosphate buffer (5 mM. ph.7.0) and 30%

acetonitryle as mobile phase, isocratic ally pumped at a low flow rate of 0.650 mL/ mn. The oven temperature was 35°C, the determination of thiamin as thiachrome was measured at an escalation wave length of 360 nm and an emission wave length of 430nm.Riboflavin was assessed by Florescence technique [11]. Minerals such as iron and calcium were determined by atomic absorption spectrophotometer method [11].

Atomic spectroscopy is one of the most widely used methods for quantitative elemental analysis. In atomic spectroscopy, a substance is vaporized and decomposed into gaseous atoms in a flame, furnace, or plasma (a gas hot enough to contain ions and free electrons). Concentrations of atoms are measured by absorption or emission of specific wavelengths of radiation. Atoms exhibit line absorption and emission spectra because electronic transitions are quantized and atoms lack the many rotational and vibrational states of molecules. There are a number of situations where elemental composition is important – *e.g.*, how much iron in an ore sample, how much lead in your drinking water, calcium in intracellular fluids. In a sense, it's the simplest type of analysis, since there are only about 120 possible analytes. But to do the analysis, the sample has to be completely destroyed (chemically and physically) and reduced to individual gas phase atoms (or related species, like ions) in well defined states that you can do spectroscopy on. Twenty milliliter sample solution was neutralized with NaOH. To this 1 ml of (1:1) H_2O_2 was added followed by 5 ml of the buffer pH 1.5 and 4 ml of xylenol orange. The resulting solution was heated to 40–45°C. Subsequently it was cooled and a complex of purple color was formed. The complex has a λ_{max} at 585 nm, on a Shimadzu UV-160, UV–Visible spectrophotometer. Stock solution of 100 ppm was given different dilutions to prepare varying concentration of iron (0.5–5 ppm).With the help of standard solution a calibration curve was auto-established in quantitative mode of Shimadzu spectrophotometer and concentration of iron was determined in the sample solution directly.

Amylase activity was estimated by the standard procedure [13] where in the reducing group liberated from starch were measured by reduction of 3, 5; dinitrosalicylic acid. Carbohydrate content was calculated by difference and energy values were computed.

3. RESULTS AND DISCUSSION

The organoleptic scores (average of triplicates) for the products prepared with ragi malted mix and wheat malted mixes are given in Tables 1 and 2.

Results from the Tables 1 & 2, indicate that the malted ragi and wheat mixes are very good. Maximum sensory scores were obtained for ragi malted mix (5.0), Ragi roti (5.0) and Ragi kheer (5.0) in the present study. Vaideh et al [14] reported that the overall acceptability of the biscuits prepared with ragi malted mix and wheat malted mix were good, is comparable with the present study. Whereas, burfi prepared from genotype malted finger millet flour showed that the overall acceptability scores ranged from fair to good [15].

Physical parameters for native and malted grains (ragi, wheat and green gram) were assessed and the results are given in Table 3. Malting resulted in decrease of 1000 kernel weight by 4.5 to 19.0 g and the volume by 5.0 to 31.2 ml which is a noticeable level of decrease. Grain hardness was also observed less in all grains after malting because the malts of the grains had shrunken appearance and their hardness was considerably lower (1.1 to 5.9 kg/cm^2) than the native grains. Malting helped to reduce the bulk density of the malted mixes. Similar observations in weight, volume and hardness of native and malted grains were reported by several authors [16,17,18,15] .

Table 1. Mean organoleptic scores for the products prepared with malted ragi mix

Products	Sensory attributes*				
	Appearance	Colour	Flavor	Texture	Over all acceptability
Ragi malted mix	4.90±0.42	4.75±0.44	4.90±0.31	4.85±0.37	5.00±0.00
Porridge	4.65±0.48	4.75±0.44	4.70±0.47	4.80±0.41	4.85±0.36
Roti	4.60±0.50	4.70±0.47	4.80±0.41	4.85±0.36	5.00±0.00
Kheer	4.75±0.44	4.65±0.48	4.85±0.36	4.70±0.47	5.00±0.00
Laddu	4.75±0.44	4.80±0.41	4.85±0.36	4.70±0.47	4.79±0.30

**5- very good, 4- good, 3- fair, 2- poor and 1- very poor*

Kolusheva and Marinova [19] conducted a study to determine the optimal conditions for enzymatic hydrolysis of starch through the use of thermally resistant bacteria-amylase, produced from strain of Bacillus subtitles XK-86 in the plant Biovet, Peshtera, Bulgaria. The study revealed that the optimal concentration was 12 enzyme units per ml suspension, since the use of higher concentration did not lead to an increase in the rate of enzymatic reaction. The study concluded that an alpha amylase gives high reaction rate of hydrolysis and thus high concentration of reducing sugar content (Table 4 in a relatively short period of time (4 hours and 15 minutes).

Influence of amylase content on the viscous behavior of low hydrated molten starches with various amylase contents (0-70%) were processed on a twin screw extruder equipped with a special slit die rheometer. Relationships between thermo mechanical treatment and starch macromolecular degradation are defined and flow curves are discussed.

The role of moisture content, product temperature, and mechanical energy on malt viscosity reveal that the viscous behavior is described by a power-law expression. Viscosity is more sensitive to moisture content and macromolecular degradation at lower amylase contents. Using multiple regression analysis, expressions for the different starches were proposed to describe the influence on the viscosity of amylopectin, which is the macromolecular component with short chain branching. The main differences observed when decreasing the amylase content were a lower viscosity and less pronounced shear thinning.

The chemical composition of native grains, germinated grains and malted mixes is given in Table 5.

It is evident from Table 5 that, wheat malted mix had significantly higher ($p<0.00$) content of fat (2.27g), carbohydrate (98.0g) and calories (396 k.cal). Ragi malted mix had significantly higher ($p<0.05$) content of calcium (440mg), thiamine (0.7mg), riboflavin (0.9mg) and amylase activity (169 mg %) when compared to native and germinated grains. Aisien [20] and Glennie [21] reported that significant decrease in fiber on malting due to cell wall degradation during sprouting process. The results in the present study can be comparable with the results reported by Malleshi and Desikachar [18].

Table 2. Mean organoleptic scores for the products prepared with malted wheat mix

Products	Sensory attributes*				
	Appearance	Colour	Flavor	Texture	Over all acceptability
Wheat malted mix	4.85±0.44	4.90±0.36	4.79±0.48	4.80±0.41	4.95±0.38
Roti	4.75±0.44	4.80±0.41	4.70±0.47	4.65±0.48	4.75±0.44
Kheer	4.80±0.41	4.80±0.41	4.70±0.47	4.85±0.37	4.90±0.31
Laddu	4.65±0.48	4.70±0.47	4.80±0.41	4.85±0.36	4.90±0.31

**5-Very good, 4-good, 3-fair, 2-Poor and 1-Very Poor*

Table 3. Physical parameters of native and malted grains (Ragi, Wheat and Green gram)

Sl. No.	Parameters	Ragi	Wheat	Green gram
1	Colour	Cream white	Brown	Green
2	Texture	Hard	Hard	Hard
3	Length (cms)			
	a) Native	0.65	-	0.55
	b) Malted	0.75	-	0.5
4	Width (mm)			
	a) Native	0.35	-	0.45
	b) Malted	0.34	-	0.4
5	1000 – Kernel wt (g)			
	a) Native	52.00	28.7	44.0
	b) Malted	47.90	24.2	25.0
6	1000 – Kernel volume(ml)	35.50		
	a) Native	33.70	28.0	64.5
	b) Malted		23.0	33.3
7	Hardness kg/cm^2			
	a) Native	13.10	19.0	7.6
	b) Malted	7.20	8.0	3.5

Table 4. Influence of substrate and enzyme concentrations on the level of enzymatic starch Hydrolysis (30 min after the beginning of hydrolysis)

Influence of the substrate concentration		Influence of the enzyme concentration	
Concentration of the substrate g.l^{-1}	% hydrolysis of starch	Enzyme concentration: units per ml suspension	% hydrolysis of starch
100	18.2	6	8.2
150	22.5	8	12.4
200	27.1	10	25.2
250	30.1	12	30.1
300	30.3	14	30.2

Table 5. Nutrient composition of native, germinated grains and malted mixes (per 100g)

Parameters	Native flours			Germinated grains			Malted Mixes	
	Ragi	Wheat	Green gram	Ragi	Wheat	Green gram	Ragi malted mix	Wheat malted mix
Moisture (g)	11.9	11.5	9.4	17.0	43.5	90.10*	8.0	7.6
Protein (g)	7.8	12.25	23.75	10.3	14.0	33.0*	15.3	19.0
Fat (g)	1.5	1.2	1.6	1.3	2.0	2.1	1.8	2.27*
Fibre (g)	3.3	2.0	4.0	1.8	10.5	11.5*	2.0	8.0
Ash (g)	2.6	2.4	3.6	2.0	2.0	4.5*	2.9	1.8
Carbohydrates (g)^{b}	72.6	71.0	57.0	75.0	82.1	60.8	94.51	98.0*
Energy (k.cal)^{c}	336	348	228.0	390.0	403.0	380.0	376	396*
Calcium (mg)	350	30.0	320.0	242.0	54.0	109.0	440*	193.2
Iron (mg)	3.9	3.5	4.0	7.7	5.0	8.0*	4.4	5.9
Vitamin C (mg)	-	-	-	8.7	5.0	157.8*	25	14.1
Thiamine (mg)	0.19	0.17	0.28	-	0.9	0.37	0.7*	0.4
Riboflavin (mg)	0.42	0.45	0.43	-	1.23	0.31	0.9*	0.5
Amylase activity $^{@}$	10.0	6.0	3.0	169*	145	132	117	102

@ mg maltose released by 1g of malt flour when acted on 1ml of 1% starch at 37^{0}C for 30 min.
**- Significant at 5% level*
b- Calculated; c- Computed

However, germinated green gram had significantly higher ($p<0.05$) content of Protein (33.0g), Fiber (11.5g), Iron (8.0g) and Vitamin C (157.8mg). The incorporation of malted green gram mix and skimmed milk powder to the wheat malted mix and ragi malted mix help to improve nutritional status of vulnerable segments of population with regard to protein, energy, iron, calcium and 'B' complex vitamins status.

A study was conducted by Ibeanu [17] to assess the energy and nutrient densities and the rate of reduction in viscosity of formulated amylase rich infant complementary blends based on local staples. In the study, the amylase rich infant complementary blends based on local staples were prepared, chemically analyzed and combined in different ratios to supply 20g of protein per day. The energy and nutrient densities of the composites were calculated before being used to prepare porridges. Amylase rich flour (ARF) made from germinated sorghum was added to the porridges to produce gruels. The rate of reduction in viscosity of the porridges after the addition of ARF was also calculated. The results indicated that the energy and protein densities of the blends were high and ranged from 0.7 to 0.8 kcal and 0.7 to 1.1g/100kcal, respectively. The densities of vitamin A, calcium and iron were low, while those of folate and iodine were high. All the porridges had over 600cP viscosity before the addition of ARF. The viscosities of the porridges were reduced on addition of ARF and the percentage reduction ranged from 1.4% in plantain: cowpea (PCo) blend to 88.6% in maize: cassava: soybean (MaCS) blends. The formulated complementary gruels were energy, protein, folate and iodine dense but low in vitamin A, iron and calcium. The gruels are however, capable of bridging the energy/nutrient gap between breast milk and the introduction of adult family diet if supplemented with vitamin A, iron and calcium from other rich sources. The results of the present study also coinciding with the results reported in the above study.

Malting is the controlled germination followed by controlled drying of the kernels. The main objective of malting is to promote the development of hydrolytic enzymes, which are not present in non – germinated grain [22]. Other benefits of the malting process include increased vitamin C content, phosphorus availability and synthesis of lysine and tryptophan, calcium content [23,15]. Further more, amylases are elaborated and as a result the viscosity of gellated starch decreases [24]. Malting also includes the inhibition of growth of pathogens through the fermentation process.

4. CONCLUSION

In conclusion, with regard to physical parameters of native and malted grains (ragi, wheat and green gram), decrease in grain length, width, kernel weight, volume and hardness of the malted grains was observed. Thus the malted grains help to reduce the bulk density of the malted mixes. ARMMs formulated were found to be nutritionally dense when compared to native and germinated grains. Hence the incorporation of malted green gram mix and skimmed milk powder to the wheat malted mix and ragi malted mix help to improve nutritive value of the malted mixes, specially protein, energy, iron, calcium and 'B' complex vitamins .

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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