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Formulation and quality evaluation of ricebean (*Vigna umbellata*) based convenient food multi mixes

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Abstract
In the twenty first century, the vagaries of malnutrition like chronic hunger, starvation and micronutrient deficiency continue to affect millions of individuals throughout the developing world; especially among South Asian countries like India, Bangladesh and Burma. Food diversification or food borne strategies like food fortification can be employed to prevent hunger, starvation and micronutrient deficiencies on a long term basis. One of the best food based approach to address these issues is by development of new value added products like Food Multi-Mix (FMM) from local ingredients for better affordability, accessibility and availability among the vulnerable section of our community. WHO and FAO focus on utilization of underutilized legumes which are of high quality protein, dietary fiber and other micronutrients having numerous health benefits. In the present study FMM was developed using underutilized legume (Ricebean), millet (Konidhan), Flex seed, Rice (luit variety) and tomato powder. The objective of the study was to evaluate the nutritional quality of the developed FMM. In the present study two FMM were developed namely FMM-I from unmalted ricebean and FMM-II from malted rice bean. Rice, millets and flex seeds were toasted and ground to fine powder individually. Tomato was oven dried and ground to fine powder. As such half part of Rice bean seeds were malted and other were slightly toasted and ground to fine powder separately. FMM-I and FMM-II were formulated based on energy density (ED) value between 15.12-18.90kJ/g by mixing all the ingredients at appropriate amount. Nutritional evaluation revealed that the anti-nutritional factors like Phytate phosphorus and saponins significantly decreased after malting. The nutritional components increased after malting. From the present study it can be concluded that the FMM developed from malted Ricebean with low level of anti-nutritional factors and high nutritional composition can be used for development or incorporation of different value added products like cakes, cookies and savoury items to enhance food and nutritional security.

Keywords: Underutilized legumes, malting, antinutritional factors

Introduction
The sustainable developmental goal emphasized a holistic approach to achieve zero hunger, good health and wellbeing till 2030. To achieve such goal, food diversifications like food fortification can be employed to prevent hunger, starvation and micronutrient deficiencies on a long term basis. One of the best food based approach to address these issues is by development of new value added products like Food Multi Mix from local functional ingredients for better affordability, accessibility and availability among vulnerable section of our community and also for reducing the major risk factors for non-communicable diseases. WHO and FAO also focused on utilization of some underutilized legume or pulses in recent years. Ricebean (*Vigna umbellata*) is a protein rich underutilized legume, which distribution is mainly confined to North Eastern Hills, Western and Eastern Ghats in Peninsular India and often in hill tracts and usually grown as an intercrop, particularly with maize, and has also been grown on residual water after rice cultivation FMM is a blend of locally available, affordable, culturally acceptable and commonly consumed foodstuffs mixed proportionately, drawing on the 'nutrient strengths' of each component of the mix in order to optimise the nutritive value of the end-product without the need for external fortification (Zotor *et al* 2015) [28]. Literature documented that very less research has been done to develop various value added food products from underutilized legumes especially rice bean. There is a need to enhance postharvest processing and value- addition of underutilized legumes to meet the future demand for processed and value-added foods, such as ready to cook dhal, snacks, bakery products,

convenient Food Multi Mixes and complementary food mixes to optimize ease of nutritious consumption. Hence, the present study entitled "Formulation and quality evaluation of Ricebean (*Vigna umbellate*) based convenient Food Multi Mixes" has been undertaken to develop value added convenient Food Multi Mixes from ricebean, an underutilized legume, with following objectives:

Objectives

1. To develop ricebean based convenient FMM's
2. To assess the nutritional and antinutritional properties of the developed convenient FMM's
3. To study the storage stability of the developed convenient FMM's.

Methodology

Fresh samples of ricebean and other functional ingredients was procured from the local market of Diphu, Jorhat and Majuli district of Assam. The raw ingredients were processed to make them ready for development of Food Multi Mixes. All the raw ingredients i.e., rice, ricebean, foxtail millet, flaxseed and tomato were processed into flour in order to incorporate them for development of FMM I and FMM II. All the ingredients except tomato and ricebean were slightly toasted and ground to fine powder. Tomatoes were oven dried at 90 °C for 3 days and ground to fine powder. Half part of Ricebean seeds were malted and other were slightly toasted and ground to fine powder separately. FMM-I and FMM-II were formulated based on energy density (ED) value between 15.12-18.90kJ/g by mixing all the ingredients at appropriate amounts. All the powdered ingredients were then sieved through 100 meshed sized sieve separately.

Nutritional composition of functional ingredients

Nutritional composition like moisture, carbohydrate, protein and fat of all the ingredients were determined with standard procedures.

Formulation of FMM I and FMM II

Unmalted ricebean was used in FMM I and malted ricebean used in FMM II. Both the Food Multi Mixes were formulated on the basis of Energy density (ED) value of all the ingredients. For the determination of ED value and product categorization, the carbohydrate, protein and fat content of all the ingredients were estimated. A total of three test samples were formulated using different level of incorporation. The total energy content of the formulations were determined by adding the estimated value of carbohydrate, protein and fat of all the ingredients for each test samples and then multiplying with a constant factor of 16.8 KJ for carbohydrate and protein and 37.8 KJ for fat, as outlined by Cameron and Hofvender (1987) [25]. The ED value of the each formulations should be between 1512.00-1890.00 kJ (360-450 kcal) per 100 g of sample. The formulation with high ED value of 1523 KJ (364 Kcal) with incorporation of 30:40:10:10:10:10 (Rice: ricebean: foxtail millet: flexseed: tomato) has been selected and subjected to further experimentation.

Nutritional and antinutritional composition of FMM I and FMM II

Nutritional composition like moisture, carbohydrate, protein, fat, and antinutritonal components like phytate and saponins of both FMM I and FMM II were determined with standard procedures.

Storage studies

Organoleptic evaluation of the developed convenient FMM I and FMM II were assessed through standard procedures.

Results and Discussion

Nutrient composition of the functional ingredients used for formulation of Food Multi Mixes (FMM's)

Moisture

Moisture content of rice, raw ricebean, malted ricebean, foxtail millet, flexseed and tomato were 3.23 g/100g, 3.58 g/100g, 4.95g/100g, 4.87 g/100g, 6.21 g/100g and 7.4 g/100g respectively. Moisture content of food ingredients influences the taste, texture, weight, appearance, and shelf life of foodstuffs. Even a slight deviation from a defined standard can adversely impact the physical properties of a food material. Food samples which are too dry could affect the consistency of the end product. On the other hand, the rate of microbial growth increases with high moisture content of samples, possibly resulting in spoiled product (Appoldt and Raihani 2017) [6]. The decreased moisture level helps in minimizing deterioration during storage which in turn increases the shelf life of the product and also increases the concentration of the nutrients and can make some nutrients more available (Morris *et al* 2004) [29].

Carbohydrate

Carbohydrate content of rice, raw ricebean, malted ricebean, foxtail millet, flexseed and tomato were 78.0 g/100g, 77.54 g/100g, 86.81 g/100g, 63.48g/100g, 24.32g/100g and 1.06g/100g respectively. Researchers have used a variety of experimental paradigms to investigate how the macronutrient constituent like carbohydrates of foods affects energy density of a food product. A number of studies have investigated the effects of dietary carbohydrate on enhancing the energy content of Food Multi Mixes (Rolls and Bell 1999; McAllan 2014) [5, 3].

Protein

Protein content of rice, raw ricebean, malted ricebean, foxtail millet, flexseed and tomato were 5.56 g/100g, 29.09 g/100g, 30.85 g/100g, 12.65 g/100g, 18.74 g/100g and 3.9 g/100g respectively (Table1). Functional ingredients like ricebean, foxtail millet, flexseed and tomato are excellent source of nutraceuticals, bioactive components and many other macro and micro nutrient components that are good for human health. Protein content in functional ingredients play a vital role in uplifting the nutritional quality of any food product. The results of the following study reveals that all the ingredients especially ricebean possess enough protein content to nourish the Food Multi Mixes to a nutrient dense food.

Fat

Fat is an another major nutrient which is a source of nutritional component and bioactive compound such as mono- and polyunsaturated fatty acids, tocopherols and phytosterols. Various studies has revealed that functional ingredients like ricebean, foxtail millet, flexseed and tomato comprises of potential cardioprotective constituents including phytosterols (Stigmasterol, β- sitosterol, and campesterol), tocopherols and squalene (Maguire *et al* 2004; Ryan *et al* 2006) [2, 7]. In present study, the results revealed that fat content of rice, raw ricebean, malted ricebean, foxtail millet, flexseed and tomato were 0.16 g/100g, 0.72g/ 100g, 1.02 g/100g, 3.60 g/100g, 3.63 g/100g and 0.95g/100g respectively.

Table 1: Nutrient composition of functional ingredients used for formulation of Food Multi Mix.

Functional ingredients (per 100 g of dry weight basis)	Nutrients			
	Moisture (g)	Carbohydrate (g)	Protein (g)	Fat (g)
Rice *(Oryza sativa)*	3.23 ± 0.10	78.0±0.37	5.56 ±0.17	0.16 ±0.38
Raw ricebean (*Vigna umbelleta)*	3.58 ±0.21	77.54±0.37	29.09 ±0.13	1.72 ±0.31
Malted ricebean (*Vigna umbelleta)*	4.95±0.14	86.81±0.27	30.85±0.20	0.52±0.33
Foxtail millet *(Setaria ittalica)*	4.87 ±0.16	63.48±0.32	12.65±0.25	3.60 ±0.28
Flex seed *(Linum usitatissimum)*	6.21 ±0.11	24.32±0.36	18.74 ±0.22	36.3 ±0.32
Tomato *(Solanum lycopersicum)*	7.4 ±0.26	1.06 ±0.39	3.9 ±0.41	2.25 ±0.33

Values are expressed in mean ± SD (Standard deviation)

Formulation and standardization of food multi mixes

Convenient FMM's from raw and malted ricebean along with other functional ingredients were formulated on the basis of standard principles outlined by Cameron and Hofvander (1987) [25].

Table 2: Formulation of Test samples (TS) for determination of Energy density value per 100 gm for product categorization.

Formulations of test samples (TS)/100g	Level of incorporation						Energy Density (ED) value KJ/100g
	Rice	Raw/ unmalted ricebean	Malted ricebean	Foxtail millet	Flexseed	Tomato	
TS 1	50%	20%	-	10%	10%	10%	1442 KJ (344 Kcal)
TS 2	40%	30%	-	10%	10%	10%	1486 KJ (355 Kcal)
TS 3	30%	40%	-	10%	10%	10%	1523 KJ (364 Kcal)
TS 4	50%	-	20%	10%	10%	10%	1470 KJ (351 Kcal)
TS 5	40%	-	30%	10%	10%	10%	1670KJ (399 Kcal)
TS 6	30%	-	40%	10%	10%	10%	1868 KJ (446 Kcal)

Out of the six test samples, TS1, TS2 and TS3 were developed using raw ricebean along with other ingredients like rice, foxtail millet, flexseed and tomato. Out of these three test samples, TS3 had the highest ED value of 1523 KJ or 364 Kcal/100g sample. TS3 was formulated by 30:40:10:10:10 incorporation of rice, raw ricebean, foxtail millet, flexseed and tomato powder respectively and was categorized as FMM I. TS4, TS5 and TS6 were developed using malted ricebean along with other functional ingredients.T6 had the highest Energy Density value of 1868 KJ or 446 Kcal/100g. T6 was also formulated with 40% incorporation of malted ricebean and was categorized as FMM III which was subjected to probiotification and was categorized as FMM IV. All together four products namely FMM I, FMM II, FMM III and FMM IV was subjected to further in-vitro and in-vivo analysis.

Nutritional and antinutritional composition of FMM I and FMM II

Table 3. Nutritional and antinutritional composition of FMM I and FMM II

Nutritional and antinutritional composition	FMM I	FMM II
Nutrient composition		
Moisture (g)	4.23±0.33^{b}	6.13±0.42^{b}
Carbohydrates (g)	63.28±0.43^{d}	62.01±0.44^{b}
Protein (g)	16.81±0.23^{b}	17.86±0.17^{a}
Fat (g)	4.73±0.43^{a}	3.91±0.41^{c}
Antinutrient composition		
Phytates (%)	2.41±0.34^{d}	2.09±0.52^{c}
Saponins (%)	0.26±0.55^{c}	0.23±0.35^{b}

Values are expressed in mean ± SD (Standard deviation); Means within rows separated by Duncan's multiple range test P = 0.01; Means followed by the same letter shown in superscript(s) are not significantly different.

Moisture

The moisture content of Food Multi Mixes developed from raw ricebean (FMM I) and malted ricebean (FMM II) were 4.23 ± 0.33g and 4.78 ± 0.54g per 100g of sample respectively (table 3). The significant increase moisture content of FMM II developed using malted ricebean compared to FMM I developed using raw ricebean was due to the fact that during germination and malting, the whole grains absorbs moisture from the soaking medium for their physiological and biochemical changes. Seed germination starts with the imbibition of water by dry seed coat which become permeable during steeping and as a result an enormous increase in moisture content occurs (Luo *et al* 2014) [24]. In 2006, Khatoon and Prakash also reported that the increase in moisture content in food mix developed from malted millets and legumes was due to the water uptake during germination process. Many studies revealed that dry legumes absorbs water more rapidly than other grains which is influenced by the physical structure of the legume seeds. The mucilages extruded from seed coats increases imbibition and further the moisture was again trapped by cellulose and pectins present in the cell walls. (Rusydi *et al* 2011; Kavitha and Parimalavalli 2014) [22, 21]. The uptake of water by dry legumes was commonly used for respiration and synthesis of cells prior to development of embryo within the seeds as reported by Nanogaki in 2010.

Both the developed Food Multi Mixes in the present study had moisture content below 10 g per 100g of sample, which were within the permissible limit recommended by FAO/WHO 2013.

Carbohydrates

The carbohydrate content of Food Multi Mix developed using raw ricebean (FMM I) and Food Multi Mix developed from malted ricebean (FMM II) were 63.28 ± 0.43g and 64.09 ± 0.39g per 100g of sample respectively. The significant increase in carbohydrate content in FMM II was due to addition of malted ricebean as during germination the starch molecules in cotyledon breakdown into smaller molecules such as glucose and fructose to provide energy for cell division while the seeds mature and grow. Similarly in 2005, Ohtsubo *et al* also revealed that during germination the α and β amylase activities during malting hydrolyzes the starch into simple carbohydrates thereby increasing the carbohydrate content.

The developed Food Multi Mixes provide an average 63 g of carbohydrates per 100 g of Food Multi Mix which is sufficient to meet approximately 51% of daily carbohydrate requirement in adult man and woman as per Recommended Dietary Allowances. FAO/WHO recommended that a multi mix should provide more than 45% of carbohydrate per 100g of sample. Thus, it can be concluded that all the Food Multi Mixes developed were at par with the recommended carbohydrate values.

Protein

Table 3 revealed that the protein content of Food Multi Mix developed using raw ricebean (FMM I) was 16.81±0.23g and malted ricebean (FMM II) was 17.52±0.43g. Protein content of Food Multi Mix developed using malted ricebean was significantly higher ($P \leq 0.01$) than Food Multi Mix developed using raw ricebean. Increase in protein content was due to synthesis of new proteins (Proteases) during mobilization of stored proteins in the cotyledons necessary for the growth of sprouts during germination (Tarasevicienе *et al* 2009; Savage and Footitt, 2017) [18, 19]. Chaudhary and Vyas in 2014 also found that during germination moisture was imbibed in seed and hormones were activated which also resulted in synthesis of new proteins. Furthermore, the amino acids produced by hydrolysis of the protein reserves are not solely used as a source of energy during synthesis of new components, which raises the protein content during germination and malting. Similarly, Pandhare *et al* in 2011 [16] also found that a significant increase in protein content of germinated grain flours, was attributed to increased water activity as a result of induction of hydrolytic enzymes viz. Gibberellin. Gibberellin induces the synthesis of α amylase in the aleurone layer, which makes the endosperm starchy and increases its protein content. (Nonogaki 2010; Yan *et al* 2014) [14, 15].

The developed Food Multi Mixes provides an average 17g of protein per 100 g of Food Multi Mix is sufficient to meet approximately 26-30% of daily protein requirement in adult man and 29-30% of daily protein requirement in adult woman as per Recommended Dietary Allowances. FAO/WHO recommended that a multi mix should provide more than 20% of protein per 100g of sample. Thus, it can be concluded that all the Food Multi Mixes developed were at par with the recommended protein values.

Fat

The fat content of Food Multi Mixes containing raw ricebean (FMM I) and malted ricebean (FMM II) were 4.73±0.43g and 4.42±0.38g respectively. The higher value of fat content in FMM I was might be due to in-situ composition of food ingredients viz. rice, raw ricebean, foxtail millet, flexseed and tomato. The values significantly decreased at $P \leq 0.01$ in FMM II which may be because of processing loss during malting of raw ricebean. In 2013, D'souza also found significant decrease ($P \leq 0.01$) in fat content of a composite flour developed from mixed cereal grains and germinated field beans which was due to loss of fat content in steeping water and the use of stored fats for sprouts to grow during germination Many researchers have also revealed that the total fat loss during processing of grains were due to total solid loss (vitamins, minerals, cellulose and lignins) and also due to use of fat as energy source during sprouting (Wang *et al* 1997; El-Adawy 2002; Hahm *et al* 2009) [12, 11, 10].

The developed Food Multi Mixes provides an average 4 g of fat per 100 g of Food Multi Mix is sufficient to meet approximately 15-16% of daily fat requirement in adult man and 19-20% of daily fat requirement in adult woman as per Recommended Dietary Allowances. FAO/WHO recommended that a multi mix should provide more than 15% of fat per 100g of sample. Thus, it can be concluded that all the Food Multi Mixes developed were at par with the recommended fat values.

Saponin

Saponins are composed of a lipid-soluble aglycon consisting of either a sterol or more commonly a triterpenoid and water soluble sugar residues differing in type and amount of sugars (Singh and Basu 2012) [9]. It lowers nutrient availability (West and Greger, 1978) [8] and decrease enzyme activity. From Table 3 it has been observed that the saponin content of developed FMM I and FMM II 0.26% and 0.23% respectively. FMM II contained lower value of saponin content ie. 0.23% than FMM I (0.26%) which is due to incorporation of malted ricebean in FMM II. In 2013 Luo *et al* revealed that phytate reduction was significantly affected by such processing techniques like germination and malting as a result of the action of endogenous phytases obtained during germination stage. They also found that germination and malting degrades the phytic acid content into inorganic phosphorus, inositol and its other intermediate forms.

Phytate

Phytates occur in several vegetable products specially legumes. Their presence may affect bioavailability of minerals, solubility, functionality and digestibility of proteins and carbohydrates in human diet (Salunkhe *et al*., 1990) [26]. Table 3 revealed that the phytate content significantly decreased in FMM III containing malted ricebean compared to FMM I containing raw ricebean. In 2009 Akapapunam *et al* found similar results on soyabeans and bombara beans that malting significantly decreased the phytate content after germination and malting. The mechanism behind reduction of phytates in legumes was due to activation of the enzyme phytase which neutralizes and reduce phytates (also called phytic acid). Some grains contain high amounts of phytase making it much easier to neutralize the phytates in them, while others have a lot lower.

Organoleptic evaluation of FMM I and FMM II

Organoleptic evaluation of Food Multi Mixes for a period of 45 days are performed with sensory evaluation by a panel of 15 judges. The colour and appearance of all the FMM's remained unchanged till the end of storage period. The scores for organoleptic characters like taste, texture and flavour of FMM's were slightly lowered with the increase in storage period (Table 4). The overall acceptability scores of Food Multi Mixes although gradually decreased throughout the storage period from 15th day onwards, but none of the products were unacceptable.

Table 4: Mean scores of organoleptic evaluation of developed Food Multi Mixes.

Storage period (days)	Organoleptic evaluation					
	Colour	Apperance	Taste	Texture	Flavour	Overall acceptability
FMM I						
0	8.40±0.56	7.63±033	6.7±0.45	8.54±0.48	8.33±0.38	8.34±0.43
15	8.40±0.45	7.63±0.43	6.7±0.45	8.53±0.37	8.33±0.43	8.20±0.58
30	8.40±0.45	7.63±0.56	6.7±0.67	5.53±0.39	8.33±0.37	8.18±0.54
45	8.40±0.43	7.63±0.35	6.6±0.46	8.53±0.46	8.33±0.38	8.18±0.32
FMM II						
0	8.65±0.59	7.74±0.55	7.23±0.38	8.43±0.45	8.63±0.49	8.36±0.62
15	8.65±0.48	7.74±0.55	7.22±0.29	8.43±0.65	8.63±0.38	8.34±0.57
30	8.65±0.48	7.74±0.55	7.22±0.34	8.42±0.43	8.61±0.47	8.33±0.33
45	8.65±0.32	7.74±0.57	7.22±0.42	8.42±0.36	8.61±0.46	8.33±0.56

Results are mean value of three replicates ± standard deviation

Conclusion

The Food Multi Mix (FMM) concept have been an effective tool in developing food products from underutilized crops like rice bean (*Vigna umbellate*) as a nutrient dense convenient product for all age groups and also for supportive purposes or therapeutic uses including in pregnancy, weaning and community-based nutrition rehabilitation for protein energy malnutrition in developing countries.

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