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Nutritional and Microbiological Evaluation of Ricebean (*Vigna umbellata*) Based Probiotic Food Multi Mix Using *Lactobacillus plantarum* and *Lactobacillus rhamnosus*

Daisy Kameng Baruah[1], **Mamoni Das**[1] **and Rajeev Kumar Sharma**[2]

[1]*Department of Food Science and Nutrition, Assam Agricultural University, India*

[2]*Department of Veterinary Microbiology, Assam Agricultural University, India*

***Corresponding author:** Daisy Kameng Baruah, Department of Food Science and Nutrition, Assam Agricultural University, Jorhat, India, Tel: 0376 234 0001; E-mail: Daisy_kameng@yahoo.com

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Abstract

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 noncommunicable diseases. WHO and FAO also focus on utilization of some underutilized legume or pulses in recent years. The use of microbial food additives in improving productivity of functional foods is currently generating a great deal of interest in scientific food industry. The objective of the study was to standardize ricebean based probiotic FMM and to evaluate its nutritional quality. Two FMM were developed namely FMM I and FMM II. All the ingredients were preprocessed before mixing together. FMM I was formulated based on energy density value between 1512.00-1890.00 kJ (360-450 kcal) per 100 g of sample and further by mixing all the ingredients at appropriate amount. Subsequently FMM II was formulated by inoculating probiotic bacteria viz *Lactobacillus plantarum* and *Lactobacillus rhamnosus* in FMM I both individually and in combination in different test samples. The test sample containing highest microbial viability after 30 days of storage was designated as FMM II. Both the Food Multi mixes had optimum macro and micronutrients. Results showed that probiotification played an important role in enhancing the nutrient content in FMM II. The Food Multi Mixes also had good physical properties in terms of bulk density, viscosity, water holding and fat holding capacity. Thus, the FMM concept had been an effective tool in developing food products from underutilized crops to bridge the gap between protein energy malnutrition in developing countries.

Keywords: Food multi mix; Probiotification; Underutilized legumes

Introduction

Legumes constitute a major crop group in the Asia-Pacific region because of their unique features including their role in human nutrition, nitrogen fixation and adaptation to stress condition.

In the present century legumes with high quality protein which are not extensively utilized as food have become a primary target to address the issue of food security [1]. According to WHO and FAO "Food security and right to food" can be achieved by utilization of legumes, specially underutilized legumes as they contain high quality protein, dietary fibre and other micronutrients along with numerous health benefits [2]. These seeds have an average of twice as much protein as cereals and the nutritive value of the proteins are usually of high quality [3]. The importance of food legumes, especially in the diets of the population of developing nations is well established. Legumes not only add to the variety in human diet, but also serve as an economical source of supplementary proteins for a large human population in developing countries like India [4].

One of such underutized legume of north east India is 'ricebean' (*Vigna umbellata*) locally termed as 'bejiamah'. It is a less known and underutilized legume, which has recently emerged as a potential legume because of its nutritional values. The nutritional quality of rice bean specially protein (30.45) is higher as compared to many other legumes of Vigna family [2].

The FMM concept is a growing concept that is gaining wider recognition and acceptance amongst nutrition scientists, being a simple sensible scientific approach in harnessing of nutrient sources to meet human needs. It is a food-based approach using traditional methods of food preparation like Malting with locally available, cheap and affordable staples (fruits, pulses, vegetables and legumes). It has been used to develop food products for clinical and non-clinical population groups (i.e., weanlings, infants, pregnant women, individuals infected with HIV/AIDS and type 2 diabetes mellitus) [5].

The use of microbial food additives in improving productivity of functional foods is currently generating a great deal of interest in scientific food industry. Food grade probiotics are being the most potential alternative to such application. The choice of cereal-legumebased FMM for the development of probiotic food products is motivated by increase in consumer vegetarianism, lactose intolerance, cholesterol content, and economic reasons that are associated with dairy products.

Hence the present study entitled "Standardization of ricebean (*Vigna umbellata*) based probiotic Food Multi Mix" has been undertaken to develop value added convenient probiotic Food Multi Mix from ricebean, an underutilized legume, with following objectives:

Objectives:

To standardize convenient FMM I from locally available ingredients.

To inoculate strains of food grade probiotic bacteria in FMM I to develop FMM II.

To evaluate nutritional and physical properties of FMM I and FMM II.

Methodology

Fresh samples of ricebean and other functional ingredients will be 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 Mix. 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. All the ingredients except tomato were slightly toasted and ground to fine powder. Tomatoes were oven dried at 90°C for 3 days and ground to fine powder. All the powdered ingredients were then sieved through 100 meshed sized sieve separately.

Formulation of food multi mix (FMM I)

FMM I was formulated on the basis of Energy density (ED) value of all the ingredients. For the determination of ED value for 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 [6]. 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.

Formulation of FMM II

Since lactic acid bacteria have a long and safe history of use in food fermentations and have been best proved for their probiotic potentials, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* were selected for the study. The strains were purchased from American Type Culture Collection (ATCC) 10801 University Boulevard Manassas, Verginia, USA and supplied by Jaldhara, Guwahati, Assam. They were in lyophilized condition, in an ampoule amount of sterile hydrating fluid along with a cotton swab (Kwik-Stik). They are stored at -20°C to -80°C in a deep freezer. The FMM I was inoculated with 10^8-10^9 CFU.g^{-1} (8 to 9 log CFU.g^{-1}) of probiotic bacteria in three different test samples (Figure 1):

FMM II A=FMM I+*Lactobacillus plantarum*

FMM II B=FMM I+*Lactobacillus rhamnosus*

FMM II C=FMM I+*Lactobacillus plantarum* and *Lactobacillus rhamnosus*

The samples were then kept for fermentation at 30°C for 24 hours in tightly packed condition to avoid contamination. After 24 hours the fermented samples were freeze dried for 10 hours at 40°C till it comes to fully powdered form. The survival of strains in all three probioticated mixes (FMM II A, B and C) during the storage period of 0, 15 and 30 days were assessed by determining the viable cell count through standard spread plate method. MRS agar was used to enumerate both lactic acid bacteria (*L. plantarum* and *L. rhamnosus*) individually and in combined. The mix which was estimated with maximum viable cell count during 30 days of storage was selected and regarded as FMM II.

Determination of pH

The pH of probioticated FMM II (A, B and C) was measured with Digital pH meter (Eutech Instruments, Germany).

Physical properties

Physical properties like bulk density, viscosity, water and fat holding capacity of both FMM I and FMM II were recorded with standard procedures.

Nutritional composition

Nutritional composition like moisture, carbohydrate, protein, fat, crude fibre and energy of both FMM I and FMM II were determined with standard procedures.

Results and Discussion

In direct microscopic counts (cell counting using haemocytometer) where all cells, dead and living, are counted, but CFU measures only viable cells (Table 1). For convenience the results are given as CFU/mL (colony-forming units per milliliter) for liquids. CFU was calculated using miles and misra method.

The CFU/ml can be calculated using the formula:

cfu/ml=(no of colonies × dilution factor)/volume of culture plate

The total cfu/ml of diluted sample were enumerated with standard spread plate technique.

Viable counts Cfu/0.1 ml	FMM II A	pH	FMM II B	pH	FMM II C	pH
0 days	4.5×10^8	5.67	3.9×10^8	5.28	6.3×10^8	6.81
15 days	4.2×10^8	5.03	3.1×10^8	4.62	5.9×10^8	6.00
30 days	2.3×10^6	3.30	1.9×10^6	3.84	5.5×10^7	5.27

Table 1: Microbiological evaluation of FMM II (A, B and C) over zero, fifteen and thirty days. FMM II A=FMM I+*Lactobacillus plantarum* FMM II B=FMM I+*Lactobacillus rhamnosus* FMM II C=FMM I +*Lactobacillus plantarum*+*Lactobacillus rhamnosus*.

Viability of probiotic bacteria (the number of viable and active cells per g or ml of probiotic food products at the time of consumption) is the most critical value for these products because it determines their

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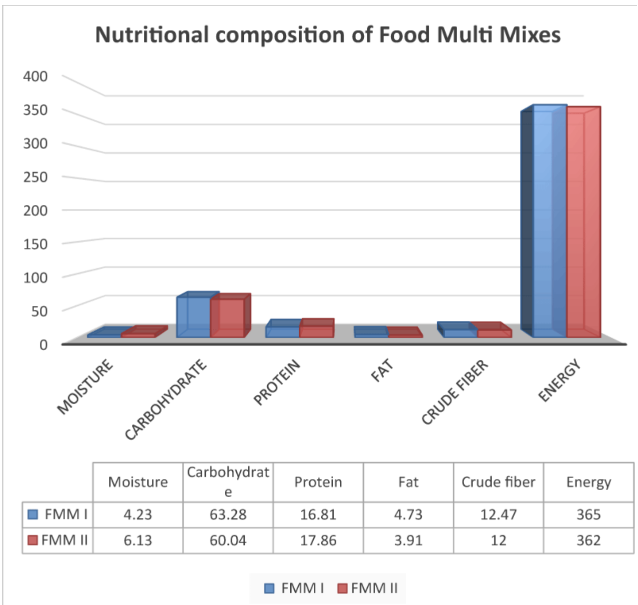
healthful efficiency. Therefore, it is important to ensure a high survival rate of probiotic bacteria during production as well as during storage.

pH of probiotic products considerably affects cell survival of probiotic microorganisms. Very low or high pH restricts the growth and stability of probiotic bacteria in fermented products. The optimum pH for survival of strains of *Lactobacilli* in fermented food products is 5.5-6. [7].

The microbiological evaluation shows the survival of probiotic strains *L. plantarum* and *L. rhamnosus* during 30 days of storage period. The initial cell counts in set A, B and C were 4.5×10^8, 3.9×10^8 and 6.3×10^8 respectively which subsequently decreased to 4.2×10^8, 3.1×10^8 and 5.9×10^8 respectively in FMM II A, B and C respectively after 15 days. There was a marked decrease in cfu of FMM A and B (2.3×10^6 and 1.9×10^6) in 30 days of storage. While it was observed that FMM II C had no such loss in total number of viable cell counts (5.5×10^7 cfu) till 30 days of storage with optimum pH (5.27) ranging from 5.5-6.0 [7] which ensures best non diary probiotic Food Multi Mix recommended by ICMR in 2014. Thus, test sample FMM II C was considered as FMM II.

Physicochemical properties of FMM I and FMM II

Food Multi Mixes are the food products based on the recipes developed from effective combination of individual locally available and commonly consumed food ingredients (super 5 ingredients), making use of their 'nutrient strengths' which when blended together and serves as 'natural fortificants', thus providing a nutrient-enriched end product at relatively low cost without the need for external fortification. The results of nutritional composition like moisture, carbohydrate, protein, fat, crude fiber and energy of developed FMM I and FMM II were presented in Figure 1.



	Moisture	Carbohydrate	Protein	Fat	Crude fiber	Energy
FMM I	4.23	63.28	16.81	4.73	12.47	365
FMM II	6.13	60.04	17.86	3.91	12	362

Figure 1: Nutritional composition of developed FMM I and FMM II per 100 gram (AOAC 925.09 19th edition).

Moisture

The moisture content of FMM I and FMM II were 4.23 ± 0.33 g and 6.13 ± 0.42 g per 100 g of sample respectively (Figure 1). Moisture content of a food mix greatly influences its taste, texture, weight, appearance, and shelf life properties. The rate of microbial growth increases with high moisture content of food mixes, possibly results in low shelf life [8]. The moisture level of a product 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 [9]. The present study showed increased moisture content in Food Multi Mix inoculated with combination of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* after fermentation and freeze drying. The increase in moisture content was also reported by Simwaka et al. [10] after fermentation of a complementary food from millet, sorghum, pumpkin and amaranth seed flours with lactic acid bacteria which might due to the availability of free bond water in the fermentation medium, the high rate of diffusion of the solute into the fermenting medium, the autolysis action of the microorganisms and the presence of large volume of water in the medium. The moisture content of the untreated samples was significantly higher than fermented, roasted and fermented/roasted samples though all were within FAO/WHO recommended safe limit (<10 g) as higher moisture can affect the storage quality.

Carbohydrate

Present data revealed that the carbohydrate content of FMM I and FMM II were 63.28 ± 0.43 g and 60.04 ± 0.44 g respectively as shown in Figure 1. The carbohydrate content tend to decrease after probiotification (Figure 1). Osman in 2011 also found significant decrease in carbohydrate content after fermentation of traditional Lohloh preparation with pearl millet. Osman observed that total carbohydrates decreased between 8 and 12 h of fermentation but as the period of fermentation was further prolonged, there was an unexpected, gradual increase of total carbohydrates to the initial level. However at the end of fermentation (24 h) there was a significant decrease in carbohydrates level probably due to the utilization by microorganisms. Many studies reveals that glucose is the major soluble sugar in a cereal-legume based food product and there was a gradual significant increase in glucose content during the first 16 h of fermentation. At the end of fermentation there was a sharp decrease in glucose content. The increase in glucose content during the first 16 h and the corresponding decrease in total carbohydrate may be due to the increase in microbial amylase activity. The sharp decrease in glucose content at the end of fermentation could be due to utilization of glucose by the microorganisms [10-13].

According to revised Recommended Dietary Allowance (RDA) for Indians-2013, the daily carbohydrate requirement for a normal weight man and woman is 130 g/day. The developed Food Multi Mixes (FMM I and II) provide an average 60-63 g of carbohydrates per 100 g of sample and that 100 g of Food Multi Mix is sufficient to meet almost 49% of daily carbohydrate requirement in adult man and woman as per RDA. While FAO/WHO in 2011 [14] recommended that a multi mix should provide more than 45% of carbohydrate per 100 g of sample. Thus, it can be concluded that all the Food Multi Mixes were at par with the recommended carbohydrate values.

Protein

The composition of Food Multi Mixes is the result of individual nutrient composition of ingredients used to formulate the mix. Present data revealed that the protein content of FMM I and FMM II were 16.81 ± 0.23 g and 17.86 ± 0.17 g respectively (Figure 1). The protein content of FMM II increased after Probiotification. In 2016, Ambani found increased protein content from 11.06 g to 12.03 g in lactic acid inoculated fermented cowpea for the development of value added products. Because during fermentation process the micro-organisms in food utilizes the carbohydrate content in food samples to synthesize amino acids needed for their growth and development. In 2009, Food Safety and Standards Authority of India (FASSAI) also stated that "Fermentation improves the nutritional quality of food products, particularly protein content.

Many researchers found that the increase in crude protein content after fermentation was attributed to the vigorous multiplication of live microorganisms within the food by secreting certain extracellular enzymes such as amylases, linamarases and cellulases during their breakdown in the fermentation medium [15,16]. These Enzymes lowers the protein required for a reaction to occur, without being used up in the reactions [17,18]. In 2012, Amoo [19] found another factor of increase in protein content in FMM II by the ability of the inoculated microorganisms to synthesize amino acids during fermentation process. *Lactobacillus rhamnosus* and *Lactobacillus plantarum* are the most monitored probiotic strains which were clinically studied and found to enhance human natural resistance and healthy digestive system and inhibits the adhesion of some pathogenic bacteria [14]. These probiotic strains also have the potentialities to enhance the nutrient content in food like protein which is attributed to microbial synthesis of proteins from metabolic intermediates during their growth in fermentation [20]. *L. rhamnosus* and *L. plantarum* is used in food industry not only as probiotics but also as a protective culture in fermented and nonfermented dairy products, nondairy food mixes, beverages, ready-to-eat foods, dry sausages, and salads.

According to revised Recommended Dietary Allowance (RDA) for Indians-2013, the daily protein requirement for a normal weight man is 60 g per day while women need 55 g of protein per day. The developed Food Multi Mixes (FMM I and II) provide an average 16-17 g of protein per 100 g of sample and that 100 g of Food Multi Mix is sufficient to meet 28-29% of daily protein requirement in adult man and 29-30% of daily protein requirement in adult woman as per RDA. While FAO/WHO in 2011 [14] recommended that a multi mix should provide more than 20% of protein per 100 g. Thus, it can be concluded that all the Food Multi Mixes were at par with the recommended protein values.

Fat

Fat is a concentrated source of energy which increases the energy density of a diet [21]. In the present study the fat content of FMM I and FMM II were 4.73 ± 0.43 g and 3.91 ± 0.41 g respectively (Figure 1). The values subsequently decreased in FMM II which may be because of processing loss during probiotification. This result of decrease in fat content was also reported by Ojokoh and Orekoya in 2016 [22] in their study on effect of lactic acid fermentation on the Proximate Composition of the Epicarp of Watermelon and found that the fat content of the fermented sample (6.82 ± 0.37) was lower than the unfermented sample (7.04 ± 0.74). The observed decrease of fat content in the fermented sample could be as a result of the breakdown of fatty acid and glycerol by lipolitic microorganisms present in the sample that are able to decompose vegetable and animal fats with the release of a considerable amount of energy during fermentation [23].

Crude fiber

The crude fiber content of FMM I and FMM II were found to be 12.47 ± 0.37 g and 12.00 ± 0.25 g respectively (Figure 1). The higher fibre content in all the FMM I in the present study can be attributed to the in situ composition of the food grains. Ricebean, foxtail millet and flexseed are always known for their high fibre contents. Many researchers reported that intake of functional ingredients like legumes, millets and oilseeds in the diet had a tremendous mode to increase daily dietary fiber consumption [24-27]. The crude fiber content decreased in FMM II which is due to the process of probiotification with *Lactobacillus* strains. Technologically, *Lactobacillus rhamnosus* and *Lactobacillus plantarum* adheres well to fibers present in the food for their growth and multiplication, which creates a possibility of decreased fiber content in cereal and legume based probiotic food products [28]. In 2018 similar results were also observed by Ogodo et al. [29] on fermented bombara groundnut seed flour and found that the fibre content significantly decreased during fermentation with lactic acid bacteria from 11.02 ± 0.05 g to 10.44 ± 0.12 g per 100 g of sample. The decrease in the crude fibre content could be also due to the ability of the fermenting organisms (Lactic acid bacteria) to metabolize the available fibre by enzymatically breaking down during fermentation and then utilizing them as a carbon source [30].

As per American Diabetic Association (ADA) 2002, the dietary fiber intake recommended by Food and Nutrition Board 2015 is an intake of 30-40 g fiber per day which are associated with significantly lower prevalence rates for Diabetes mellitus, Coronary Heart Disease, stroke and Peripheral vascular disease [31,32]. Thus it can be revealed that 100 g of FMM I and II is sufficient to meet 42% and 40% respectively of daily dietary fiber requirement for reduced prevalence rates of Diabetes mellitus and Coronary Heart Disease. On the other hand, as per the revised Recommended Dietary Allowance (RDA) for Indians- 2013, the daily fiber requirement for a normal weight man is 30 g per day while women need 21 g of fiber per day. Thus, it can also be revealed that 100 g of Food Multi Mixes (FMM I and II) will nourish approximately 44% and 62% of daily fiber intake for an adult man and woman respectively.

Energy

The energy values of FMM I and FMM II were found to be 365 ± 0.55 and 362 ± 0.29 kcal respectively per 100 g of sample as shown in Figure 1. Findings revealed that a food multi mix should provide more than 360 Kcal of energy as per PAG recommendation [33,34] and 300-400 Kcal according to BIS specifications. Composite mix in the present investigation provides 362-380 Kcal, thus satisfying both the recommendations. The recommended total energy intake of an adult man (18-39 years) with normal BMI with body weight of 55-60 kg is 2320 kcal/day (sedentary worker), 2730 kcal (moderate worker) and 3490 kcal (heavy worker). Thus by comparing with present findings, an average of 100 g of each Food Multi Mix will be sufficient to meet 15 to 20% of daily total energy intake of the recommended dietary allowances for an adult man of sedentary, moderate and heavy worker. Similarly, the recommended total energy intake of an adult woman (18-39 years) with normal BMI and body weight of 55-60 kg is 1900 kcal/day (sedentary worker), 2230 kcal (moderate worker) and 2850 kcal (heavy worker). Thus by comparing with present findings, an average of 100 g of each Food Multi Mix will be sufficient to meet 18 to 22% of daily total energy intake of the recommended dietary allowances for an adult woman of sedentary, moderate and heavy worker.

Physical properties of food multi mixes (FMM I and FMM II)

The physicochemical characteristics of Food Multi Mix is important for developing value added food products. Since the choices of consumers are getting diverse in this new millennium, the food manufacturers are increasingly demands best base product which imparts good functional properties to the food, apart from nutritional quality. Physical properties of foods are very crucial in product development, process design, shelf life and quality. Knowledge of physical properties is important in handling, preparing, processing, preserving, packaging, storing and distribution of foods. The data regarding the results for physicochemical characteristics like bulk density, viscosity, water holding and fat holding capacity of both FMM I and FMM II are presented in Table 2.

Physical properties	FMM I ± SD	FMM II ± SD	CD 5%
Bulk density (g/ml)	0.48 ± 0.11	0.46 ± 0.38	0.42
Viscosity (centipoise)	4.70 ± 0.45	4.80 ± 0.22	NS
Water holding capacity (g)	2.09 ± 0.70	2.02 ± 0.51	0.47
Fat holding capacity (g)	2.32 ± 0.23	2.32 ± 0.63	0.45

Table 2: Physical properties of Food Multi Mixes (FMM I and FMM II). Values are expressed in mean ± SD (Standard Deviation) NS-Not Significant FMM I-Food Multi Mix with raw ricebean. FMM II-Probioticated Food Multi Mix with raw ricebean.

Bulk density

From Table 2 it was evident that the bulk density of FMM II (0.46 ± 0.38 g/ml) decreased after the process of probiotification of FMM I (0.48 ± 0.11 g/ml). Similarly, Adebowale and Maliki [35] found that the Bulk density values decreased gradually with fermentation process in pegion pea flour which is due to the breakdown of starch during fermentation. This reduction in starch content after its breakdown leads to decrease in the bulk density [36]. The bulk density is a reflection of the load of the flour samples to carry, if allowed to rest directly on one another. The bulk density of a flour sample influences the amount and the strength of packaging material; texture, and mouth feel [37]. Bulk density of food is reported to depend on combined effects of interrelated factors such as intensity of attraction between inter-particle forces, individual particle size and their number of contact points [38]. Hence, a change in any one of these characteristics of powdered flour may result in significant changes in its bulk density. Reports have shown that decrease in bulk density of fermented flours would be an advantage in the preparation of infant foods. In 2017 Codex Alimentarious stated that the action of microbial organism results in the predigestion of the starchy portion of the food (dextrinization) thus reducing the bulk of the food, and ultimately increasing the nutrient density of the food. Thus, fermentation has been reported as a useful and traditional method for the preparation of low bulk weaning foods [39-41]. The low value of bulk density obtained from this study also makes the samples desirable for packaging and transportation [42].

Viscosity

Viscosity is an important constraining factor and also a rheological property related to the quality of the liquid multimix which invariably depends on various factors like composition, total solid content and temperature. The cold paste viscosities of developed Food Multi Mixes slurries were 4.70 ± 0.45 (FMM I) and 4.80 ± 0.22 (FMM II) centipoise (Table 2). The results showed increase in viscosity of fermented Food Multi Mix (FMM II). Many researchers revealed that the decrease in pH during fermentation is the main cause of increase in viscosity [43-45]. As pH decreases the acid hydrolysis of starch results in thinning of starch gel which leads to increase in viscosity. Another factor resulting in higher viscosity could be the gradual degradation of the endosperm matrix in which the starch granules are embedded [9]. In 2004 Nout [46] found that irrespective of pH, *L. plantarum* has a viscocity increasing effect on fermented maize samples. Many researchers had also revealed that a combination of malting and fermentation is known to be better than malting alone because this combination not only reduce viscosity, but the fermentation components also impart colour and flavour along with increase in product's shelf life [47-49].

Water holding capacity

Water-holding capacity (WHC) is an important protein-water interaction that occurs in various food systems. WHC is the ability of a protein matrix to absorb and retain bound, hydrodynamic, capillary, and physically entrapped water against gravity [50-52]. The data reveals that the WHC of Food Multi Mix (FMM II) decreased after fermentation and freeze drying as shown in Table 2. This decreased value of WHC was may be due to increased moisture content of the FMM II after fermentation and freeze drying. In 2018, Msheliza et al. [53] also found that fermentation of Weaning Food Produced from Blends of Sorghum and Soybean had higher moisture content than unfermented blends which significantly decreases the WHC in fermented blends. Though after prolonged freeze drying of FMM II, they contained more of moisture content and was not free flowing like FMM I. Similar results were also reported by Igbabul et al. [54] in 2014 that WHC of Mahgany bean (*Afzelia africana*) flour decreased after fermentation and freeze drying.

Fat holding capacity

Fat holding capacity is an important property in food formulations because fats improve the flavour and mouthfeel of foods. The fat holding capacity of FMM I and FMM II were 2.32 g ± 0.23 and 2.32 g ± 0.63 respectively (Table 2). It has been observed from the data that both the mixes had comparatively similar fat holding capacities. No or less change in fat holding capacity after processing, indicated their suitability for low-fat snacks food formulations [55].

Conclusion

The Probiotic Food Multi Mix (FMM) concept have been an effective tool in developing food products from underutilized crops for supportive purposes and therapeutic uses including in pregnancy, weaning and community-based nutrition rehabilitation for protein energy malnutrition in developing countries.

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