

A Nutritious *Medida* (Sudanese Cereal Thin Porridge) Prepared by Fermenting Malted Brown Rice Flour with *Bifidobacterium Longum* BB 536

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ABSTRACT

The nutritive value of spontaneously fermented brown rice flour *medida*, a Sudanese cereal thin porridge, is low. This study was carried out to improve the nutritional quality of *medida*. The flour was soaked and malted at 30°C to optimise the protein content. Flour malted for two days had the highest protein content. Skim milk was added to the malted brown rice flour *medida* and fermented using *Bifidobacterium longum* BB 536. Maximum count of *B. longum* BB 536 up to 9 log CFU/ ml was attained at 4.6 final fermentation pH. The resultant viscosity was similar to that of the spontaneously fermented brown rice flour *medida*. There was significant ($P < 0.01$) increase in both the energy density and the protein content, having increased 12 folds and 24 folds, respectively. The essential amino acids including lysine and methionine were highly augmented. The resultant *medida* have stable flowing characteristics and meet the whole protein and energy requirements for infants and children aged 1 – 10 years old.

INTRODUCTION

Cereal grains are utilised as food worldwide. In Africa, the majority of cereal-based foods are consumed in the form of porridge and naturally fermented products. *Medida*, the Sudanese cereal thin porridge, is prepared for fasting, sick or convalescent people, nursing mothers, and weaned infants. The word *nasha* is used to replace the word *medida* in urban areas (Dirar, 1993). Chemical analysis of sorghum *medida* (*nasha*) showed that the

product contained 95% water, 3.2% starch, 1.3% protein, 0.3% crude fibre, 0.4% ash, 0.2% fat and only 11.5 kcal per 100 ml (Monawar & Badi, 1987). Therefore, inadequate and poor intakes of protein and energy have been reported among those who depended mainly on cereal porridges (El Mahdi, 1985). This is because the volume of traditional *medida* that has to be ingested to meet the energy and protein needs would be too large. The addition of 0.4% synthetic lysine and 20% full-cream skim milk has been found to be suitable for

improving the nutritional value of food (Monawar & Badi, 1987). In addition, the use of cereal grains in combination with legumes or skim milk has been shown to improve overall protein quality (El Tinay, El Mahadi & El Soubki, 1985; Graham *et al.*, 1986).

When cereal flour is cooked into porridge, starch gelatinisation results in a bulky product with high viscosity. Malted flour is added to reduce the viscosity (Hansen *et al.*, 1989; Gopaldas, Suneeta & Cinnamma, 1988). Many desirable changes occur during the malting of cereal grains due to the breakdown of complex compounds into simple forms (Subramanian *et al.*, 1992; Chaturvedi & Sarojini, 1996) and the transformation into essential constituents. The resulting product is less viscous after cooking and suitable for fermentation with lactic acid bacteria such as *bifidobacteria*.

The microbial groups involved in spontaneous fermentation of cereal flour to produce *medida* include *Lactobacillus spp.*, *Acetobacter spp.*, and *Saccharomyces cerevisia* (Abdel-Gader & Mohamed, 1983). Recently, instead of the traditional lactic acid bacteria, the use of probiotics bacteria such as *bifidobacteria* to improve the therapeutic quality of food has gained considerable interest. *Bifidobacteria* are beneficial for human beings of all ages, as they are a predominant member of the endogenous intestinal flora, capable of improving the balance of the intestinal microflora by preventing colonisation of pathogens, activating the immune system and increasing protein digestion (Ishibash & Shimamura, 1993). *Bifidobacterium longum* is one of the *Bifidobacterium* species found mainly in human faeces (Benno & Mitsuoka, 1986) and has been most used industrially. *B. longum* strain BB 536 was isolated from the faeces of a healthy baby in 1969. Many physiological effects of the strain (Ishibash, Yaeshima & Hayasawa, 1997) and its protective effects against pathogens (Namba *et al.*, 2003) have been report-

ed. The strain has been used commercially for various food applications in several countries.

Among cereal grains, rice has the highest calorie content (Watt & Merrill, 1963). In addition, rice contains a better balance of essential amino acids. Thus its lysine content can be supplemented to an optimum level at a lower cost than for wheat, corn, millet, or sorghum. In this study, a modified technology was used to produce *medida* by fermenting malted rice flour with probiotic bacteria *Bifidobacterium longum*. The resulting fermented *medida* had stable flowing characteristics, a high calorie density and protein content per unit volume.

MATERIALS AND METHODS

Paddy rice

Paddy rice (MR 219) grown in Malaysia was provided by Komplek Bernas Sekinchan, Selangor, Malaysia.

Unmalted brown rice flour

Cleaned paddy rice was de-husked using a Satake rice machine (Satake Engineering Co. LTD, Japan), then the brown rice was ground into flour by a hammer mill and sieved through a 355 µm screen for analytical use.

Spontaneously fermented unmalted brown rice flour *medida*

Traditional *medida* was formulated using un-malted brown rice flour. The starter culture of the spontaneously fermented dough (*ajin*) was prepared following the traditional method (Abdel Gadir & Mohamed, 1983). 100 g brown rice flour was blended with 150 ml distilled water and incubated at 37°C until the initial pH dropped to around 4. The fermented dough was prepared using 25 g brown

rice flour blended with 500 ml distilled water, followed by addition of 10% starter culture and fermented overnight at 37°C. The precipitate of the fermented dough was discarded and the remaining part was added slowly to 1.25 L boiling water on a hot plate with continuous stirring for 5 - 8 min to produce the *medida*.

Malting of paddy rice

Malting of paddy rice was carried out according to the method of Hamad & Field (1979) with some modifications. Cleaned paddy rice was washed twice with distilled water and soaked for 12, 24, 36 and 48 hours in twice the volume of distilled water in 2 L beakers, which were placed in a temperature-controlled water bath (Yihder Bu-420, Taiwan R. O. C) at 30°C. The water in the beakers was renewed every 12 hours during the soaking period to avoid any changes by fermentation.

For germination, the paddy was spread on aluminum dishes and incubated at 30°C for 1, 2, 3, and 4 days. During the germination period, the paddy was turned and rinsed every 16 hours with distilled water to promote aeration and prevent mould development. Paddy rice soaked for 36 hours and malted (germinated) for 2 days was dried in an oven at 50°C for 48 hours. After that the roots were removed and the remaining portion of the paddy was de-husked using a Satake rice machine. The malted brown rice was ground into flour by a hammer mill and sieved through a 355 µm screen and used for the analysis.

Fermentation of malted brown rice flour with *Bifidobacterium longum* BB 536

Preparation of starter culture

Bifidobacterium longum BB536 (Morinaga Milk Industries, Japan) from the stock of the probiotic laboratory, Faculty

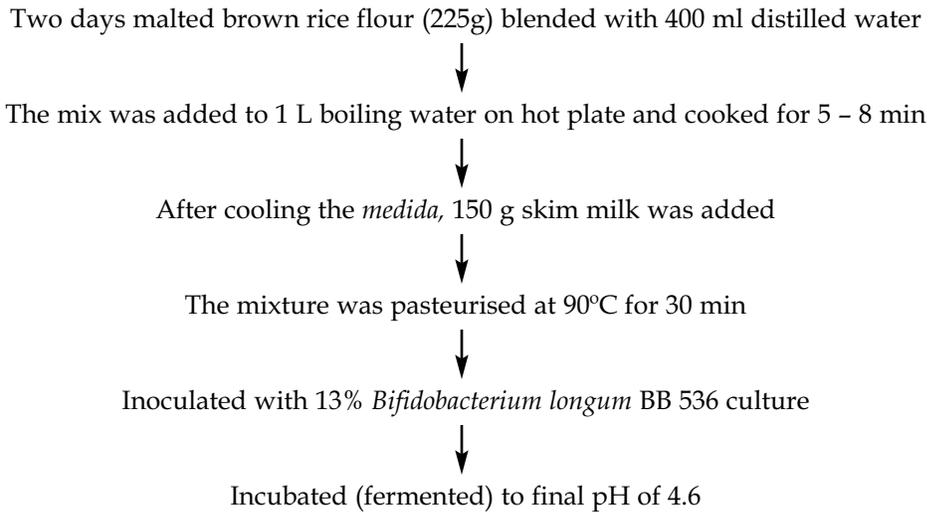
of Food Science and Biotechnology, Universiti Putra Malaysia was used.

The freeze-dried *B. longum* BB 536 cultures were regenerated by transferring into 10% sterilised (121°C for 15 min) skim milk and incubated anaerobically (using anaerobic jar) at 37°C for 48 hours. The regenerated culture was further subcultured in 10% pasteurised (90°C for 30 min) skim milk. The stock culture was produced by inoculating the last culture in 10% pasteurised skim milk followed by anaerobic incubation as described before.

Fermentation of *medida*

Medida was fermented as shown in Figure 1. Two days malted brown rice flour (225 g) was blended with 400 ml distilled water using a commercial Warring blender for 2 min. The resulting mix was added slowly to 1 L boiling water on a hot plate with continuous stirring for 5-8 min. After cooling, 150 g of skim milk was combined with the mixture.

Fermentation was carried out in a batch system using a 2 L Bioreactor with a temperature-controlled water bath (Model Jeio Tech Desk Top, Korea) and an electronic stirrer (Model G gas-Col, Terre Haute, USA). To create the anaerobic condition, oxygen-free nitrogen (OFN) (Malaysia Oxygen Berhad) was used. The Bioreactor was autoclaved for 45 min at 121°C. After cooling, pasteurised (90°C for 30 min) combined mixture of the *medida* and the skim milk was aseptically added and agitated until its temperature was equilibrated to 37°C, followed by inoculation with 13% *B. longum* BB 536 culture. The initial pH of the substrate was set to 6.7 using food-grade sodium metabiphosphate or sodium bicarbonate. The fermentation was run to a final pH of 4.6.

Figure 1. Flow diagram for *medida* production (modified technique)**Enumeration of *B. Longum* BB536**

The media used for plate count enumeration was de Man Rogosa Sharpe Medium agar (Merck, Germany) with added L-cysteine. It was prepared using distilled water and sterilised by autoclaving for 15 minutes at 118°C.

For the total count of *bifidobacteria*, 1 ml fermented malted brown rice flour *medida* was serially diluted in 9 ml peptone water. 0.1 ml of each dilution was aseptically plated on MRS agar plates. The plates were then incubated anaerobically at 37°C for 48 hours and colony-forming units (CFU) per ml were counted.

Viscosity and the total solids of fermented *medida*

Viscosity was measured using programmable DV-11+ viscometer (Brookfield, USA), whereas total solids was determined using an AGO Hand Refractometer (Japan).

Analytical methods**Chemical analysis**

To determine the moisture content, samples were oven-dried at 105°C for 24 hours and the loss in weight was considered as moisture. Total fat was extracted with petroleum ether, while the total fat of *B. longum* BB 536 fermented *medida* was determined using chloroform and methanol with a ratio of 70:30. Total protein was determined by micro-kjeldahl method using conversion factor of 6.25 x N. Fibre was determined by method 978:10 (AOAC, 1990). For the determination of ash content, method 923:03 (AOAC, 1990) was used. Carbohydrate content was calculated as the difference between total weight and the sum of moisture, fat, protein, fibre and ash content. Energy value was calculated by the Atwater System (Watt & Merrill, 1963) using factors of 3.47 kcal/g for protein, 8.38 kcal/g for fat and 4.2 kcal/g for total carbohydrate content.

Determination of the essential amino acids of fermented medida

The essential amino acids of spontaneously fermented brown rice flour *medida* and *B. longum* BB 536 fermented malted brown rice flour *medida* was determined by Pico.Tag method using reversed-phase high performance liquid chromatography (RP-HPLC). Qualitative identification was performed by comparing retention times of 250 pmol of pierce H standard. Quantification was accomplished by using the internal standard alpha amino butyric acid (AABA). A Waters HPLC system was used, comprising a 501 pump, a 484 tunable absorbance detector set at 250 nm, and an oven column set at 37°C. Pico.Tag^R column (3.9 mm X 15 cm) was used. Separation of all amino acids was conducted under gradient condition. Mobile phase consisted of sodium acetate trihydrate buffer, pH 5.7 (A) and acetonitrile (B), delivered at a flow rate of 1 ml/min. The injection volume for the standard and the blank was 8 µL, whereas that for samples was 20 µL.

Determination of the mineral content of fermented medida

Determination of Ca, Fe, Mg and Zn was carried out using atomic absorption spectrophotometry following the procedure of McGary & Young (1976).

Statistical analysis

All the experiments were replicated twice and the data were statistically analysed by T test using MINITAB statistical software 2000.

RESULTS & DISCUSSION

Steeping period

The length of steeping had a significant influence on enzyme development

during the malting process (Eneje *et al.*, 2004). Therefore, paddy rice was steeped for different periods to obtain maximum absorption of water. The percentage of water absorption was calculated based on the volume of paddy before and after steeping. The increase in volume after the end of the steeping period is considered the amount of absorbed water. The water absorption percentage after 12, 24, 36 and 48 hour steeping periods were 17.69 ± 0.14 , 21.11 ± 0.08 , 24.34 ± 0.01 and 24.34 ± 0.03 , respectively. A steeping period of less than 24 hours required frequent rinsing with water. Moreover, the appearance of rootlets needed longer time. On the other hand, the steeping water turned slimy at 48 hours steeping period. Maximum water absorption was achieved at 36 hours of steeping.

The effect of length of steeping periods on the protein content of paddy rice was also investigated. It was observed that protein content of paddy rice increased with longer steeping periods. The percentage of protein was 7.78 ± 0.71 and 9.16 ± 0.16 for samples steeped for 24 hours and 36 hours, respectively.

Steeping at 36 hours appeared to be most appropriate, producing maximum water absorption and protein content and was thus used for further study. Following the steeping, paddy rice was incubated at 30°C during the two days germination period as recommended by the Association of Official Seed Analyst (1970).

Kilning temperature

Drying virtually follows germination in the malting process of cereal grains. It involves drying of the malted grains in an oven until the rootlets become brittle and the moisture content is around 12%. At low (37°C) drying temperature of malted paddy rice, malt growth was observed with germination, continuing for 4 days due to the high humidity and low temperature, which encouraged the growth of

malt. At drying temperature higher than 50°C, the smell of flavours appeared, due to the hydrolytic enzymes. It has been reported that an elevated drying temperature of malted grains permit greater enzyme survival because the malt is less moist (Briggs *et al.*, 1981). Previous studies have also kilned cereal grains at 50-66°C (Hamad & Fields, 1979; Okafor & Aniche, 1980). Therefore, an elevated temperature of 50°C was used for kilning.

Proximate composition of rice flour

During malting, seeds undergo pronounced metabolic changes and the structure profile of the various organic components is altered. Sprouting of cereal grains causes increased enzyme activity, loss of dry matter, as well as a slight increase in crude fat and fibre. However, most of the nutrient increases are not true increases, as they are simply due to the loss of dry matter, mainly in the form of carbohydrates, due to respiration during sprouting. As the total carbohydrates decrease, the percentage of other nutrients increases (Lorenz, 1980).

The results of proximate analysis of unmalted and malted brown rice flour are summarised in Table 1. The ash and protein contents of malted paddy rice increased in the first two days of malting, and then decreased as the malting period continued. Lorenz & Valvano (1981) also

reported increases in ash and protein during the first two days of soft wheat germination. However, their results showed that protein content continued to rise thereafter.

The carbohydrate content of malted paddy rice decreased in the first two days of germination, and then increased with the length of malting period, due to the decrease in the other nutrients including protein ash, fibre and fat. The fat and fibre decreased as the germination period increased, recording the minimum content on the fourth day. However, Lorenz (1980) reported a slight increase in crude fat and fibre during malting of cereal grains. The decrease of fat and fibre in paddy rice may be due to the development of enzymes, which broke them down into fatty acids and soluble fibre. Beevers & Spittstoesser (1968) indicated that the breakdown in protein and fat occurred during malting of cereal seeds. The result of this study regarding the decrease in the protein content of paddy rice grown for 72 hours is in agreement with the study of Hamilton & Vanderstoep (1979) in which similar changes in protein content of germinated alfalfa seeds were observed.

Proximate composition of fermented *medida*

Table 2 shows the improvement in proximate composition of malted brown

Table 1. Proximate composition of un-malted and malted brown rice flour

<i>Type of rice flour</i>	<i>Protein</i>	<i>Fat</i>	<i>Ash</i>	<i>Fibre</i>	<i>Carbohydrate</i>
Unmalted brown	7.10 ± 0.10	1.26 ± 0.16	1.05 ± 0.15	1.17 ± 0.13	89.42 ± 0.04
1 day malted	8.24 ± 0.16	0.97 ± 0.03	1.26 ± 0.24	1.03 ± 0.03	88.50 ± 0.02
2 days malted	9.16 ± 0.16	0.79 ± 0.11	1.39 ± 0.11	0.71 ± 0.17	87.95 ± 0.55
3 days malted	7.99 ± 0.04	0.53 ± 0.04	1.29 ± 0.04	0.52 ± 0.01	89.67 ± 0.05
4 days malted	7.47 ± 0.26	0.29 ± 0.09	1.22 ± 0.03	0.35 ± 0.06	90.67 ± 0.08

Mean value ± S.D. of duplicates on dry bases

Protein content was calculated using the conversion factor of 6.25 x N.

Table 2. Proximate composition of spontaneously fermented brown rice flour *medida* and *B. longum* BB 536 fermented malted brown rice flour *medida*

Components	Spontaneously fermented <i>medida</i>	<i>B. longum</i> BB 536 fermented <i>medida</i>
Protein	8.64 ± 0.34	18.08 ± 0.38
Fat	0.64 ± 0.01	5.56 ± 0.08
Ash	1.64 ± 0.07	6.17 ± 0.25
Fibre	2.00 ± 0.01	3.52 ± 0.27
Carbohydrate	87.08	71.82

Values are mean ± S.D. of duplicate measurements on dry basis

Table 3. The energy value, protein content and essential amino acids per 100 ml of spontaneously fermented brown rice flour *medida* and *Bifidobacterium longum* BB 536 fermented malted brown rice flour *medida*

	Spontaneously fermented <i>medida</i>	<i>Bifidobacterium longum</i> BB 536 fermented <i>medida</i>
Energy value	7.35	84.99
Protein	0.16	3.80
Lysine	0.44 ± 0.03	51.75 ± 1.06
Methionine	0.29 ± 0.02	19.56 ± 0.00
Phenylalanine	0.60 ± 0.05	39.55 ± 2.04
Isoleucine	0.53 ± 0.04	34.60 ± 4.16
Valine	0.85 ± 0.02	52.64 ± 8.44
Leucine	0.98 ± 0.04	60.08 ± 2.70
Threonine	0.42 ± 0.01	22.24 ± 1.12

Values are mean ± S.D. of duplicate fermentation batches.

The energy value was calculated using factors of 3.47 kcal/g for protein, 8.37 kcal/g for fat and 4.2 kcal/g for total carbohydrate.

The amino acids were determined using HPLC system

Table 4. Comparison of Ca, Fe, Mg and Zn in spontaneously fermented brown rice flour *medida* and *B. longum* BB 536 fermented malted brown rice flour *medida*

Type of <i>medida</i>	Ca (mg/L)	Fe (mg/L)	Mg (mg/L)	Zn (mg/L)
Traditional fermented	3.70 ± 0.53	4.07 ± 0.58	78.33 ± 3.21	0.96 ± 0.12
<i>B. longum</i> BB 536 fermented	378.67 ± 2.52	2.59 ± 0.07	5.40 ± 1.73	1.31 ± 0.03

Values are mean ± S.D. of duplicate fermentation batches.

Minerals were determined using the atomic absorption spectrophotometer.

rice flour *medida*, fermented to final pH of 4.6 using *B. longum* BB 536 up to a count 9 log CFU/ml. Compared with spontaneously fermented brown rice flour *medida*, the increases in nutrient contents are as follows: protein content by 2.1 fold, fat 8.69 fold, ash 3.76 fold and fibre 1.76 fold. The low carbohydrate content of *B. longum* BB 536 fermented brown rice flour *medida* was due to the high content of the other nutrients.

The energy value of fermented *medida*

The energy value of spontaneously fermented brown rice flour *medida* was low (Table 3). Children 1 - 10 years old would need to ingest at least 19 - 35 L of this *medida* to meet the daily energy requirements of 1,360 - 2,600 kcal (5,692.96 - 10,883.6 kJ) as recommended by FAO/WHO/UNU (1985). However, due to the significant increase ($P < 0.01$) of up to 12 fold in energy value of *B. longum* BB 536 fermented malted brown rice flour *medida*, the amount of this porridge needed to meet the daily energy requirements for children of this age group is only 0.76 - 1 L.

The protein content of fermented *medida*

The protein content of spontaneously fermented brown rice flour *medida* was 0.16 per 100 ml. Children 1 - 10 year old would need to ingest at least 10 - 18 L of this *medida* per day to meet the total protein requirements of 13 to 14 g per day (FAO/WHO/UNU, 1989).

Skim milk has occupied an important place in human nutrition as a rich source of protein in cereal-based diets in a majority of populations worldwide. In this study, the combination with skim milk significantly ($P < 0.01$) improved the protein content of *B. longum* BB 536 fermented malted brown rice flour *medida* by 24-fold (Table 3). Thus the consumption

of 421 - 737 ml of this *medida* is sufficient to provide the total recommended daily protein intake of these children.

The essential amino acids of fermented *medida*

In nutritional terms, there are two main factors of prime importance in relation to protein, namely the total protein content and the concentration of essential amino acids in the food. Lysine is the first limiting amino acid in cereals. The use of skim milk in the fermentation of *medida* resulted in noticeable improvement in the profile of the essential amino acids. Moreover, the high cell number of *B. longum* BB 536 in fermented *medida* has contributed to improving the amino acid profile. The higher content in some essential amino acids of cheese was related to the higher count of *lactobacilli* (Skeie, Lindberg & Narvhus, 2001).

As shown in Table 3, compared with spontaneously fermented brown rice flour *medida*, lysine and methionine of *B. longum* BB 536 fermented *medida* were increased significantly ($P < 0.01$). The increases in phenylalanine, leucine and threonine were also significant ($P < 0.05$). However, the increases in isoleucine and valine were not significant. Cysteine was not determined in this study since it was not included in the mix of the standard amino acids.

The Ca, Mg, Fe and Zn content of fermented *medida*

Skim milk has been recognised for a long time as an excellent source of minerals, especially calcium. In various countries, skim milk-based products are a principle source of calcium, providing 60-75% of total calcium intake (Flynn & Cashman, 1997). As shown in Table 4, due to the addition of skim milk, the calcium content of *B. longum* BB 536 fermented malted brown rice flour *medida* is much higher compared with spontaneously

fermented brown rice flour *medida*. The Mg and Fe content were below the levels in spontaneously fermented brown rice flour *medida*. They may have been utilized by *B. longum* BB 536 during fermentation.

CONCLUSIONS

To maintain better protein content in malted paddy rice, it is necessary to steep the paddy for 36 hours followed by germination for 2 days, as shorter steeping period and more than two days of germination may result in rice flour with low protein content. Findings of this study have shown that processes such as malting and controlled fermentation can produce a more nutritious *medida*.

The flowing characteristic of *medida* is of importance. The malted brown rice flour with added skim milk and fermented with *B. longum* BB 536 has stable flowing characteristics. Moreover, at the similar viscosity as that of spontaneously fermented brown rice flour *medida*, there was an 11-fold increase in the total solids (21%). The high content of total solids was attained by malting the flour and combining it with skim milk. During the fermentation of *medida*, the skim milk improved the growth of *B. longum* BB 536, although cereal media has been said to be inappropriate for *bifidobacteria* fermentation. Furthermore, the addition of skim milk significantly improved the protein and calcium levels of the fermented *medida*.

At the same time, the delivery of *Bifidobacterium longum* BB 536 as a component of fermented *medida* is preferable. In addition to the high *B. longum* BB 536 (probiotic) cell numbers, the fermented *medida* is a nutrient-dense food source, providing nutrients including high quality protein, energy, calcium and additional healthful attributes, such as antimicrobial fermentation end-products and physiologically active peptides and protein. Therefore, the resultant *medida* is more suitable as

nourishment for adults and children of all ages.

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