INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

CHARACTERIZATION AND GLYCEMIC LOAD OF READY TO COOK (RTC) PREBIOTIC FLOUR MIXTURE

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ABSTRACT
The ready cook (RTC) prebiotic flour mixture was a mixture of whole cereals such as wheat, sorghum, oatmeal, finger millet, proso millet; pulses such as whole green gram flour, dehusked soya flour; amla fruit powder; drumstick leaves powder; spice mix consists of fenugreek, cumin and coriander seeds. The physical, functional, nutritional, thermal and sensory profile of RTC prebiotic flour mixture was determined for its characterization. The *in vivo* glycemic load of formulated RTC prebiotic flour mixture was investigated among 15 normal subjects. The RTC prebiotic flour mixture had high bulk density (1.154 gm/ml) and better hydration capacity compared to wheat. The conclusion gelatinization temperature was 91.5°C with the gelatinization temperature range of 12°C. One hundred gram of RTC prebiotic flour mixture contains 16.8 gm of protein, 6.6 gm of crude fiber, 33 mg of vitamin C, 7 mg of iron and 159.3 mg of calcium; mean total sensory score was in highly acceptable range (19-25) and grouped under high glycemic index food (mean GI = 77). If this food consumed along with buttermilk, it was considered as good synbiotic food.

KEYWORDS: Food groups**,** Properties, Nutritional composition, Glycemic index, Sensory score.

INTRODUCTION

The human micro flora is a complex ecosystem that plays a part in health and disease of the host. The human body contains approximately 1014 aerobic and anaerobic bacteria found on the skin and mucous surfaces of the oral cavity, upper respiratory tract, urinary tract and vagina, the largest population inhabits of the gastrointestinal tract (Simon, 1986). Therefore, it could be assumed that dietary manipulation of gut micro flora, in order to increase the relative number of "beneficial bacteria" could contribute to the well-being of the host (Gibson and Roberfroid, 1995). The different approach to bacteria, dating back to the early twentieth century is gaining popularity approximately 100 years later and may affect the way many diseases are treated in the future. This approach uses the idea of prebiotic, a general term for nutritional supplements containing non-digestible carbohydrate that, when introduced to a human, have a beneficial impact on the host by selectively stimulating the growth or activity of one (or) a limited number of bacteria in the colon (Girdhari and Ghosh, 2006). The prebiotic compared with probiotic which introduce exogenous bacteria in to the colonic microflora, a prebiotic aims at stimulating the growth of one or a limited number of the potentially health promoting indigenous microorganism, thus modulating the composition of the ecosystem (Macfarlane and Isolauri, 1997) It was felt desirable to develop foods based on known and commonly available food ingredients containing protective as well as therapeutic nutrients such as dietary fiber, blood sugar regulators and micronutrients suitably to provide total nutritional support and that too at price affordable by the needy including consumers belonging to low income group (Simon, 2002). In view of these facts, a ready to cook (RTC) prebiotic flour mixture was formulated and investigated for its properties, glycemic index and synbiotic nature.

MATERIALS & METHODS

Materials

On the basis of nutraceutical property, fiber content and complete protein nature, whole wheat (25%), whole white sorghum (10%), oat grain (10%), finger millet (10%), proso millet (10%), whole green gram (10%), dehusked soya flour (10%), amla fruit powder (5%), drumstick leaves powder (5%) and spice mix (5%) containing fenugreek, coriander and cumin seeds were selected also by considering the inclusion of food from each food group, processed accordingly mixed to prepare RTC prebiotic flour mixture.

Methods

Processing of ingredients

All ingredients were cleaned for impurities, destoned and graded for its maturity. Whole wheat was soaked in water for one hour, sun dried and milled in to flour. Whole white sorghum, finger millet, proso millet were soaked in water for 24 hours, sun dried and milled into flour. Soya bean was also soaked in water for 24 hours, dehusked, steamed for 20 minutes, sundried and milled into flour. The fully matured ripened amla was washed in cold water, removed the seeds, sliced, oven dried at 60°C for 48 hours and ground into powder. The fenugreek seeds, coriander seeds and cumin seeds in equal proportion were roasted and

milled in to flour. The processed ingredients were mixed in mentioned proportion and homogenized to obtain RTC prebiotic flour mixture.

Characterization of RTC prebiotic food

Physical properties

Particle size distribution

One hundred gram of RTC prebiotic flour mixture was passed through the test sieves with mesh size of 22, 30, 44, 52, 62, 72 and 85 BSS unit arranged in ascending order, shaken by rotating in clockwise and anticlockwise direction. The quantity of flour sample remaining in each test sieve was weighed and noted as percentage of flour retained on mesh screen.

Bulk density

Three gram of flour mixture was poured in 10 ml measuring cylinder, tapped for 10 times on a rubber sheet and the volume occupied by the flour measured (Wang and Kinsella, 1976).

$$\text{Bulk density (gm/ml)} = \frac{\text{Weight of the flour (gm)}}{\text{Volume after tapping (ml)}}$$

True density

True density was determined by liquid displacement method in which a known mass of (1gm) sample added in a 10ml graduated a measuring cylinder containing a known volume of (5 ml) toluene and the increase in volume was noted (ASAE, 2001).

$$\text{True density (gm} \qquad \frac{\text{Weight of the flour (gm)}}{\text{Volume of toluene displaced by flour (ml)}}$$

Porosity

Porosity of flour mixture was calculated by following formula (Thompson and Issac, 1967).

$$\text{Porosity} = \frac{\text{True density} - \text{Bulk density}}{\text{True density}} \times 100$$

Falling number

Amylase activity was measured using the Hagberg falling number test. Seven gram of flour mixture was suspended in 25 ml of distilled water using a standard viscometer glass tube. The tube was placed in the falling number apparatus, where the suspension was heated in a water bath at 100^0C and stirred for 60 seconds. The α-amylase breaks down the starch suspension, causing a reduction in the viscosity which was measured using the time taken for a plunger to fall through the suspension. The time in seconds was taken as the Hagberg Falling number (Khetarpaul *et al*., 2005).

Sedimentation value

Sedimentation value measured by sedimentation shaker method used to assess the gluten quality and bread making potential of the flour by observing the way in which a ground wheat or flour suspension coheres and settles. Weighed 3.5gm of flour mixture, mixed with 50ml of bromo phenol solution and shook for 3 minutes in the sedimentation shaker. Then again added 25 ml of reagent solution, allowed it to shake for 5 minutes, rested for 5 minutes and the sedimentation value of the flour mixture was noted. Hard wheat flour having high content of gluten showed high sedimentation value as compared to soft wheat flour (Khetarpaul *et al*., 2005a).

Functional properties

Water/Oil absorption capacity

One gram of flour was weighed (W_s) in to a measuring centrifuge and the content was weighed (W_i). To one gram of flour, 10ml of distilled water/gingelly oil was added and mixed well until the flour gets thoroughly wetted. The tube was kept in a water bath at 30°C for 30 minutes. The tube was then centrifuged at 3000 rpm for 20-30 minutes. The supernatant was measured for cross verification of the result. The weight of the centrifuge tube with the soaked sediment was weighed (W_f) (Janicki and Walczak, 1960; Sosulski *et al*., 1976).

$$\text{Water/Oil absorption capacity (gm/gm)} = \frac{W' - W'}{Ws}$$

Swelling power

One gram flour was weighed (W_s) in a measuring centrifuge and the volume was noted (V_i). To one gram of flour 10ml of distilled water was added and mixed well until the flour was thoroughly wetted. The tube was kept in water bath at 65°C for 30 minutes and then centrifuged at 3000 rpm for 20-30 minutes. The increase in volume of the soaked sediment was noted (V_f) (Leach *et al*., 1959).

$$\text{Swelling power (ml/gm)} = \frac{V_f - V_i}{W_s}$$

Foaming capacity and stability

Thirty ml of aqueous dispersion contain one gram of flour mixture was mixed thoroughly using an ultra –turrax T25 homogenizer at 9500rpm for 30 minutes in a 250 ml graduated cylinder and the total volume of the liquid was measured immediately after 30 seconds. The difference in volume was expressed as the volume of the foam. Foam stability was estimated by measuring the fall in volume of the foam after 60 minutes (Bernardi don *et al*., 1991).

Emulsification capacity

Two gram of flour sample and 23 ml of water were blended for 30 sec using an electric blender at 1600 rpm. After complete dispersion, refined groundnut oil was added continuously from a burette and blending continued

until there was a phase separation. This was observed visually. Emulsification capacity is expressed as milliliters of oil emulsified by gram of flour mixture (Beuchat *et al*., 1975).

Thermal properties

RTC prebiotic flour mixture was analyzed for onset gelatinization temperature (T_o in °C), conclusion gelatinization temperature (T_c in °C), onset gelatinization time (t_o in min), conclusion gelatinization time (t_c in min) using the procedure described by Sethi and Rao (2005).

Nutritional composition

Moisture by hot air oven method, ash by gravimetric method, vitamin C by AVC method, total sugar by Phenol sulphuric acid method, reducing sugar by Lane and Eynon method, non-reducing sugar by phenol sulphuric acid method, total carbohydrate by Anthrone method, crude fiber by acid and alkali digestion method, protein by microkjeldhal method, fat by soxhlet method, iron by Wong's method, calcium by Mccance and Shipp method and phosphorus by Fiske and SubbaRow method (Ranganna, 2004; Sadasivam and Manickam, 2005) were determined.

Sensory profile

Sensory characteristics such as appearance, color, taste, texture and flavor of RTC prebiotic flour mixture and in its synbiotic form (as porridge) was analyzed by 5 point numerical score card in which score 1 indicates poor quality of that criteria and score 5 indicates good quality; considered acceptable if their mean total score was ≤11. Sensory evaluation was done by 20 semi trained panel members from department of Food Science and Nutrition, Periyar University, Salem, Tamil Nadu, India.

Isolation and enumeration of *lactobacillus* species

One gram of RTC prebiotic flour mixture was mixed with 5ml of distilled water and also with 5ml of buttermilk obtained from 6 hours fermented curd. From this one ml was taken, serially diluted (10^{-5} to 10^{-7}) and inoculated on to MRS agar media by pour plate method under aseptic condition. The plate were also incubated at 28±2°C for two days under anaerobic condition (anaerobic condition was established by placing the plates inside jar along with hydrogen and carbon dioxide generator envelope in the presence of palladium catalyst) to determine the total *lactobacillus* count (Schoaafsma, 1996).

$$\text{CFU/gm} = \frac{\text{No. of colonies} \times \text{dilution factor}}{\text{Quantity of sample (gm)}}$$

Determination of glycemic load

Totally 15 adult men and women aged between 20-25 years who had BMI within 20-23 kg/m^2 and fasting blood sugar level between 70-110 gm/dl; clinically free from any nutritional deficiency symptoms were selected to determine the glycemic load of RTC prebiotic and as synbiotic flour mixture. On the first visit, the selected subjects were subjected to oral glucose tolerance test using 25gm glucose load. On a subsequent visit, 50 gm. of RTC prebiotic flour mixture containing 25gm of carbohydrate was given in the form of dosa prepared without oil and as synbiotic food with buttermilk.

$$\text{Glycemic index (GI)} = \frac{\text{incremental area under curve of test food}}{\text{incremental area under curve of standard food}} \times 100$$

$$\text{Glycemic load (GL)} = \frac{\text{Carbohydrate in gm} \times \text{GI}}{100}$$

Cost ratio

The total cost calculation of the product (TCP) per kg was calculated by assessing fixed cost (FC), variable cost (VC) and net profit ratio (NPR) as 10% of sum of fixed and variable cost.

$$\text{TCP} = \text{FC} + \text{VC} + \text{NPR}$$

RESULTS & DISCUSSION

Physical properties

The quantity of flour passed through 62 and above size meshes was 70.77% which indicted the small to medium sized particles. Wankhede and Andrew (1983) suggested that the medium particle sized flour revealed greater water absorption capacity and reduces the dough development time. Premavalli *et al*. (2006) reported that smaller starch granules pack more efficiently and have large surface area available for non-covalent bonding with endosperm protein matrices which results in higher bulk density. This was further proved by high bulk density of the RTC prebiotic flour (Table 1). The falling number of RTC prebiotic flour (Table 1) revealed that flour was less viscous and had high α-amylase activity. The sedimentation value (Table 1) indicates the suitability of RTC prebiotic flour mixture for preparation of bread as per khetarpaul *et al.* (2005) who suggested that the sedimentation value of flour > 60 ml is suitable for bread preparation.

Functional properties

The water absorption capacity (Table 1) of developed RTC prebiotic flour mixture was comparable to wheat cultivars (1.8 to 2.0 gm/gm) and swelling power (Table 1) was significantly less than wheat cultivars (7.6-9.8ml/gm). Premavalli *et al*. (2006) suggested that increased water absorption capacity and swelling power reflects the susceptibility for hydration and better gelatinization. The developed RTC prebiotic flour mixture showed negligible foaming capacity (Table 1) which was stable only for 1.5 minutes. One gram of flour in 25ml of colloidal suspension could capable of emulsifying 14 to 15 ml of gingelly oil.

TABLE 1. Characteristics of RTC prebiotic flour mixture

Characteristics	Values
Physical properties	
Particle size distribution as percentage of flour retained	
22	0
30	5.1
44	12.55
52	19.37
62	26.05
72	31.73
82	33.27
Bulk density (gm/ml)	1.54±0.12
True density (gm/ml)	1.44±0.32
Porosity (%)	0.71±0
Falling number (sec)	110±0
Sedimentation value (ml)	63.5±1.5
Functional properties	
Water absorption capacity (gm/gm)	1.66±0.11
Oil absorption capacity (gm/gm)	1.37±0.04
Swelling power (ml/gm)	4.16±0.00
Foaming capacity (%)	0.65±0.15
Foaming stability (min)	1.53±0.00
Emulsification capacity (ml)	15.14±0.92
Thermal properties	
Onset gelatinization temperature (°C) T_o	81.5±1.5
Conclusion gelatinization temperature (°C)T_c	91.65±0.935
Onset gelatinization time (min) t_o	1.08±0.04
Conclusion gelatinization time (min) t_c	1.3± 0.007
Gelatinization range (T_c - T_o)	12.02±0.01
Nutritional composition	
Moisture (gm %)	8.9±0.2
Total carbohydrate (gm %)	52.8±0.7
Protein (gm %)	16.80±0.0
Fat (gm %)	4.10±0.03
Crude fiber (gm %)	6.64±2.60
Total ash (gm %)	1.44±0.02
Vitamin C (mg %)	33.11±0.95
Iron (mg %)	7.03±0.10
Calcium (mg %)	159.3±3.1
Phosphorous (mg %)	43.115±0.055
Total sugar (gm %)	4.485± 0.055
Reducing sugar (gm %)	1.3±0.1
Non – Reducing sugar (gm %)	3.12±0.02

Values are the average of two determinants.

Thermal properties

The higher conclusion gelatinization temperature (91.65^0C) predicted the low amylose content of RTC prebiotic flour mixture as per Sasaki *et al*. (2007) who reported that starch with higher amylose content showed lower conclusion gelatinization temperature. Flipse *et al.* (1996) also revealed that amylopectin plays a major role in starch granule crystallinity and presence of amylose indirectly lowers the melting point of the crystalline region. The gelatinization temperature range of RTC prebiotic flour mixture was similar to kodo millet, proso millet and barnyard millet flour which revealed the range between 12 to 13^oC reported by Sajeev and Moorthy (2006). Frediksson *et al*. (1998) stated that the range of gelatinization temperature indicates the quality and heterogeneity of starch crystallites and Yuan *et al*. (1993) suggested that the lack of homogeneity of crystallite order structure inside the starch granules might cause a broader gelatinization range. The gelatinization range of RTC prebiotic flour revealed better homogeneity of starch crystallite order.

Nutritional composition

One hundred gram of RTC prebiotic flour mixture (Table1) could able to provide 25% RDA of crude fiber and protein; 75% RDA of vitamin C; 40% RDA of calcium for adult person recommended by ICMR (2010). This proves that RTC prebiotic flour mixture could be considered as balanced multi nutrient supplementary food for both normally and diseased subjects. Simon (2002) suggested that the development of any formulation with concentration of carbohydrates ranging 50 and 60 %, protein ranging between 12 and 17%, lipids ranging

between 4 and 9%, dietary fiber, vitamins and minerals concentration ranging between 12-20% is an embodiment of the invention. The developed RTC prebiotic flour mixture also revealed the concentration of all nutrients within these specified range.

Sensory characteristics

The mean total score (Table 2) of porridge prepared from RTC prebiotic flour mixture and its synbiotic form was in highly acceptable range (19-25).

TABLE 2. Mean sensory score of RTC prebiotic flour mixture and synbiotic form

Criteria	RTC prebiotic food	RTC synbiotic food
Appearance	4.05 ± 0.9	3.85 ± 0.8
Color	3.8 ± 1.0	3.8 ± 0.8
Taste	3.7 ± 0.6	3.75 ± 0.6
Texture	4.1 ± 0.7	4.1± 0.7
Flavor	4.0 ± 0.7	3.8 ± 0.7
Total	19.65 ± 1.9	19.35 ± 2.0

Values are the average of twenty determinants.

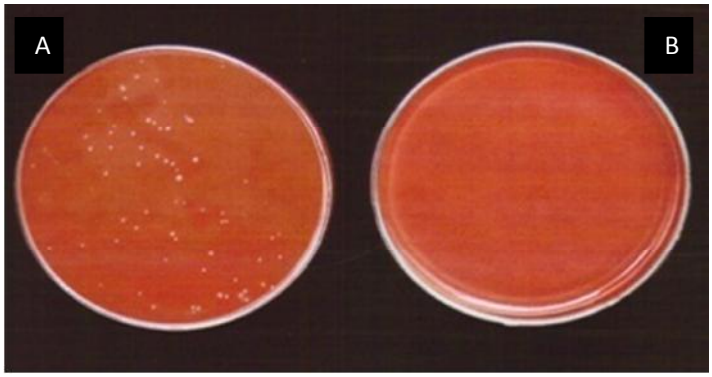

FIGURE 1 Isolation and enumeration of *Lactobacillus* count in RTC synbiotic (A) and prebiotic (B) flour mixture.

***Lactobacillus* count**

Lactobacillus count was negligible in RTC prebiotic flour mixture. But the addition of butter milk to convert it as synbiotic form revealed *lactobacillus* count of $58x10^5$ CFU/ml (Fig.1).

Glycemic load

According to Brand-Miller (2007), the developed RTC prebiotic flour mixture in the form of dosa without oil and seasonings and its synbiotic from was grouped under high glycemic index food. The mean glycemic response, area under curve, glycemic index and glycemic load (Table 3) revealed that the glycaemic index was similar to the range of finger millet based preparations (75 to 85) and lower than rice flour based preparations (79 to 83) reported by Urooj *et al*. (2006).

TABLE 3. Mean glycemic response, area under curve, glycemic index and load of RTC prebiotic flour mixture and synbiotic form

Food items	Blood glucose levels(mg/dl)				Area under curve	Glycemic index	Glycemic load
	0 hour	1 hour	2 hours	3 hours			
Glucose	89.46±22.28	128.93±31.84	112.33±27.57	93.06±22.43	351.0±15.19	100.0±0.00	50.0±0.0
Prebiotic food	84.73±21.79	102.33±25.29	84.866±20.66	79.86±19.29	269.5±15.97	76.89±5.36	38.4±2.0
Synbiotic food	93.67±23.25	144.53±35.03	123.53±29.75	97.80±23.50	280.1±09.96	77.03±3.00	38.5±1.0

Values are the average of fifteen determinants.

Holm *et al.* (1989) reported that the starch digestibility of food vary greatly depending on factors such as degree of starch gelatinization, extent of amylase -lipid complexation, presence of dietary fiber, anti-nutrients, protein and processing technique.

Cost of production

The total cost of production per kg of RTC prebiotic flour mixture was Rs. 25.00 (Table 4).

TABLE 4. Cost of production of RTC prebiotic flour mixture

Expenses/kg	Amount (Rs.)
Personel (A)	**5.00**
Raw ingredients (B)	
Whole wheat	1.5
Sorghum	0.24
Oatmeal	1.9
Finger millet	0.45
Proso millet	0.4
Whole green gram flour	0.8
Dehusked soya flour	0.9
Amla fruit powder	0.004
Drumstick powder	0.2
Fenugreek	0.325
Coriander seeds	0.475
Cumin seeds	1.2
Total (B)	**13.394**
Utilities (C)	
Power	1.00
Water	2.00
Fuel	2.00
Total (C)	**5.00**
Contingency Expenses (D)	
Transport	0.50
Publicity, postage, telephone and stationary	0.50
Depreciation on building @5%	0.25
Depreciation on machine @10%	0.50
Interest on capital investment @12%	0.75
Total(D)	**1.5**
Total cost of production (E)	20.894
Average yield loss cost as10% (F)	2.08
Net profit ratio@10% (G)	2.08
Cost of product per kg (E+F+G)	**25.054**
Valid cost of production per kg	**25.00**

CONCLUSION

The developed RTC prebiotic flour mixture was considered as balanced multi nutrient rich supplementary food or health mix for normal and diseased adult individual. It could be used as wheat flour substitute for the preparation of nutrient enriched bread, biscuits, noodles *etc.* as per its physical and functional properties. Further collaborative research with food industries to develop various nutritious snacks and ready to use foods using RTC prebiotic flour mixture can be explored.

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