Studies on physico-chemical analysis of probioticated malted cereals with *Lactobacillus casei* & their possible applications

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Abstract - The objective of this study was to find out the suitability of different malted cereals for probiotication by using *Lactobacillus casei*. Phyto-chemical analyses of cereals like ragi, pearl millet and jowar were carried out using the standard methods. The carbohydrates, flavonoids, saponins, phenols and glycosides were present where as the alkaloids and steroids were absent in all the above malted cereals. Generally these foods are nutritious and health beneficial. We have chosen them as raw material for our work due to the presence of several macro and micro nutrients. The malted powders of cereals were used to study the probiotication using *Lactobacillus casei* and phyto-chemical and physico-chemical properties were carried out using the stranded methods. Quantitative analysis of antioxidants was carried out using DPPH method, revealed the presence of good amount of antioxidants in all malted and probioticated cereals. The results showed that there is a clear decrease in the amount of total sugars, increase in the flavonoid content, protein content and antioxidant activity. The probioticated cereal food products have more nutritive values than the normal grains. These are beneficial to children, teenagers, pregnant women, lactating women and anemic patients.

Key words: Malted cereals, phyto-chemical analysis, probiotication, physico-chemical analysis, nutritional values.

INTRODUCTION

Ragi (*Eleusine coracana*) is an important staple food in the eastern and central Africa as well as some parts of India (Majumder et al., 2006). It is rich in protein, iron, calcium, phosphorus, fibre and vitamin content. The calcium content is higher than all cereals and iodine content is said to be highest among all the food grains. Ragi has best quality protein along with the presence of essential amino acids, vitamin A and phosphorus (Gopalan et al., 2004). Thus ragi is a good source of diet for growing children, women, old age people and patients. Ragi provides highest level of calcium, antioxidants properties, photochemical, which makes it easily and slowly digestible. Hence it helps to control blood glucose levels in diabetic patients very efficiently. The bulkiness of the fibres and the slower digestion rate makes us feel fuller on, fewer calories and therefore may help to prevent us from eating excess calories. Pearl millet (*Pennisetum glaucum*) is a nutritious cereal grown on about 10 million ha in India, which is the largest producer of this crop in the world. Pearl millet is the most widely grown type of millet Pearl millet grains are usually made up of 70% carbohydrates and consist almost exclusively of starch. The starch itself is composed of two third amylopectin and one-third amylase. It has no husk, no tannin, contains 5-7% oil and has higher protein and energy levels than maize or sorghum. Pearl millet is also rich in vitamins, potassium, phosphorus, magnesium, iron, zinc copper and manganese (Baker, 2003). Jowar or sorghum (*Sorghum bicolor* (L.) Moench) is one of the major staple food grain crops in India. Out of the total area under jowar cultivation in India, 50% is cultivated in Maharashtra. Pearl millet, like sorghum, is generally composed of 9-13% protein but large variations in protein content, ranging from 6-21%, have been observed (Baker, 2003).

Germination is a natural process occurred during growth period of seeds in which they meet the minimum condition for growth and development (Sangronis et al., 2006). During this period, reserve materials are degraded, commonly used for respiration and synthesis of new cells prior to developing embryo (Vidal-Valverde, 2002). The process starts with the up-take of water by the quiescent dry seed and terminates with the emergence of the embryonic axis, usually the radical (Bewley and Black, 1994). Several studies on the effect of germination on legumes found that germination can increase protein content and dietary fiber; reduce tannin and phytic acid content and increase mineral bioavailability (Rao and Prabhavathi, 1982; Ghavidel and Prakash, 2007). Germination also was reported to be associated with increase of vitamin concentrations and bioavailability of trace elements and minerals found that germination improves calcium, copper, manganese, zinc, riboflavin, niacin and ascorbic acid content (El-Adawy et al., 2004). One of the greatest constraints in the popularization and commercialization of food grains has been its branding as poor man's food, samples prepared with combinations of malted grains were rich in calcium, iron, phosphorus, and crude fiber. The main objective of this study was to determine the malted cereals act as fermented media for the growth of probiotic *Lactobacillus casei* and study of the physico-chemical properties of probioticated malted cereals product.

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MATERIALS AND METHODS

Malting and preparation of flour from the cereals

The malting of cereal grains was carried out using the following methodology (Fig.1). The cereal grains were washed with water five times and soaked in water for 24 h. Later excess water drained and seeds were tied in a muslin cloth and weight was kept on it. The seeds were kept for 36 h at 27±3°C and allowed to germinate. The germinated seeds were dried in shade for 2 days and grounded to make malt flour by using the electric grinder.

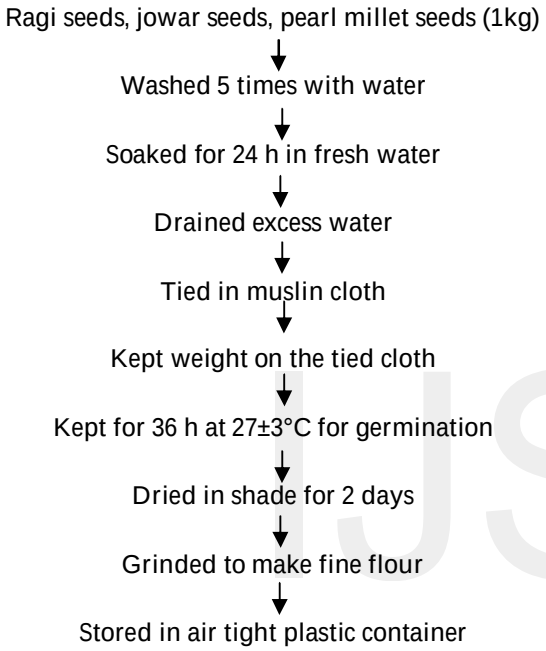

Fig. 1 Flow chart of malting process of cereals.

Phyto-chemical analysis

The analysis was carried out by using the standard methods (Harborne, 1992).

Test for carbohydrates

Carried out using the Fehling's and Benedict's test. The germinated powered filtrates were hydrolyzed with dilute HCl neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugar. Filtrates were also treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Detection of flavonoids

Carried out using alkaline reagent test. The germinated and non-germinated powdered were treated with few drops of NaOH solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Detection of tannins

Carried out using gelatine test. To the fruit juice, 1% gelatin solution containing NaCl was added. Formation of white precipitate indicates the presence of tannins.

Detection of glycosides

Carried out using Legal's test. Malted powders were treated with sodium nitropruside in pyridine and NaOH. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of alkaloids

Carried out using Wagner's test. Malted powders were treated with Wagner's reagent (iodine in potassium iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of saponins

Carried out using foam test. 5 ml of germinated crude extract was shaken with 2 ml of water. If foam produced persists for ten minutes indicates the presence of saponins.

Detection of proteins

Carried out using 2 ml of 0.2% solution of crude sample when boiled with ninhydrin, violet colour appeared indicates of the presence of amino acid and protein.

Detection of iron

Carried out using 2 ml of test samples and add few drops of concentrated nitric acid boil the solution then cool and add 2-3 drops of potassium sulpho cyanide solution.

Detection of calcium

Carried out using 2 ml of test samples add ammonium chloride and ammonium hydroxide. Filter the solution and to the filtrate add 2 ml of ammonium oxalate solution white precipitation indicates the presence of calcium.

Determination of pH

It was measured by digital pH meter (Eutech, Japan) pre calibrated with buffers of pH 4.0 and 7.0.

Determination of TSS

Total solid substances was determined using hand refractometer (0-30) (Erma, Japan) in terms of Bx (Brix).

Titrable acidity was carried out by the method of Ranganna et al., (2001).

Preparation of fermentation media

Fermentation media was prepared by dissolving 5 g of each malted powder (ragi, jowar, pearl millet) in 100 ml distilled water. The starch present in the fermentation media was first gelatinized and partially hydrolyzed by keeping the suspensions in a water bath at 95±5°C for 1 h. The media was cooled to room temperature and then filtered. After malted suspensions were autoclaved at 121°C. Then aseptically inoculated (>10^5 CFU/ml) with 24 h old *Lactobacillus casei* MRS broth culture, All fermentations were carried out at 37°C. The absorbance was read at 600 nm for cell growth. Physico-chemical properties were determined by different time intervals.

Determination of the total flavonoids content of malted cereals and probioticated cereals

The total flavonoid content of malted cerelas was measured using colorimetric method described by Chang et al., (2002). Aqueous and ethanolic extracts that has been adjusted to come under the linearity range and different dilution of standard solution were added to 10 ml of volumetric flask containing 4 ml of water. To the above mixture 0.3 ml of 5% $NaNo_2$ was added. After incubating 5 min 0.3 ml of 10% $AlCl_3$ was added. After 6 min, 2 ml of 1M NaoH was added and the total volume was made up to 10 ml with distilled water. Then the solution was mixed well and the absorbance measured against a freshly prepared reagent blank at 415 nm. The total flavonoid content of the samples was estimated from the standard curve prepared using rutin and expressed as mg rutin (mg RE/L).

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity

The assay was carried out according to the method described earlier (Vijayakumar et al., 2013) with some modifications. A stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and then stored at 20°C. The working solution was obtained by mixing 10 ml stock solution with 45 ml methanol to obtain an absorbance of 1.1±0.02 units at 517 nm using a spectrophotometer. Different volumes of various probioticated cereals and malted cereals (50-200 µl) were allowed to react with DPPH solution (final volume 4 ml) and were shaken vigorously and allowed to stand for 30 min in dark at room temperature. Methanol was used as a blank. BHT (butylated hydroxyl toluene) was used as a standard. A control sample with no added cereal aqueous extract was also analyzed and radical-scavenging activity (% inhibition) was calculated using the following equation.

DPPH free radical scavenging activity (%) = [(A control - A sample) /A control] × 100 (A = absorbance value at 517 nm)

Table 1. Phyto-chemical analysis of malted and probioticated cereals.

S. No	Test name	Ragi	Pearl millet	Jowar	P.R	P.P	P.J
1	Carbohydrates						
	(a) Fehlings test	+	+	+	+	+	+
	(b) Benedict's test	+	+	+	+	+	+
2	Phenols and tannins	+	+	+	+	+	+
3	Flavonoids (alkaline reagent test)	+	+	+	+	+	+
4	Saponins	-	-	-	+	+	+
5	Glycosides (Salkowskis test)	+	-	-	+	-	-
6	Steroids	-	-	-	-	-	-
7	Iron	+	+	+	+	+	+
8	Calcium	+	+	+	+	+	+
9	Proteins	+	+	+	+	+	+

(- indicates absence; + indicates presence; P.R, P.P and P.J indicates malted and probioticated cereals namely Ragi, Pearl millet and Jowar)

Fig. 2 Germinated cereals and their malted flours.

Ragi

Perl millet

Jowar

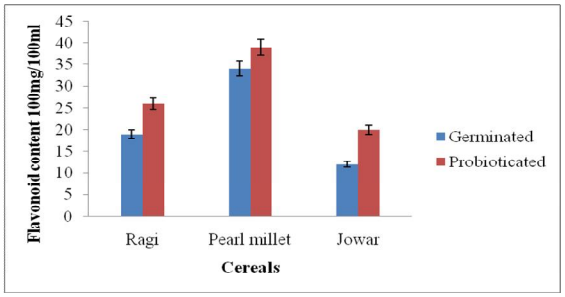

Fig. 3 Flavonoid content of malted and probioticated cereals.

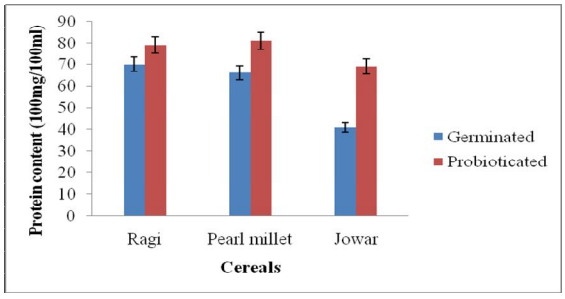

Fig. 4 Protien content of malted and probioticated cereals.

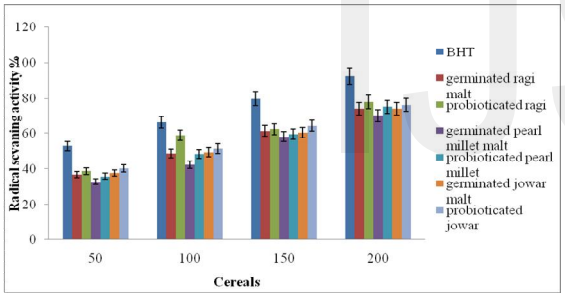

Fig. 5 Antioxident activity of malted and probioticated cereals.

Table 2. Physico-chemical properties of malted and probioticated cereals at differnet time intervels.

Name of the grains	Time Interval (h)	TSS Brix %	Titrable acidity mg/100ml	pH	Reducing Sugars mg/100ml
Ragi	24	3.0	11.3	4.5	680
	48	2.5	13.2	3.5	410
	72	2.1	15.4	2.6	120
Pearl millet	24	4.0	9.5	4.7	490
	48	3.0	10.5	3.6	370
	72	2.5	12.8	2.7	170
Jowar	24	1.5	12.3	4.8	180
	48	1.0	13.5	3.8	120
	72	0.5	14.1	2.5	50

*Values are mean of three replicates (± standard deviation).

RESULTS AND DISSUSSION

In this study we have taken three types of cereals namely ragi, pearl millet and jowar. Generally these foods are nutritious and health beneficial due to the presence of several macro and micro nutrients. To increase the amount of nutrients in these cereals have carried out a process called malting, in which the seeds undergo germination for 48 h of time. Malting increases the enzyme activities and helps to breakdown of macro substances into micro substances. This in turn helps in easy digestion.

The results of the phyto-chemical analysis of malted and probioticated cereals were presented in Table. 1 clearly indicates the improvement in the nutrients. The results presented in Table. 2 indicates the physico-chemical properties of malted and probioticated cereals at differnet time intervels. Fermented foods are good source of beneficial microorganisms like lactic acid bacteria. After fermentation the nutritive values of the particular cereals are increased. In all cereals (ragi, perl millet and jowar) during probiotication the amount of flavoniods (Fig. 3), proteins (Fig. 4) and antioxidants are increased (Fig. 5), while reducing sugars and pH are decreased (Table. 2).

Antioxidants are important as free radical scavengers and the proteins are helpful for tissue repair. Recently, attempts have been made for fortification of finger millet flour with iron and zinc. In germinated mung and kidney beans and decreased significantly in germinated white, black, red and brown rice ($p< 0.05$). Vidal-Valverde et al. (2002) explained that during germination, carbohydrates were used as source of energy for embryonic growth which could explain the changes of carbohydrate content after germination. The α-amylase activities were found to parallel with the pattern of starch breakdown. An increased radical scavenging and reducing power activity has been shown in roasted little millet (Pradeep & Manisha, 2011). Generally these foods are nutritious and health beneficial.

Our results shown that growth of *L. plantarum* is significantly enhanced the media containing malted cereals. The antioxidant activity of free and bound phenolic acid mixture was found to be significantly lower than that of synthetic antioxidants such as BHT (Fig. 4). However, no correlation was made between the phenolic acid content and antioxidant activity in some varieties of barley (Schoonen et al., 2002). Similar observations were also made in the case of probioticated malted grains for which the antioxidant activity of methanolic extracts was found to be lower than that of BHT and gallic acid (Schoonen et al., 2002). The antioxidant activity of the free phenolic acid mixture, in the present study, was found to be higher compared to that of the bound phenolic acid (Fig. 4). This is

in contrast to the results reported for some malted cereals, for which higher antioxidant activity was reported in bound phenolic acids (Imeh et al 2002).

Further studies like organoleptic properties and storage studies are under progress. We are also aiming to develop fermented products and that are formulated with other supporting LAB strains to develop a novel probiotic product acceptable by consumers. The non-digestible components in the fermented cereal media should also be studied for their potential as probiotics. One of the greatest constraints in the popularization and commercialization of food grains has been its branding as poor man's food, samples prepared with combinations of malted grains were rich in calcium, iron, phosphorus and crude fiber. Higher mineral and fiber is useful for easy digestion. Hence the malted grain cereals prepared by supplementation with malted ragi, pearl millet and jowar will be beneficial to growing children, teenagers, pregnant women, anemic patients and used for diabetic patients.

CONCLUSION

The results of this study indicate that the *Lactobacillus casei* exhibited better growth and survived at 37°C, which could be attributed to its chemical composition in probiotication of malted cereal media. It was also observed that the low pH could be the main limiting factor for the growth of *L. casei*.

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