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Study on nutritional changes in ragi koozh under controlled fermentation with probiotics

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Abstract
Ragikoozh was prepared from ragi grains by natural fermentation and by inoculating probiotics, *Lactobacillus acidophillus and Bifidobacterium sp*. The purpose of the investigation was to reveal the nutritional beneficial changes brought during fermentation and compare the probiotics fermented and naturally fermented ragikoozh. Ragikoozh fermented for 24 hours under natural condition and probiotic inoculated together with unfermented control were analysed for the proximate composition, vitamins like thiamine, riboflavin, niacin and essential amino acids. Physical parameters like pH, acidity, moisture and total solids were also determined.
Processing of ragi into ragi koozh by fermentation brought about favourable nutritional changes. Protein, reducing sugars, iron, riboflavin and niacin were increased while thiamine concentration reduced considerably. Amino acids were found to be increased especially the lysine content which is a limiting factor in cereal. On comparison the probiotic fermented ragikoozh performed better than naturally fermented ragi koozh. Ragi koozh prepared with probiotic was found to be more acceptable than natural fermented ragi koozh on sensory evaluation.

Keywords: Ragi, probiotics, *Lactobacillus acidophilus and Bifidobacterium sp*

Introduction
Food is power. Food is celebration of plenty". Food is at the very of centre of human development. The right to food is the most fundamental of human rights. Without foods nothing happens; no economical endeavors, no science or engineering, no music or literature not even procreation. Without proper food, people can surely survive but will remain deprived of fruitful living. A balanced food is considered as a merit for life (Bhagavat Gita)
The coarse grains are comparable to major cereals with respect to nutritive value, but their consumption is restricted only to people from socioeconomically weaker section of society. Ragi koozh is one of the traditionally fermented beverages which are consumed extensively in Tamil nadu. The drink is believed to be a good health drink and as not only an ethnic value but also a religious touch. However, the drink is prepared under uncontrolled and unhygienic condition involving undefined micro flora. The present concept of food consumption is not only for energy or Pal ability but also for sustaining health of the individuals. So health oriented food and neutraceuticals are major concerns of today's consumers. In this context, the trend is to develop food with health promoting microorganisms called as probiotics. The incorporation of *Lactobacillus acidophilus and Bifidobacterium sp*. in yogurt produced a milk product with an excellent therapeutic value (Kailas apathy and Rybka, 1997). Therefore the objective of the present study was to find out the nutritional improvement by the probiotic inoculation in ragi koozh on comparison with naturally fermented ragi koozh. The present investigation compared a food chemical evaluation of unfermented control, naturally fermented ragi koozh and probiotic fermented ragi koozh.

Materials and methods
Raw materials
Ragi grains were obtained from departmental stores in Coimbatore.

Preparation of Ragi Koozh and Unferemented Control
The ragi grains were cleaned and powdered and sieved. The sifted flour was cooked by boiling with water with continuous stirring. To the cooked ragi flour sterilized water was added and inoculated with probiotics *Lactobacillus acidophilus and Bifidobacterium* culture for probiotic fermented ragi koozh (T3) while in T2 it was left for natural fermentation. In unfermented control the similar process was followed and refrigerated. The fermentation in the two treatments was carried out for twenty four hours.

Analytical Methods

Protein (Microkjeldhal, nitrogen 6.25; AOAC 2000) [1] carbohydrate (Hedge and Hofrieter 1962) [10] reducing sugars (Somogyi, 1952) [14] phosphorus and potassium (Jackson 1970) were determined according to standard procedure. Moisture content and acidity of the sample was analysed according to AOAC method 2000 [1]. Titrable acidity is expressed in terms of lactic acid and volatile acid in terms of acidic acid. Total solids of fermented products were determined by drying weighed sample of the homogenate at 50^0c in vacuum for 24 hours. Iron was determined by atomic absorption spectrophotometery. Vitamins like thiamine, riboflavin and niacin were determined according to AOAC method 2000 [1]. Quantitative aminoacids analysis was performed by paper chromatography method described by (Hemantharao and Subramanian 1970) [9].

Survival Study of Probiotics

The survibality of the introduced probiotics in ragi koozh up to twenty four hours of fermentation was carried out. Samples were taken every five hours and population of the both the culture were enumerated by plate count method in MRS medium for Lactobacillus and TPY medium for Bifidobacterium.

Sensory Evaluation

The sensory evalution of food was determined by 9 point hedonic scale (Larmond, 1977) and was judged by ten untrained persons to know the quality of the prepared food.

Safety of the Fermented Food

The fermented food developed was tested for the presence of *Clostridium*, *Streptococcus* and *Bacillus cereus* in Sulphite agar medium, and Baird parker and Nutrient agar medium by pour plate method.

Result and Discussion

Physiochemical properties of the unfermented ragi koozh (T1), naturally fermented ragi koozh (T2) and probiotic feremented ragikoozh (T3) are elucidated in table 1. Amino acid content is elicited in table2. Vitamin content is illustrated in figure 1.The survival study of the probiotics is presented in Table3.

The difference between the unfermented control and ragi koozh fermented under natural and probiotic fermentation were attributable to the effect of fermentation. Due to fermentation the protein content was found to increase significantly in both naturally fermented ragi koozh (10.10g/100g) and probiotic fermented ragi koozh (10.50 g/ 100g). The reducing sugar content was also found to increase significantly under both conditions of fermentation. The carbohydrate content was found to be reduced after fermentation. The dramatic reduction in carbohydrate content may be due to the utilization by the micro flora as carbon and energy source. The increase in protein may be related to reduction in carbohydrate and partly by the biomass produced during fermentation. This corroborates well with Basappa 2002 [2]. There was no change in phosphorous and potassium while the iron content was found to increase after fermentation. Comparatively the probiotic culture fermented the grain efficiently than the natural fermentation. This may be due to the higher overall initial concentration of the starter.

Table 1: Physicochemical properties of ragi koozh

Treatment	Uninoculated control	Naturally fermented koozh	Naturally fermented koozh
Moisture %	42.0	83.0	80.0
pH	6.5	3.5	3.7
Titrable acidity g/100g	0.0	3.0	2.8
Volatile acidity g/100g	0.0	0.03	0.03
Total solids g/100g	58.0	18.0	20.0
Protein g/100g	7.3	10.10	10.50
Carbohydrate g/100 g	72.0	35.0	39.0
Fat %	1.2	1.6	1.8
Potassium mg/100g	0.270	0.270	0.270
Iron mg/100g	0.003	0.0046	0.0046
Calcium mg/100g	0.32	0.35	0.035

Thiamine content was found to increase under naturally fermented ragi koozh while it decreased under probiotic fermented ragi koozh. This is may be due to the fact the probiotic culture did have the synthetic capacity for thiamine. Three fold increased in riboflavin and niacin content was observed in fermented ragi than uninoculated control. Similar result was obtained by Steinkraus (1994) [15] in the production of kefir beverage where riboflavin and niacin content almost doubled. Fermentation generally enhances the nutritive value of the food by increasing thiamine, nicotinic acid and riboflavin as the result of microbial activity. B group vitamin content generally show increase on fermentation of cereals.

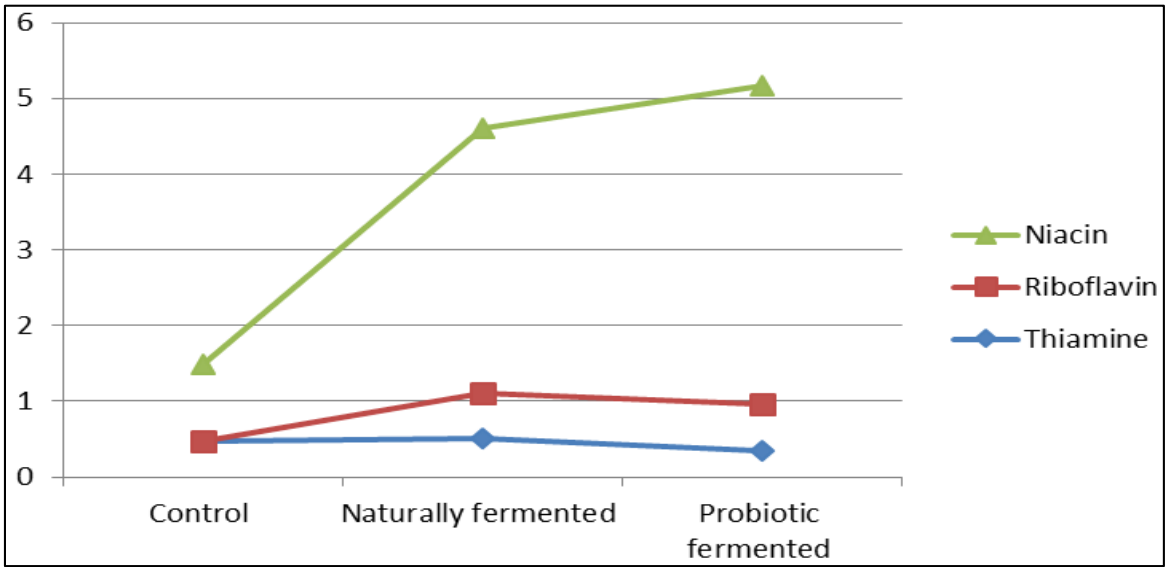

Fig 1: Vitamin content of ragi koozh

The ragi koozh fermented with probiotic yielded more amino acids than natural fermentation. In general the fermented ragi koozh under both natural and probiotic condition yieded more aminoacid content with high concentration of valine, threonine, leucine and phenylalanine. Lysine which is a limiting aminoacids was found to increase by 19 times its initial content. Hamad and Fields (1979) reported that natural fermentation of cereals increases the relative nutritive value and available lysine. Increase in amino acids may be due to synthetic capacity of microbial population. This finding corroborates well with Cronk *et al* (1977) [5]. Lysine is reported to be excreted by strains of *Lactobacillus plantarum* (Newman and Sands 1984). Bacterial fermentation involved in proteolytic activity is reported to increase the biological availability of essential amino acids more than yeast fermentation which mainly degrades carbohydrates (Chavan and Kadam 1989) [4].

Table 2: Amino acid content of Ragi koozh

Amino acid g /100g	Control	Naturally fermented	Probiotic fermented
Arginine	1.2	4.3	6.7
Histidine	0.5	5.5	5.5
Lysine	0.1	1.9	1.9
Phenylalanine	0.0	4.8	7.1
Tyrosine	0.1	1.0	0.7
Methionine	0.6	1.2	1.4
Threonine	0.3	5.8	6.0
Cystine	0.0	2.3	1.7
Leucine	0.1	1.0	2.0
Isoleucine	0.2	1.5	1.5
Valine	0.7	3.0	3.0

The pH decreased after fermentation with increased acidity. The total solid content was reduced considerably after fermentation. The increased acidity is due to production of lactic acid by probiotic culture in T2. The increased acidity under natural fermentation may due to the acid secreted by the undefined micro flora. The solid content decreased due to increase in volatile solids.

The survival study of the introduced probiotic population was found to increase upto 15 hours of fermentation and declined further. Lee (1977) reported that lactic acid bacteria grown in kimchi could survive in acid and bile juice. After fifteen hours the reduction in population may be due to the less availability of nutrients and increased in acidity. The sensory evaluation revealed the acceptance of both fermented ragi koozh. Probiotic fermented ragi koozh was found to be more preferable than naturally fermented.

Table 3: Viability of probiotics in ragi koozh

Duration of fermentation (hrs)	*Lactobacillus acidophilus* log cfu / ml	*Bifidobacterium Sp* log cfu / ml
0	2	2.5
5	6	6.0
10	6.5	6.0
15	8	7
20	8	7
24	6.5	6.0

On the safety aspect fermented ragi koozh revealed negative for the presence of *Staphylococcus, Clostridium and Bacillus*. In ragi koozh prepared under natural and probiotic fermented condition lactic acid bacteria predominates. An important feature of lactic acid bacteria apart from lactic acid production is their ability to produce secondary metabolite like bacteriocin and other antimicrobials which prevents pathogens and spoilage microorganisms. The role of the live lactic acid bacteria in preventing pathogenic and spoilage bacteria is well known. The bacteriocin produced by lactic acid bacteria has gained much attention as a potential useful food additives against food pathogens. Nisin for instance is a 34 residue peptide that is very active against gram positive bacteria including spore formers like clostridium, botulinum (Mouri 1991; Flevery *et al.,* 1996) [13, 6].

Conclusion

This investigation of ragi koozh serves largely to substantiate the earlier findings concurring nutritional improvement by fermentation. Maximum nutritional advantage of higher content of protein, reducing sugar, iron, higher level of riboflavin, Niacin and more amount of amino acids especially lysine were gained in ragi koozh fermented with probiotic culture on comparison to natural fermentation. Both ragi koozh was found to be authentically and organoleptic ally acceptable and could pave way for yet another better method of utilizing small millet like ragi.

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