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Chemical and biological evaluation of the effects of fermentation on the nutritive value of foods prepared from rice and grams

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1. Studies were made of the effects of fermentation on the chemical composition and nutritive value of Idli, a product prepared from milled rice and dehusked black gram, and also of Khaman, a product prepared from dehusked and milled bengal gram.

2. Fermentation brought about an increase in thiamine and riboflavine contents and a decrease in phytate content in both materials.

3. Weanling rats fed on fermented Idli and Khaman were found to be superior to those fed on the corresponding unfermented product with regard to weight gain, nitrogen retention, thiamine and riboflavine contents of the liver and the activities of liver xanthine oxidase and succinic dehydrogenase and haemoglobin content of blood.

After cereals, pulses are the chief sources of proteins in the Indian diet. They not only have a high protein content but also supplement the low lysine content of cereal proteins. However, they are not used to any great extent in the feeding of infants because of the belief that they are not easily digestible. Boiled legumes of the 'dry' type are not easily accepted and tolerated by infants. Thin soups are more suitable, but their nutrient content is diluted. Fermented foods such as Idli and Khaman based on legumes or cereal–legume mixtures are very popular. It should be possible to incorporate fermented foods made from cereal–legume combinations or legumes into infant diets, but convincing evidence is lacking of their superiority to unfermented foods.

The reported changes during fermentation include an increase in free sugar, non-protein nitrogen (Desikachar, Radhakrishnamurthy, Rama Rao, Kadkol, Srinivasan & Subramanayan, 1960), free nicotinic acid (Rajalakshmi, Nanavaty & Gumastha, 1964), methionine and choline in Idli (Radhakrishna Rao, 1961), thiamine, riboflavine and nicotinic acid in fermented cattle feed (Gunther, 1960), and a breakdown of phytate in bread dough (Davidson, Meiklejohn & Passmore, 1963) and of trypsin inhibitors in certain fermented legume preparations (Aykroyd & Doughty, 1964). These changes would be highly significant for practical nutrition if they represent the general pattern of changes during fermentation.

The improved value of fermented foods has been indicated by a number of animal experiments. The protein in tempeh, a fermented preparation from soya beans, was found to have a greater biological value for rats than unfermented soya beans (György, 1961). The protein efficiency ratio (PER) of tempeh was found to match that of skim-milk powder. Prolonged feeding with an unfermented soya-bean preparation produced hepatic injury in rats which did not occur in animals given tempeh. Fatty infiltration of the liver caused by feeding rats with a high-fat, low-protein diet could

be more effectively treated by Idlis prepared from the fermented batter than by those from unfermented batter (Radhakrishna Rao, 1961). Substitution of Idli for cooked rice in a poor rice diet increased the nutritive value (Kantha, Narayana Rao, Indiramma, Swaminathan & Subramanayan, 1961). However, in the latter study the Idli diet differed not only with regard to fermentation but also in legume content.

The present paper describes studies on the effects of fermentation of Idli and Khaman on their chemical composition and nutritive value for rats.

EXPERIMENTAL

Preparation of Idli and Khaman

Approximately 55 ml of water were poured over approximately 12 g black gram dal and the first washing was discarded; 110 ml more water were added and the dal was steeped in this for 1 h. The dal was then rubbed lightly with the fingers and the washing drained off into a beaker to be used as inoculum. The inoculum was added to a mixture of 20 g coarsely ground rice, 10 g finely ground black gram dal and 2 g common salt and the batter prepared by adding water to a volume of 60 ml. This formed a batter for the preparation of fermented Idli. Approximately 12 g sago were washed with water and the washing was discarded; 55 ml more water were added and the washing was drained off after 1 h to be used as inoculum. Coarsely ground bengal gram (30 g) was mixed with 2 g common salt and a loose dough was prepared by the addition of the washing from sago made up to 50 ml with water.

The batter in both instances was mixed thoroughly and beaten with a rotary motion so as to provide aeration, kept covered and allowed to ferment at 37° for 15 h. At the end of incubation, the batters were steamed in a steaming vessel, cooled, cut into pieces, dried in an oven at 60° to constant weight, ground to a powder, stored in aluminium containers with air-tight lids and used for chemical and biological evaluations. For the control, unfermented samples, the batters were steamed before incubation.

The fermented and unfermented samples of Idli and Khaman prepared in the above manner were analysed for moisture, crude protein, thiamine, riboflavine, total and phytate phosphorus.

Biological evaluation

For biological evaluation of the fermented foods, forty albino rats were used with a mean initial weight of 45 g. They were divided into four groups of ten animals each, matched for weight and sex. As far as possible litter-mates were assigned to the different groups. The rats were caged individually.

To 100 g of the food samples, prepared as described earlier, 10 g groundnut oil and 2 g salt mixture were added, the oil being added at the time of feeding. The salt mixture was calculated to supplement the mineral content of the test foods, as calculated from the food tables (Aykroyd, 1963), so as to meet the requirement of the rats. It was made up of (parts by weight): calcium citrate 308, calcium carbonate 137, $Ca(H_2PO_4)_2.H_2O$ 56, crude sodium chloride 80, ferric ammonium citrate 9, copper sulphate 0·59 and manganese sulphate 0·11. In addition, 3 drops of shark-liver oil, as a source of vitamins A and D, were added to 10 g of food once a week. No other

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vitamins were added, as this study was designed from the standpoint of practical nutrition and it was considered better to give the diets without supplementation.

The animals fed on the fermented Idli or Khaman were pair-fed with those fed on the unfermented product. The food intakes of the two groups fed on Idli and of the two groups fed on Khaman were found to be nearly the same, approximately 6 g/day. Weights of the rats were recorded every 3rd day. The haemoglobin content of blood obtained from the tail vein was determined at the beginning and end of the experiment by the acid haematin method. Nitrogen balance studies were carried out during a 3-day period at the end of 4 weeks. The nitrogen contents of the urine, faeces and diet were determined by the micro-Kjeldahl method.

Chemical evaluation

About a week after the nitrogen balance studies, the animals were killed by decapitation for biochemical determinations. Animals from the different experimental groups were killed simultaneously, one animal being selected from each group at a time. The abdomen was opened and the liver quickly removed and freed from adhering blood and connective tissues. A portion of the liver was used for enzyme studies and the remainder for the other determinations.

Moisture. Moisture was determined by drying at 60° to constant weight.

Crude protein. Nitrogen content was measured by the conventional micro-Kjeldahl method; for food samples 1 g was used and for liver samples about 0·2 g. Protein content was calculated as N × 6·25.

Thiamine and riboflavine. These were determined by the method described by the Association of Vitamin Chemists (1951).

Total phosphorus. The sample was ashed by the addition of concentrated sulphuric and nitric acids and made up to 50 ml with water. Phosphorus was estimated by the method of Fiske & Subbarow (1925).

Phytate phosphorus. This was estimated by the method of McCance & Widdowson (1935).

Glutathione. The nitroprusside method of Grunert & Phillips (1951) with slight modifications was used. A known amount of tissue (0·1–0·2 g) was homogenized in 3 ml 3% (w/v) metaphosphoric acid. To 1 ml of the homogenate, 6 ml saturated sodium chloride and 2 ml water were added and the mixture was incubated at 20° for 10 min. After incubation, a solution containing 20 mg of sodium nitroprusside in 1 ml water was added followed by the addition of 1 ml of 2% (w/v) sodium cyanide solution saturated with sodium carbonate. Intensity of the resulting colour was measured at 520 nm, 2 ml of 2% metaphosphoric acid being used as blank.

Xanthine oxidase. The assay system used was similar to that of Luck (1963). A 10% liver homogenate was prepared by homogenizing the liver in a Potter–Elvehjem homogenizer at 0° for 1 min with 2% (w/v) sodium fluoride in water as the grinding medium. The reaction mixture contained: xanthine, 0·5 μmole; methylene blue, 0·25 μmole; sodium phosphate buffer, pH 7·4, 200 μmoles; and enzyme extract, 1·5 ml. The mixture was incubated in Thunberg tubes at 37° and the time taken for complete reduction of methylene blue measured. The blank contained all the components

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except the substrate. The factor suggested by Luck was used to derive enzyme units from the time taken.

Succinate dehydrogenase. The assay system used was similar to that of Srinivasamoorthy & Swaminathan (1955). A 10% liver homogenate was prepared by homogenizing the liver in a Potter–Elvehjem homogenizer at 0° for 1 min, with 0·1 M-sodium phosphate, pH 7·4, as buffer. The reaction mixture contained: sodium succinate, 25 μmoles; 0·1 M-phosphate buffer, pH 7·5, 0·5 ml; 2% 2,3,4-triphenyltetrazolium choloride (TTC), 0·1 ml; and enzyme extract, 0·2 ml. The mixture was made up with water to 3 ml and incubated at 37° for 30 min. The colour was extracted with 6 ml toluene and the colour intensity of the toluene layer measured at 540 nm. The blank contained all the components except the substrate. Enzyme activity is expressed as μmoles TTC reduced per g wet tissue per h at 37°.

RESULTS AND DISCUSSION

The organism involved in Idli fermentation was identified as *Leuconostoc mesenteroides* and was found to be present in washings from black gram dal (*Phaseolus mungo* L.). The organism dominant during fermentation of Khaman was identified as a Lactobacillus type and found to be the same as that present in the washings of sago (R. Rajalakshmi & K. Vanaja, unpublished).

Table 1. *Composition of fermented and unfermented Idli and Khaman*

	Amount in 100 g dry material					
				Phosphorus (mg)		
Food	Protein (g)	Thiamine (mg)	Riboflavine (mg)	Total (1)	Phytate (2)	(2) as % of (1)
Unfermented Idli	13·9 (13·7–14·9)	0·21 (0·20–0·25)	0·25 (0·24–0·29)	262 (244–281)	174 (156–200)	67
Fermented Idli	14·0 (13·9–14·1)	0·58 (0·51–0·64)	0·54 (0·43–0·60)	241 (230–252)	113 (104–126)	47
Unfermented Khaman	21·9 (21·8–22·0)	0·53 (0·49–0·54)	0·26 (0·24–0·28)	322 (311–333)	211 (193–215)	66
Fermented Khaman	22·0 (21·9–22·4)	0·79 (0·77–0·84)	0·75 (0·70–0·80)	270 (259–282)	135 (114–156)	50

Values are means of four to seven determinations with range shown in parentheses.

Table 1 shows the chemical composition of fermented and unfermented Idli and Khaman. As expected, no changes were found in protein content. Thiamine and riboflavine contents showed large and significant increases with fermentation in both Idli and Khaman. Phytate phosphorus decreased by about 30% in both materials. The changes are of considerable importance from the standpoint of practical nutrition, particularly as cereal-based diets are often deficient in riboflavine and their high phytate content interferes with the absorption of both calcium and iron. The change in phytate phosphorus is consistent with reports of the appearance of phytase activity during fermentation of bread dough (Davidson, Meiklejohn & Passmore, 1963).

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Ananthachar & Desikachar (1962) found no change in thiamine content during fermentation of Idli, possibly because the technique of fermentation employed by them was different, with yeast and a Lactobacillus organism participating in the fermentation, whereas in our study the organism involved was *Leuconostoc mesenteroides*. In this connexion, changes in the thiamine, riboflavine and nicotinic acid contents of milk during fermentation to curd have been found to depend on the organism employed and its metabolic requirements and synthesizing ability (Rao & Basu, 1952).

Table 2. *Results for rats fed on fermented and unfermented Idli and Khaman*

(Mean values with their standard errors and ranges; ten animals/group except for the determination of glutathione for which only five animals from each group were used)

	Unfermented Idli	Fermented Idli	Unfermented Khaman	Fermented Khaman
Initial body-weight (g)	45 (41–51)	45 (40–51)	45 (41–48)	45 (41–48)
Weight gain in 4 weeks (g)	30 (24–39)	37 (24–43)	37 (34–39)	45 (38–49)
Mean food intake in 4 weeks (g)	148	150	158	156
Mean protein intake in 4 weeks (g)	18	19	29	31
Weight gain/g protein eaten (g)	1·5 ± 0·10 (1·2–2·0)	2·0 ± 0·09 (1·4–2·3)	1·3 ± 0·02 (1·2–1·4)	1·5 ± 0·05 (1·3–1·8)
Percentage of absorbed nitrogen retained	67 ± 1·7 (62·1–70·5)	75 ± 0·4 (71·4–77·9)	74 ± 1·4 (70·2–77·6)	82 ± 0·9 (80·3–84·4)
Haemoglobin (g/100 ml):				
Initial	11·1 ± 0·53 (9·2–14·2)	11·5 ± 0·54 (9·2–13·8)	11·7 ± 0·55 (9·2–14·2)	10·9 ± 0·37 (9·2–12·0)
Final	14·2 ± 0·21 (13·5–14·5)	15·4 ± 0·24 (14·1–16·5)	14·8 ± 0·36 (13·1–16·8)	15·6 ± 0·16 (14·9–16·2)
Liver content of:				
Protein (g/100 g)	15·8 ± 0·17 (15·1–16·7)	16·0 ± 0·15 (15·2–16·6)	15·8 ± 0·37 (15·1–16·1)	16·3 ± 0·32 (15·3–18·9)
Thiamine (mg/100 g)	0·68 ± 0·02 (0·59–0·76)	0·90 ± 0·15 (0·69–1·25)	0·94 ± 0·04 (0·82–1·28)	1·21 ± 0·08 (0·94–1·56)
Riboflavine (mg/100 g)	2·75 ± 0·22 (1·71–3·53)	3·42 ± 0·23 (2·39–4·94)	2·68 ± 0·14 (2·49–3·75)	3·66 ± 0·27 (2·59–4·82)
Glutathione (mg/100 g)	0·19 ± 0·04 (0·13–0·32)	0·25 ± 0·04 (0·15–0·41)	0·23 ± 0·05 (0·10–0·33)	0·39 ± 0·11 (0·22–0·64)
Xanthine oxidase*	7·9 ± 0·40 (7·2–9·9)	11·9 ± 1·02 (9·9–13·9)	6·8 ± 0·46 (5·7–8·7)	11·0 ± 1·16 (8·7–17·4)
Succinic dehydrogenase†	11·1 ± 0·97 (10·1–13·2)	23·5 ± 0·58 (13·2–29·1)	11·1 ± 0·54 (9·7–12·8)	25·8 ± 2·4 (18·6–32·7)

* Enzyme units derived from dividing 2500 by time taken (sec) for reduction of methylene blue.
† μmoles 2,3,4-triphenyltetrazolium chloride reduced per g wet tissue per h at 37° under assay conditions.

The results of the animal experiment are shown in Table 2. Fermentation increased the nutritive value for rats of both Idli and Khaman as judged by the criteria employed.

The improvement in weight gain of rats per g protein intake (in view of the differences in vitamin content, the term PER is not used) when Idli was given is contrary to the findings of Ananthachar & Desikachar (1962) and of Khandwala, Ambegaokar,

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Patel & Radhakrishna Rao (1962) but consistent with the report of Radhakrishna Rao (1961) on the greater capacity of fermented Idli to prevent fatty infiltration of the liver in rats. As already pointed out, a different micro-organism was employed in the first-mentioned study. In the study of Khandwala *et al.* (1962), water at 80° was used to prepare the batter. This may have inhibited the fermentation, as the optimum temperature is around 30°. Further, black gram dal and rice were used in the proportions of 4:1 instead of the usual 1:2. Also, Khandwala *et al.* used supplements of B vitamins and, if the difference in nutritive value of fermented and unfermented Idli is partly due to the vitamin synthesized during fermentation, this would be obscured by the administration of vitamin supplements. The need for evaluating without vitamin supplements, foods to be used in practical nutrition has been emphasized by György (1964) as the foods under test are not likely to be taken along with vitamin supplements. Further, the improvement in food conversion efficiency is consistent with the report of an increase with fermentation in methionine, which is the limiting amino acid in the rice–black gram mixture as well as in bengal gram. It could, however, be due to the greater vitamin content of the fermented products rather than to any change in the nutritional value of the protein. The improvement did not appear to be due to greater digestibility as faecal nitrogen content did not differ significantly between groups given fermented and unfermented products. The weight gain was greater with Khaman than with Idli in absolute terms, but less when considered on the basis of protein intake. This is consistent with the higher protein content of the Idli.

Significant differences were found in the thiamine and riboflavine contents of the livers of rats receiving different treatments, and these were consistent with the differences in the vitamin contents of the different diets.

The higher glutathione content of the liver in the groups of rats fed on the fermented foodstuffs was not statistically significant, but only large differences could be detected with the small numbers of animals used. Glutathione was measured mainly because of the expectation that if fermentation results in an increase in methionine content this might in turn be reflected in a higher glutathione concentration of the liver.

The livers of animals given the fermented food also showed greater activities of xanthine oxidase and succinate dehydrogenase, an observation which is consistent with the increase in nitrogen retention and riboflavine content.

Fermentation was found, accordingly, to increase the thiamine and riboflavine contents and to decrease the phytate content of both Idli and Khaman and to increase their overall nutritive value for rats.

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