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STUDIES ON GROUNDNUT FERMENTATION

1. Effect of fermentation on the nutritive value of the product

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ABSTRACT

The biological value, digestibility, fat and vitamin contents of fermented groundnut have been given along with those recorded for the raw, roasted and boiled samples of the groundnut. Fermentation has been shown to reduce the fat content by about half. In the light of results obtained, fermented variety has been suggested to be superior to other varieties for supplementing dietary proteins especially in view of its ready acceptability, digestibility and improved protein efficiency.

Groundnut is recognized as a concentrated form of food having in it a high protein (25.3%) and a very high (44-50%) fat content. It is also rich in the vitamins of the B complex, notably thiamin and nicotinic acid and to an extent pantothenic acid. But for a lack of vitamin A and some of the amino acids and minerals, groundnut would form a complete food in itself.⁷

It is well recognized that Indian diets lack in proteins and protein deficiency raises a serious problem. In view of the shortage of animal proteins, there is much in favour of the use of groundnut in supplementing deficient diets. But the principal difficulty about groundnut is its overall poor digestibility, presumably caused by high fat contents together with a lack of the fat soluble food accessories. Various attempts have in the past been made to improve its nutritive value and those aimed at supplementing with lysine and methionine⁸ and

with methionine and tryptophan¹¹ appear to be most satisfactory. Recently Subrahmanyam and coworkers^{3, 17, 18} and Lal and Rajagopalan¹⁴ have made commendable efforts towards finding ways and means of utilizing groundnut cake at supplementing our poor diets. The present investigation represents another attempt made to see if the age-old practice of eating groundnut after a sort of pickling in brine has any beneficial influence on the nutritive value of the resultant product and as the results will reveal there is something in favour of it.

MATERIALS AND METHODS

Because of the variability in the composition of the material used, the methods adopted called for careful attention. At the outset it was considered of utmost importance to ensure that in all cases a strictly representative portion of the groundnut was taken for analysis. In order that the samplings may be as homogeneous as possible, all the analyses were performed on nuts derived from one particular harvest. In all, lots from two harvests were made use of during this study. Samplings were made from various portions of the same bulk material in order to represent as fair an average of the whole as possible. The nuts chosen were of good quality, moderate in size, and were well cleaned and dried before use.

In a preliminary experiment the extent of salt, the temperature and time of boiling and the fermentation period that is required to yield the most desirable product (organoleptically) were determined. It was observed that boiling the groundnut with shells for 20 minutes in 3 per cent sodium chloride yielded the most satisfactory product. Likewise, the product that had undergone fermentation for about 2 days was declared to possess the most agreeable aroma and palatability. Therefore, samples subjected to 20 minutes boiling in 3 per cent brine and which had undergone 48 hours of fermentation at room temperature were taken up for all investigations. For control, both the raw and the boiled (but not allowed to undergo fermentation by storage in a refrigerator) samples were included. Moreover, in order to have results on a comparative basis (roasted groundnut has been the subject of extensive investigations) roasted samples were also subjected to similar analysis. Roasting was carried out in an electrically heated chamber, fitted with a stirrer, wherein the nuts could be uniformly roasted to a light brown colour to the extent that the testa of the seed, on cooling, could be removed with ease. Thus the four samples analysed were respectively, raw, roasted, boiled and fermented.

Biological value by the nitrogen balance technique:—Nitrogen balance method defined by Thomas¹⁹ and developed by Mitchell¹⁵ was adopted. Four groups of albino rats (six in each group) were maintained on a stock diet till they attained the weight of 60-80 g. The choice of rats in each group was such that gave an equal average weight. They were fed *ad libitum* a low nitrogen (4 per cent whole egg protein) diet for 7 days. Urine and faeces of the individual animals were collected during the last 3 days of this endogenous period, and the samples

preserved appropriately until they could be analysed for nitrogen. The animals were then changed over to the diets containing the test de-fatted protein fed at 10 per cent levels for the 4 groups. The feeding was continued for 7 days, and during the last 3 days, collection of excreta (faeces and urine) were made as before and their nitrogen determined. The record of food intake, and subsequently the nitrogen content of food ingested was also maintained. The weights of the animals before the experiment, before and after the test feeding, were also

TABLE I

Nitrogen balance studies for biological value and digestibility

(a) Endogenous faecal and urinary excretion of rats; protein level in the diet — 4 %

Subsequently fed with the following groundnut diets	Nitrogen excretion	
	Faecal mg.	Urinary mg.
Raw	25.08 + 2.31	14.32 + 0.75
Boiled	25.96 + 1.27	15.82 + 1.37
Roasted	23.95 + 1.75	19.96 + 1.90
Fermented	24.83 + 1.32	23.19 + 2.57

(b) Experimental diet period at 10 % protein level

Groundnut diet groups	Nitrogen intake mg.	Nitrogen excretion		Nitrogen absorbed mg.	Biologi- cal value	p*	Digesti- bility coeffi- cient mg.	P*
		Faecal mg.	Urinary mg.					
Raw	122.77 ± 1.45	38.82 ± 3.42	63.29 ± 5.49	108.72 ± 2.95	56.02 ± 5.47		88.54 ± 1.97	
Boiled	118.50 ± 4.11	33.97 ± 2.99	46.54 ± 4.80	113.85 ± 2.96	75.52 ± 6.56	0.05	93.33 ± 2.38 (-)	
Roasted	117.97 ± 2.35	35.33 ± 2.37	57.06 ± 4.02	105.73 ± 2.72	69.57 ± 2.62	0.05	89.63 ± 1.68 (-)	
Fermented	121.20 ± 1.64	32.48 ± 0.69	51.92 ± 2.78	112.92 ± 2.80	76.38 ± 0.70	0.01†	93.20 ± 1.10 (-)	

* P (-) means not significant. † Highly significant

recorded. Equal distribution of litter mates were not considered important as it has been adequately proved that rats chosen at random gave mean growth response and variance similar to those from the same stock paired with respect to litter.¹⁰ All the analytical results were statistically analysed before conclusions were drawn. The results are presented in Table I.

Protein efficiency ratios:—Four groups of young albino rats of six in each group (weighing 30-50 g.) were selected in a representative manner with due consideration to weight and sex. They were maintained on the experimental diet, each group receiving 10 per cent of the respective test protein. The rats were housed in independent cages and fed *ad libitum* and the weekly food intake was recorded. Weights of the animals were recorded every week, the rats being weighed on the day following the end of a week before food was offered. From the results obtained the protein consumed by each rat and the protein efficiency ratio were calculated. The results are presented in Table II.

TABLE II
Protein efficiency ratio at 10 % protein level

Groundnut diet groups	Initial weight g.	Final weight g.	Increase in weight g.	P*	Protein efficiency ratio	P*
Raw	38.30	64.50	26.20		1.388	
	± 1.71	± 3.45	± 3.51		± 0.138	
Boiled	38.30	62.50	24.20	(-)	1.137	(-)
	± 1.50	± 4.87	± 4.35		± 0.160	
Roasted	38.30	59.80	21.30	(-)	1.205	(-)
	± 1.39	± 2.48	± 2.98		± 0.138	
Fermented	38.30	60.10	21.80	(-)	1.373	(-)
	± 2.38	± 4.25	± 3.00		± 0.127	

* P (-) means not significant

Evaluation by enzymatic digestion of protein:—As digestibility of foods ingested is of paramount importance in a study such as this digestibility of proteins and fats was determined by employing the method of Volhard²⁰ for peptic and of Kunitz¹³ for tryptic digestibility. For fats, the method recommended by Ahmed and Sa'een¹ using lipase derived from the plant *Ricinus communis* was adopted. The results obtained are recorded in Table III.

Nitrogen determination (for calculating protein values), fat analysis and assays of thiamin and nicotinic acid were carried out by adherence to the standard methods available for the purpose. It may however be mentioned that chemical methods were followed for assays of vitamins in preference to the micro-

TABLE III
In vitro digestibility of groundnut samples by enzymes

Samples	Peptic activity ml. of N/10 NaOH	Tryptic activity colorimeter readings	Lipase activity ml. of N/10 KOH
Raw	3.72	0.091	9.91
Roasted	4.56	0.131	7.14
Boiled	3.92	0.164	11.41
Fermented	3.08	0.228	5.82

biological procedures. In as much as roasted samples did not reveal the presence in appreciable amounts of both the vitamins, they were not included in the assay work. The results recorded for vitamins in the various samples are presented in Table IV.

TABLE IV
 Thiamin and nicotinic acid contents of groundnut samples

Samples	Harvest	μ g of thiamin/g.	mg. of nicotinic acid/100 g.
Raw	A	10.45	10.50
	B	10.47	12.60
Boiled	A	8.75	12.00
	B	8.22	11.70
Fermented	A	1.64	17.15
	B	1.40	16.01

RESULTS AND DISCUSSION

As may be seen from Table I, there is no significant difference between the digestibility of the raw and roasted groundnut samples but the biological value of the latter is slightly more with a significance between 5 and 10 per cent levels. This agrees with the experiments of Cama and Morton⁶ in that moderate roasting appears to have the effect of enhancing the nutritive value of the groundnut. Further, the results obtained in the case of raw and roasted groundnut are in fair agreement with those obtained by Mitchell *et al.*¹⁶ and are in close agreement with those of Guggenheim and Buechler-Czaczkas⁹ and Armstrong and Thomas.² The digestibility of the boiled variety is only slightly or not at all significantly different from the raw variety, though apparently it appears to have been

improved by boiling. When compared with the raw, the fermented sample has a highly significant biological value between 1 and 2 per cent levels, but this is also witnessed in the case of boiled sample suggesting thereby the advantage of boiling. However, the biological value of 76.38 for the fermented variety is higher, though only slightly, but this may be considered as highly satisfactory when compared with the raw and the roasted variety and as such could be considered of help in ameliorating protein malnutrition in our country, particularly in view of the fact that we are the largest producers of this food material and therefore we do not have to depend on imports. Furthermore, the digestibility of the food on fermentation becomes very satisfactory.

The protein efficiency ratio, *i.e.*, the gain in weight of rat per gram of protein consumed does not show any significant changes due to various types of processing. Of the four samples (Table II), roasted groundnut on the whole appears to be least nutritive with respect to its protein; boiling seems to improve the biological value, but the subsequent fermentation does not improve it any further. The protein efficiency ratio obtained, though lower than that of Buss and Goddard⁵ and Jones and Widness¹² is similar to that obtained by Borchers and Ackerson⁴ for the raw groundnut. From all this it may be concluded that though the fermentation of the groundnut helps in a better utilisation of its proteins, it contributes nothing towards better digestibility and growth. It is however interesting to observe that though the overall digestibility *in vivo* is generally uniformly maintained, peptic digestibility *in vitro* is lowered and the tryptic digestibility in the fermented variety increased. This may mean that the trypsin inhibitor reported to be present in the groundnut may have been completely or partially destroyed by the "processing" applied.

Fermentation does not seem to bring about any increase in the vitamin content of the product. In fact, thiamin is partially destroyed by boiling as well as subsequently during fermentation, in contrast to nicotinic acid which is not lost during this process; if at all, a gain is registered in the fermented variety.

The digestibility of the oil in the groundnut appears to be affected by processing in that the oil from the fermented variety is rendered slightly less

TABLE V
Fat contents of the groundnut samples (per 100 g.)

Samples	Wet weight basis	Dry weight basis
Raw	57.07	60.54
Roasted	73.06	76.72
Boiled	59.69	71.64
Fermented	26.45	39.22

digestible than that derived from raw, boiled or roasted samples. However, subsequent investigation led to the finding that the oil contents of fermented variety totalled to only about half that was present in the otherwise treated samples. Examination of the fermenting liquor revealed that the oil lost from the grains had not been released therein and this led to the obvious conclusion that during fermentation, which often took a vigorous course after the first 24 hours, the oil to a large extent was catabolised by the activities of the associating micro-organisms. The results, typical of several samples analysed, are presented in Table V.

The "fermented groundnut" has yielded favourable results by the two methods of protein assessment. Moreover, the digestibility tests have indicated that fermentation of the groundnut, prior to its consumption not only destroys the trypsin inhibitor initially present therein, but does not in any way affect its digestibility. In fact fermentation seems to bring about a favourable change in its composition and helps improve absorption of its proteins. Above all, the fermented product, as judged by its palatability and acceptability tests, has a better appeal and as such should prove to be of considerable benefit under our conditions particularly because of our inadequacy in protein consumption.

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REFERENCES

1. Ahmed, B. and Sareen, R. N. . . *J. Sci. & Industr. Res.*, 1946, 4, 710.
2. Armstrong, R. M. and Thomas, B. *Brit. J. Nutrition*, 1950, 4, 166.
3. Bains, G. S., Reddy, S. K. Bhatia, D. S. and Subrahmanyan, V. *Bull. Centr. Tech. Res. Inst.*, 1952, 2, 38.
4. Borchers, R. and Ackerson, C. W. *J. Nutrition*, 1950, 41, 339.
5. Buss, L. W. and Goddard, V. R. . . *Food Res.*, 1948, 13, 506.
6. Cama, H. R. and Morton, R. A. . . *Brit. J. Nutrition*, 1950, 4, 297.
7. Daniels, A. L. and Loughlin, R. . . *J. Biol. Chem.*, 1918, 33, 295.
8. Grau, C. R. . . *J. Nutrition*, 1946, 32, 303.
9. Guggenheim, K. and Buechler-Czaczkes, E. *Brit. J. Nutrition*, 1950, 4, 161.
10. Harte, R. A., Travers, J. J. and Sarich, P. *J. Nutrition*, 1947, 34, 363.
11. Jones, D. B. and Divine, J. P. . . *Ibid.*, 1944, 28, 41.
12. ———— and Widness, K. D. . . *Ibid.*, 1946, 31, 675.
13. Kunitz, M. . . *J. Gen. Physiol.*, 1947, 30, 291.

14. Lal, B. M. and Rajagopalan, R. . . *J. Indian Inst. Sci.*, 1957, **39**, 161 & 169.
15. Mitchell, H. H. . . *J. Biol. Chem.*, 1923-24, **58**, 873.
16. ———, Burroughs, W. and Beadles, J. R. *J. Nutrition*, 1936, **11**, 257.
17. Murthy, H. B. N., Swaminathan, M. and Subrahmanyam, V. *J. Sci. & Industr. Res.*, 1950, **9-B**, 173.
18. Subrahmanyam, V., Rama Rao, G. and Swaminathan, M. *Ibid.*, 1950, **9-B**, 259.
19. Thomas, K. . . *Arch. Anal. u. Physiol. Anat. Abstr.*, 1909, 219.
20. Volhard, F. . . *Munchen. med. Wehnschr.*, 1900, **47**, 141.