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STUDIES ON GROUNDNUT FERMENTATION

2. The micro-organisms involved in the fermentation

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ABSTRACT

The nature and the density of population of micro-organisms associated with groundnut fermentation have been established by adopting suitable microbiological procedures. Evidence has been drawn to show that the microflora of the fermenting liquors are responsible for the decomposition of the fatty components of the groundnut during its fermentation.

Fermented foods and drinks have constituted valuable food items of man from times immemorial though from the biochemical standpoint the fermentation process has come to be studied only within the last century. Biochemically speaking, fermentations involve chemical changes or decompositions brought about in organic substrates by the activity of living organisms or their enzymes and from this it would be clear that there are many kinds of fermentations falling within this category. Whereas the fermentation processes industrially exploited for the manufacture of alcohols, organic acids, penicillin and other antibiotics, and vitamins represent microbiological processes wherein the maximum yields of the products are obtained under specialized conditions from singled out micro-organisms, there exist other fermentations wherein symbiotic relationships existing among organisms are taken advantage of in the preparation of the products. Among the latter category fall the pickles, sauerkraut, soysauce, *idli*, buttermilk and other varieties of foods and drinks in the preparation of which micro-orga-

nisms have a vital part to play either by way of producing acids which tend to preserve the foods or bringing about improvement in their nutritive quality.

In the previous communication, in this series[4], it was shown that fermentation of groundnut for two days in 3 per cent brine improved not only the palatability and digestibility of the product but also had the effect of enhancing the biological value of the groundnut protein. Furthermore, it was brought out that fermentation resulted in a loss of 30 per cent or more of the oil contents of the groundnut and that the loss could be accounted for as due to the catabolic activity of the micro-organisms involved in the process. It was therefore considered of interest to determine the nature and density of population of different microflora involved in the fermentation of groundnut and the results recorded are presented in this paper.

MATERIALS AND METHODS

The materials used for microbiological examination was the liquor in which the groundnut was allowed to undergo fermentation at room temperature, *viz.*, 24-27°C. The colour of the brine (3 per cent) immediately on boiling with the groundnut was amber, but during fermentation it developed a dark brown colour. Fermentation usually took a vigorous turn after 24 hours and in as much as fermentation, as has been stated in the previous communication, allowed to continue for 3 days did not yield a product of agreeable aroma and palatability, fermentation was allowed to occur only for 2 days and therefore the material used throughout the present study consisted of the liquors (1) immediately after boiling of the groundnut, (2) 24 hours after fermentation had occurred, and (3) after 48 hours of fermentation.

The microbial picture of the liquors could best be obtained by streaking suitabily diluted portions thereof onto nutrient agar plates. The choice of nutrient agar as a medium lay in the fact that boiling (of groundnut in 3 per cent brine) for 20 minutes would ordinarily result in the death of all bacteria except the sporeforming organisms and these along with the subsequent contaminants from the air, could grow well on nutrient agar.

For quantitative studies, which were made by the colony count method, 0.05 ml. each of suitably diluted liquors (2 per cent sodium citrate solution was used as a diluent) were streaked on 3 nutrient agar plates, (1) immediately after processing of the groundnut, (2) after 24 hours, and (3) after 48 hours of fermentation. Colony counts were made from the plates incubated at 37°C at intervals of 3, 7 and 10 days. Differentiation was made between sporeforming forms from the rest by examination of the colony characteristics and microscopical appearance of the organisms from the representative colonies. Each experiment was carried out in triplicate and the microfloral population was calculated in terms of colony counts that developed per ml. of the liquors. The results of two typical experiments are presented in Table I.

For the isolation and identification of the bacteria, methods and media employed by Smith *et al.*[7] and described in the Manual of Pure Culture study of Bacteria[8] were strictly adhered to. Additional tests employed by Knight and Proom[6] were also found useful. In general, the system followed for the recognition of the sporeforming bacteria was that outlined by Iyer and Bhat[5] and detailed in the Bergey's Manual.[2] In the course of this work a total of 90 isolates was examined and these were obtained from 9 different experiments. The species encountered are listed in Table II. Special attention was paid to the study of fat hydrolysis by the isolates in as much as it was established that fat content of the groundnut tended to disappear as the microfloral population increased in the liquors. For testing the power of the bacteria to split fat, Gorodkowa's agar containing tributyrin was employed. Cultures were streaked on the tributyrin agar individually and in mixed population as encountered in the liquors.

Results and Discussion

It is clear from the figures presented in Table I that as a result of heat treatment only a few hundreds of viable forms could survive in the liquors and that within 24 and 48 hours they increased to such an extent that they had to be reckoned in millions.

TABLE I

Microfloral population of fermenting groundnut liquors (average of 3 plates)

Temp. of incubation : 37° C	Initial (before fermentation)		After 24 hours of fermentation		After 48 hours of fermentation	
	Expt. 1	Expt. 2	Expt. 1	Expt 2	Expt. 1	Expt. 2
	$\times 10$	$\times 10$	$\times 10^4$	$\times 10^4$	$\times 10^6$	$\times 10^6$
Sporeforming organisms . .	468.0	296.0	586.0	188.0	162.0	88.0
Non-sporeforming organisms	6.0	4.0	8.0	4.0	12.0	10.0
Total . .	474.0	300.0	594.0	192.0	174.0	98.0
Percentage of:						
Sporeforming organisms	98.7	98.7	98.6	96.8	93.1	88.8
Non-sporeforming organisms	1.3	1.3	1.4	3.2	6.9	10.2

It is also clear that more than 95 per cent of the flora consisted of the sporeforming bacilli which alone could survive the heat treatment by virtue of the resistance of their spores and which could subsequently find conditions in the liquors suitable for their propagation and activities.

TABLE II

Names of the bacteria isolated from fermenting groundnut liquors

(Total number of isolates studied was 90)

Experiment	Species
I	*B.* megaterium, B. firmus*
II	*Alcaligenes marshalli, Serratia marcescens, B. cereus, B. subtilis, B. megaterium*
III	*B. megaterium, B. subtilis*
IV	*B. subtilis, B. megaterium, B. cereus, Micrococcus luteus*
V	*Micrococcus luteus, B. magaterium, B. subtilis, B. alvei, B. pumilus*
VI	*B. subtilis, B. cereus. B. circulans, B. alvei, B, megaterium, B. luteus, B. sphaericus* var. *fusiformis*
VII	*B. subtilis, B. megaterium, B. cereus, B. laterosporus*
VIII	*B. alvei, B. pumilus, B. megaterium, B. cereus, B. subtilis, B. circulans*
IX	*B. cereus, B. subtilis, B. megaterium*

* Sporeforming species belonging to the genus *Bacillus*

A persual of the list of the surviving bacteria (Table II) would indicate that the most dominating species, among the aerobic mesophilic flora, were *B. megaterium, B. subtilis* and *B. cereus* but this is not surpising when we realise that these three species were considered to be the dominant among the flora revealed in (1) soils and air, (2) marine conditions, (3) hay and other habitats in our environments[1]. It is however interesting to observe from results presented in Table II that the flora varies from experiment to experiment though, by and large, their activity in the fermenting liquors could be attributed to mesophilic sporeforming bacteria.

Tests for fat hydrolysis revealed that individually none of the species of sporeforming bacilli (listed in Table II) could hydrolyse tributyrin. In fact neither Bergey's Manual[2] nor the monograph by Smith *et al.*[6] refer to fat hydrolysis as a test for their identification, though *B. megaterium* and *B. cereus* are known for their fat storage characteristic which provides an useful test in their identification. The storage of fat necessarily infers the ability of the organisms to break down, at least in the present circumstance, fats from the fermentable medium. In so far as *Micrococcus luteus* and *Serratia marcescens* are concerned, the inference is obvious; these are distinctly lipolytic species. What is even more significant, aliquots of diluted or undiluted fermenting liquors when plated

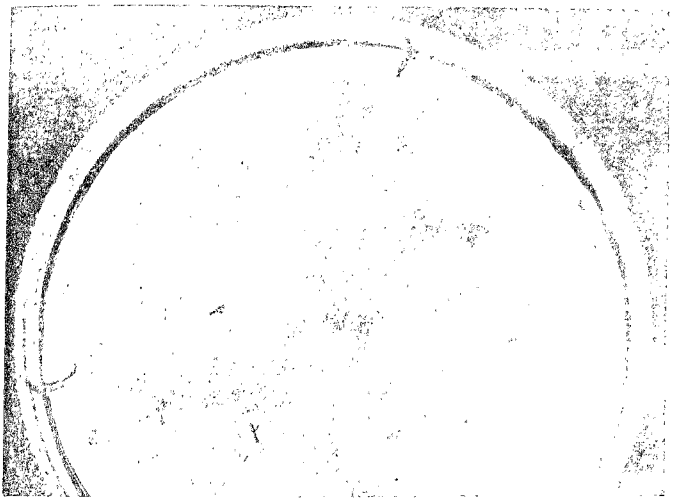

PLATE I

Growth seen on Gorodkowa's tributyrin calcium carbonate agar on streaking with fermenting groundnut liquor. Note the halos (around some of the colonies) produced as a result of dissolution of calcium carbonate by the acid formed from tributyrin by the fat hydrolysing species of bacteria.

on Gorodkowa's agar developed growths showing unmistakable halos of fat-hydrolysis (See Fig. 1) even on the second day of fermentation, suggesting thereby the preponderance of fat-hydrolysing species in the fermenting liquors. Further evidence in support of the microbial action on the oils was derived by carrying out an analysis of the oils extracted from the raw and the fermented variety of the groundnut. Whereas the molecular weights of the esters of the saturated fatty acids of the raw oil showed higher values, the corresponding values for the esters prepared from the oil of the fermented series were of a lower order and this is indicative of a breakdown in the molecular structures of the higher fatty acids due to fermentative activities of micro-organisms.[3] From all this it would appear that although the organisms isolated from the fermenting liquors did not individually demonstrate their ability to decompose fats, collectively or synergistically they displayed their power to metabolise fat, an observation which accounts for (1) the reduction in the lipid contents, (2) the improved digestibility and other characteristics reported in favour of the fermented groundnut.

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