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A STUDY OF MICRO-ORGANISMS IN BLACK TEA AND THEIR ACTIVITIES ASSOCIATED WITH STORAGE*

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The microbiological changes undergone by black tea due to firing and various storage conditions were evaluated by using standard techniques. Water proved to be the best extractant for micro organisms in tea while a 5 or 10 min shaking time gave similar extracting efficiencies. Firing of dhools did not sterilize the tea but gave a reduction only in the bacterial count. Accelerated storage with free access to air and moisture showed a steadily increasing fungal count and a constant bacterial count. Storage in high barrier packaging films showed that access to air played the most important role in controlling microbial counts of stored black tea.

INTRODUCTION

Although the chemical changes associated with storage of black tea have been studied (Stagg, 1974 ; Dougan *et al*, 1978 ; Cloughley, 1981), the microbiological aspects have been relatively neglected. Wickremasinghe (1978) points out to the significant increase in microbial populations during withering of green leaf and the effect of microbial proteins which reduce the astringency of black tea extracts, giving rise to rainy teas. However, there is a paucity of data relating to the quantitative aspects of black tea microbiology and this study is an attempt to fill in some of those gaps.

MATERIALS AND METHODS

Black tea :

All grades of tea produced at St. Coombs Factory , Talawakele (1300 m amsl) were subjected to this study. These grades were BOP (Braken Orange Pekoe), BOPF (Broken Orange Pekoe Fannings), BP (Broken Pekoe), BM (Broken Mixed), Dust, Fannings and refuse or BMF (Broken Mixed Fannings).

Media :

Tryptone soy agar (Oxoid, 1982) was used to enumerate bacteria while potato dextrose agar (Oxoid, 1982) which was acidified with 10% lactic acid (after sterilizing) was used to enumerate fungi. After preparation all media were sterilized at 120°C for 15 mins.

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Storage conditions :

Accelerated storage was carried out by exposing freshly made BOP grade tea to air and moisture in a chamber saturated with water vapour at 22°C.

Storage at various moisture levels but with no access to air was carried out by sealing 10 g samples in pre-sterilized triple laminated aluminium foil packages when the moisture level rose to 4, 6 and 8% in samples exposed to moisture.

Bulk storage in miniature tea chests was done by spraying sterile water into freshly made BOP tea in proportions to obtain moisture levels of 7.5 and 11% and then packing into the small chests.

Sampling :

Fermented dhools were sampled at the drier inlet while the ungraded black tea was sampled at the drier outlet prior to any handling. Sampling after accelerated storages and bulk storage was done aseptically after physically mixing the bulk.

Extracting :

An accurately weighed quantity of black tea was introduced aseptically to a conical flask containing 60 ml sterile extractant. The flasks were shaken for 5 or 10 min in a water bath set at 35°C before plating.

Plating :

A suitable quantity (usually 1 ml) of the extract after dilution, if necessary, was plated in tryptone soy or potato dextrose agar as the case may be. All media extractants and equipment used for aseptic work were sterilized at 120°C. All incubations were carried out at 28°C.

RESULTS AND DISCUSSION

The optimum shaking time for organism extraction was established by extracting in sterile water for 5 or 10 min and plating the extract in tryptone soy agar. BOP, dust and fannings gave differing counts after a 24 h incubation (Table 1) but after 48 h only the extracts from BM teas showed a difference. It was envisaged to use grade teas (BOP/BOPF) for storage studies and therefore a 5 min extraction time at 35°C extractant temperature was decided on followed by an incubation period of 48 h for the plates.

TABLE 1—*Determination of a better extracting medium for micro-organisms in tea**

Grade of Tea	*Counts/g (24 h incubation)*		*Counts/g (48 h incubation)*	
	Water	*Nutrient broth*	*Water*	*Nutrient broth*
BOP	1.02×10^3	1.22×10^3	4.37×10^3	6.63×10^3
BOPF	2.2×10^2	5.2×10^2	3.43×10^3	3.9×10^3
BP	5.2×10^2	3.6×10^2	2.69×10^3	2.13×10^3
BM	1.4×10^2	3.2×10^2	6.7×10^2	6.97×10^2
Dust	1.2×10^2	1.68×10^3	5.63×10^3	5.99×10^3
Fannings	4.8×10^3	4.1×10^4	3.65×10^4	2.88×10^4
Refuse (BMF)	2.4×10^3	2.87×10^4	4.85×10^4	3.12×10^4

* Mean values of determinations done in triplicate.

The better medium of extraction was determined by using either water or nutrient broth as the extractant and an extraction time of 5 min. After 24 h of incubating the dust, fannings and refuse tea gave differing counts with the nutrient broth giving the higher values (Table 2). However after 48 h the counts equalised for both media. Some problems were encountered when extracting with nutrient broth like froth formation and therefore water was decided on as the extractant of choice.

TABLE 2—*Determination of best extracting time**

Grade	*Counts/g* (24 *h. incubation*)		(*Counts/g* (48 *h. incubation*)	
	5 *min.*	10 *min.*	5 *min.*	10 *min.*
BOP	9×10^2	1.26×10^4	1.43×10^4	2.51×10^4
BOPF	9×10^2	9×10^2	1.23×10^4	1.5×10^4
BP	9×10^2	9×10^2	3.79×10^3	3.99×10^3
BM	9×10^2	9×10^2	8.25×10^3	1.50×10^4
Dust	7.91×10^3	1.08×10^4	1.77×10^4	1.9×10^4
Fannings	4.07×10^3	5×10^4	3.97×10^4	4.87×10^4
Refuse (BMF)	2.1×10^3	6.07×10^3	5.27×10^4	3.04×10^4

* Mean value of determinations in triplicate

All the grades produced at the end of a days manufacture were sampled next for bacteria and fungi both in the black tea and the tea brew. The results (Fig. 1) show that dust, fannings and refuse tea (BMF) were the most contaminated with both bacteria and fungi. They all had over 10^4 viable bacteria g^{-1} dry wt., while the others had less than 8×10^3 viable bacteria g^{-1} dry wt.

The fungal counts were about 4×10^2 g^{-1} dry wt. for BOP, BOPF, BP, BM and dust while fannings and refuse tea (BMF) contained over 5×10^3 g^{-1} dry wt. This difference between the grades could be explained in part by the degree of post-firing handling the grades received. Generally the BOP and BOPF's consist of over 80% of a days manufacture and will be subject to the least amount of handling. The off grades on the other hand are subject to much greater handling. One redeeming feature was that the boiling water extracts of all grades had somewhat similar bacterial and fungal counts which were independant of the initial counts (Fig. 1). A point of major concern that arose was the presence of gram-ive staining organisms in the off-grade teas. It was therefore decided to investigate the possible origins of such organisms. Fermented dhools were sampled at the dryer entrance as well as the dryer outlet and the absence of such gram-ive staining organisms was noted. From Fig. 2 it is apparent that firing does not sterilize tea but reduces the bacterial count by one order while having hardly any effect on the fungal count. This could be expected because actual temperatures during tea firing does not exceed 90° which

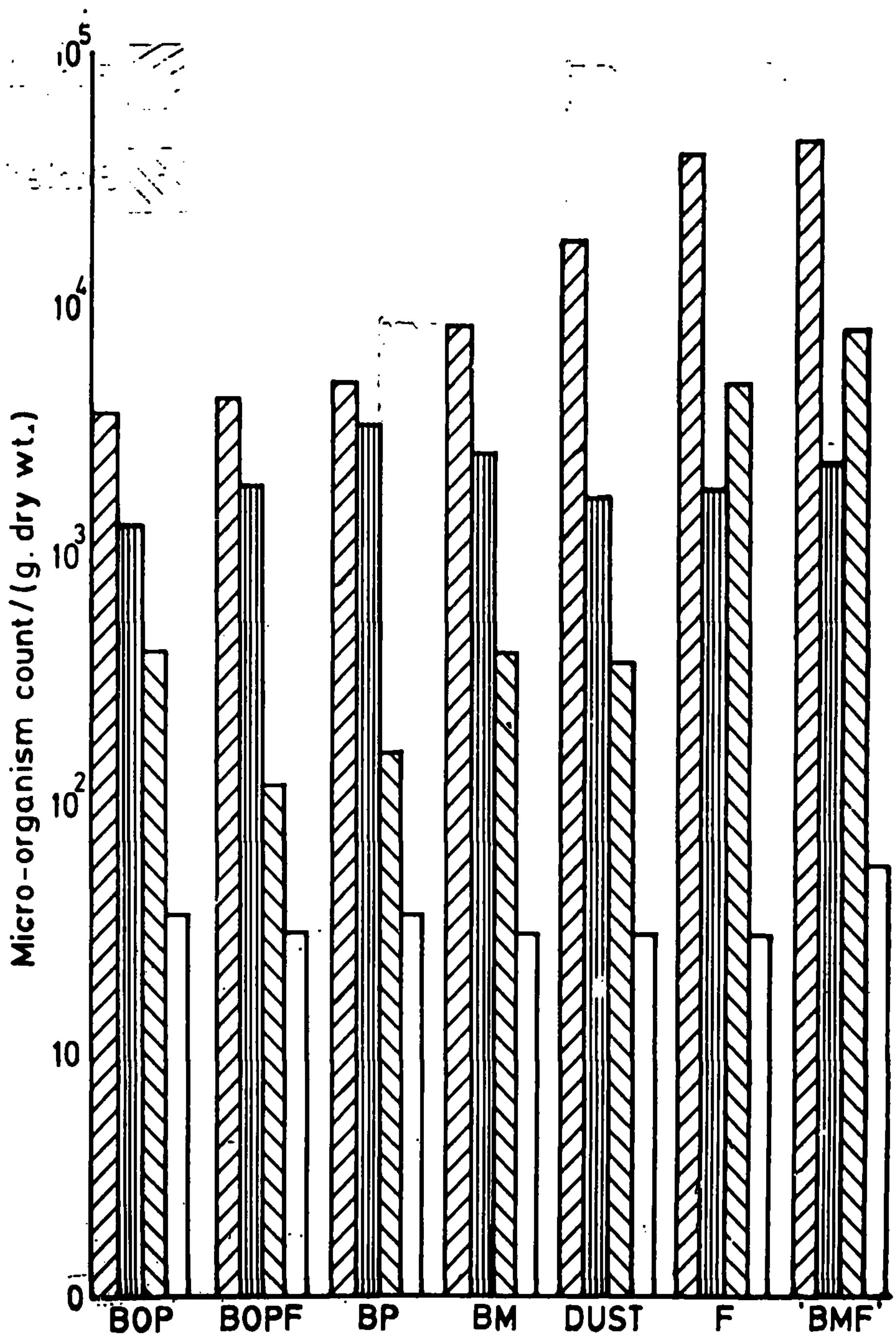
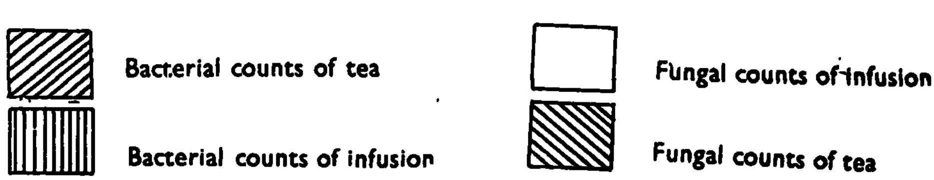


Fig. 1 — Microbial counts of different grades of teas.



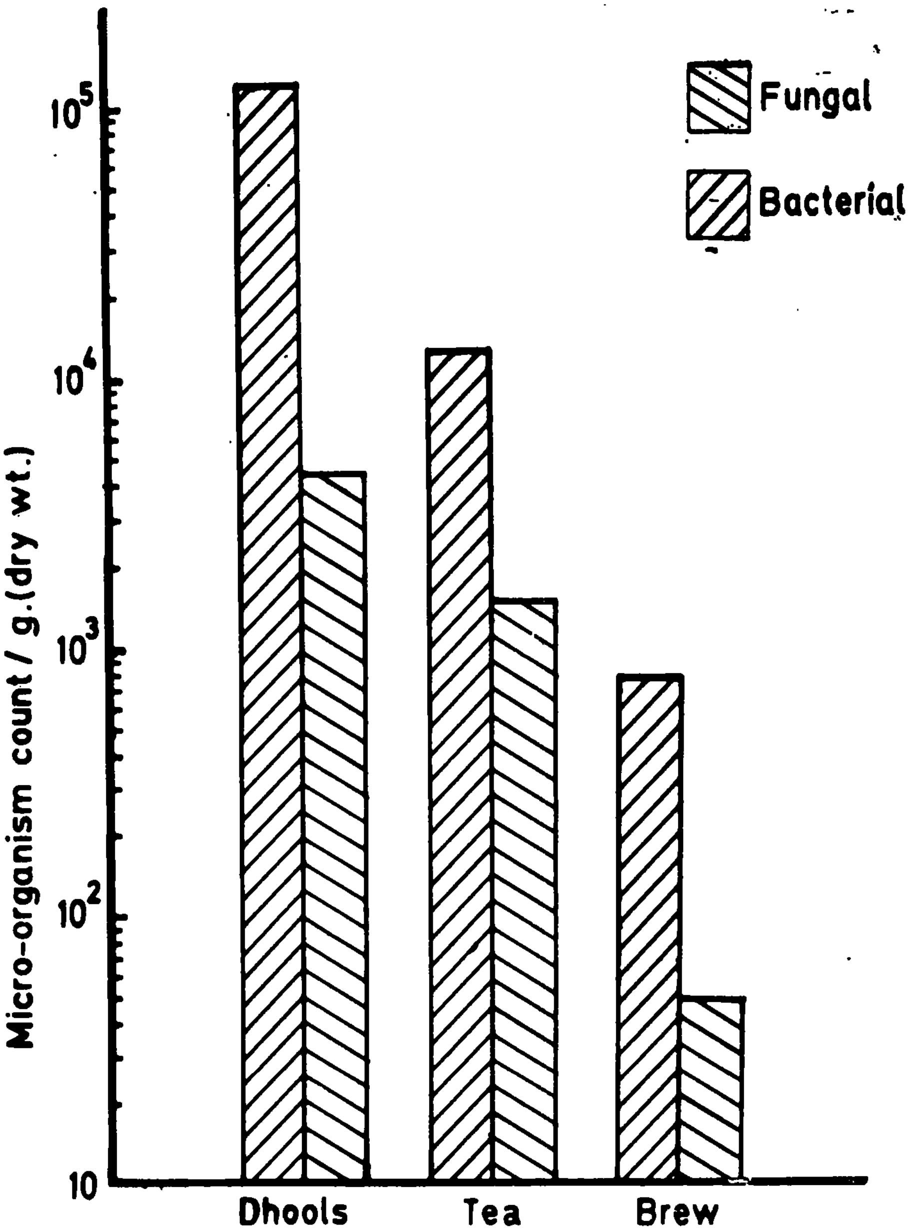


Fig. 2 — Effect of firing on microbial population

is probably too low to kill thermophiles and certainly too low to kill fungal spores. It could therefore be reasonably extrapolated to show that gram-ive organisms do not survive the tea manufacturing process but are introduced by post firing handling due to lack of hygenic conditions. Also it is interesting to note that fermented foods like yoghurt, which depend on the fermentation iteself for their flavour, have a count of about 10^8 viable organisms ml^{-1} (Pelczar *et. al.*, 1977) and that if the count in the dhools exceeds about 10^6 organisms g^{-1} there is every possibility that bacterial metabolites may be introduced to give " taint " in the fired tea, without evidence of contamination of the black tea.

On accelerated storage with free access to air and moisture, the moisture content of tea reached about 22% after 8 days with the water activity reaching 0.95, effectively providing excellent conditions for microbial multiplication. After 10 days or so visible mycelia were observed. The bacterial count stabilized at about 10^4 g^{-1} tea (Fig. 3) showing a bacteriostatic effect of black tea. This effect was reported by Das (1962) and was much more pronounced in green tea. It was obvious that fungal growth was not affected although inhibitory effects against virus (Konowalchuk and Spiers, 1978) and yeasts (Ekanayake, *et. al.*, 1986) have been observed. The fungi growing on tea under accelerated storage have been observed as follows—

Penicillium citrinum, Pencillium corylophilum, Penicillium chrysogenum, Geosmithia putterilli, Aspergillus niger and Rhizopus stolonifer.

The main chemical change noticeable was the reduction in the free amino acid level by over 60% during the 8 day period. This could be considered as further evidence for an active nitrogen utilising system such as microbial multiplication. Organoleptic evaluation of the infusions showed a noticeable softness on the 4th day and a fungal taint on the 6th day. The infused leaf also darkened in appearance from the desirable copper colour due possibly to a coating of the leaf particle surface by microbial cell debris. These changes are akin to the character inherent in " rainy teas " (Wickremasinghe, 1978). Bi-weekly sampling and plating on trypticase soy agar and potato-dextrose agar of teas stored in laminated aluminium foil pouches showed that air/oxygen was necessary for the growth of tea microflora (Figs. 4 and and 5). It was interesting to note that even at the beginning of the experiment the bacteriostatic effect of tea was obvious in the high moisture samples where presumably the mobility of the inhibitory substances were greater. But with time the available oxygen was metabolised and the aerobes could not survive, giving after 8 weeks a tea with a very low microbial count. As expected fungal growth was controlled only by the oxygen availability (Fig. 5). Organoleptic evaluation of these samples after 8 weeks showed an increasing astringency from the 6% to 8% moisture samples whereas the 4% samples still maintained the nice rounded flavour of tea. This contribution to increased astringency may come indirectly by the decrease of the amino acids which are metabolized by the microflora or by the increased gallic acid due to tannase action (of *A. niger*) on the theaflavin gallates. It has been shown conclusively (Sanderson *et. al.*, 1976) that the unique taste of tea could be altered profoundly by changing the composition of soluble tea constituents, which is the apparent result of storage.

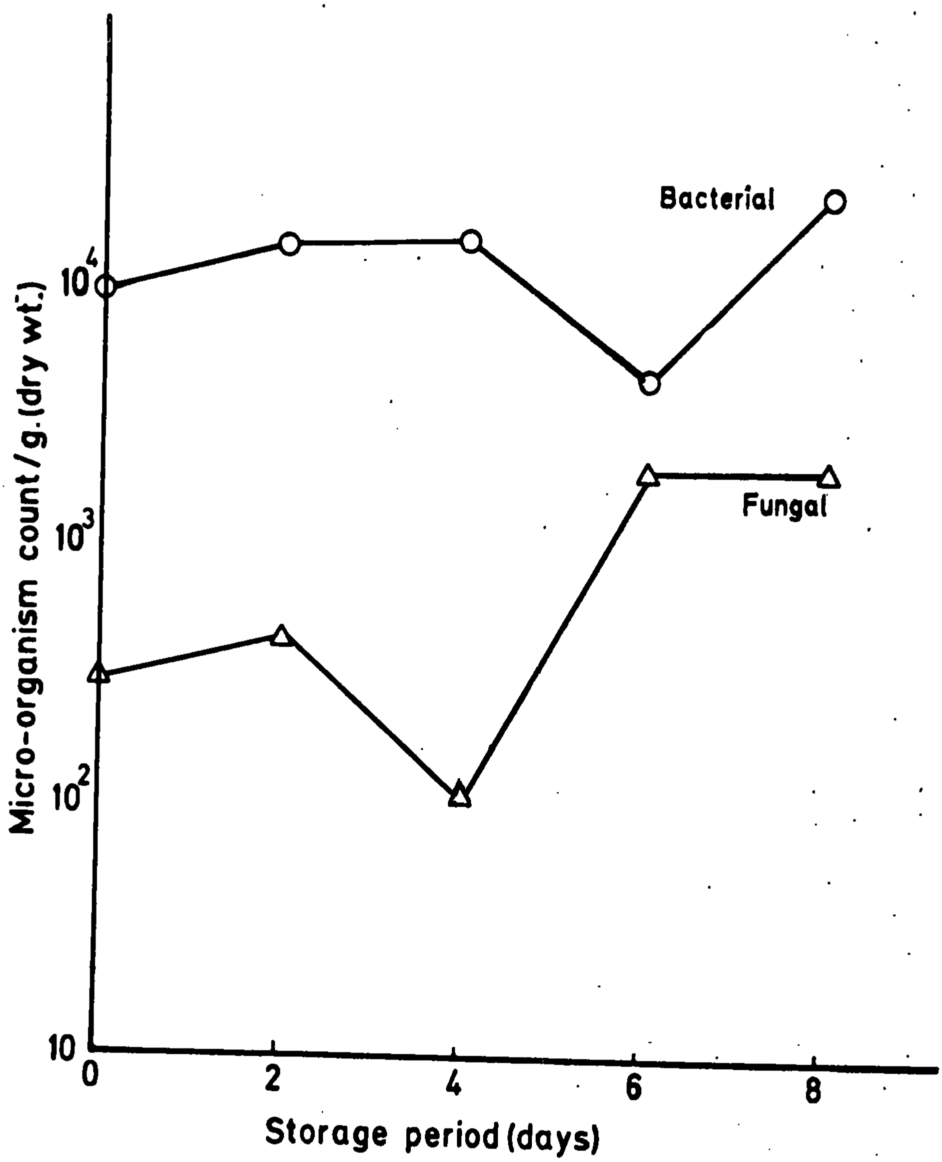


Fig. 3 — Microbial counts in samples subjected to accelerated storage.

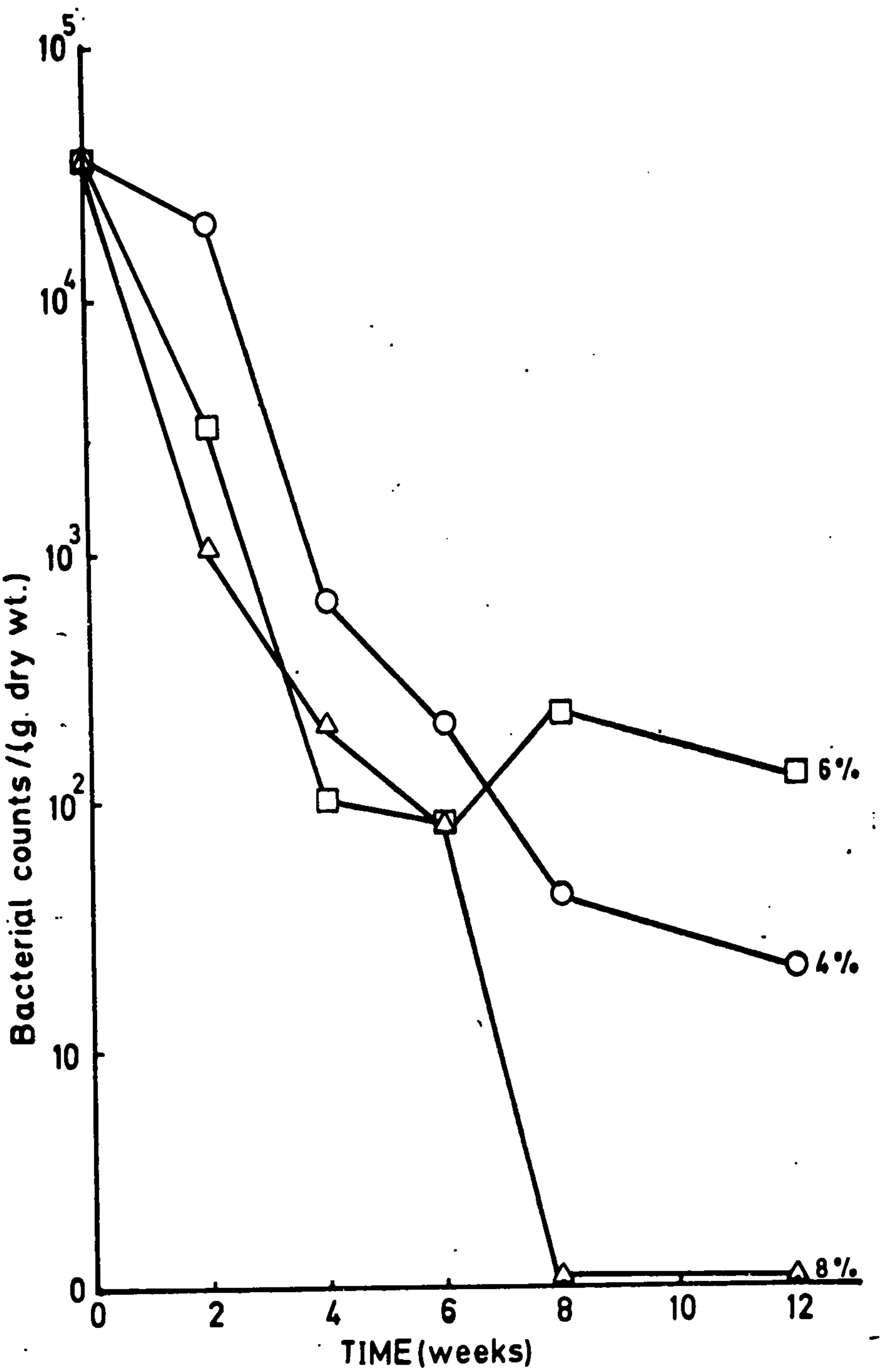


Fig. 4 — Effect of time on bacterial counts in samples stored in triple laminated aluminium foil pouches.

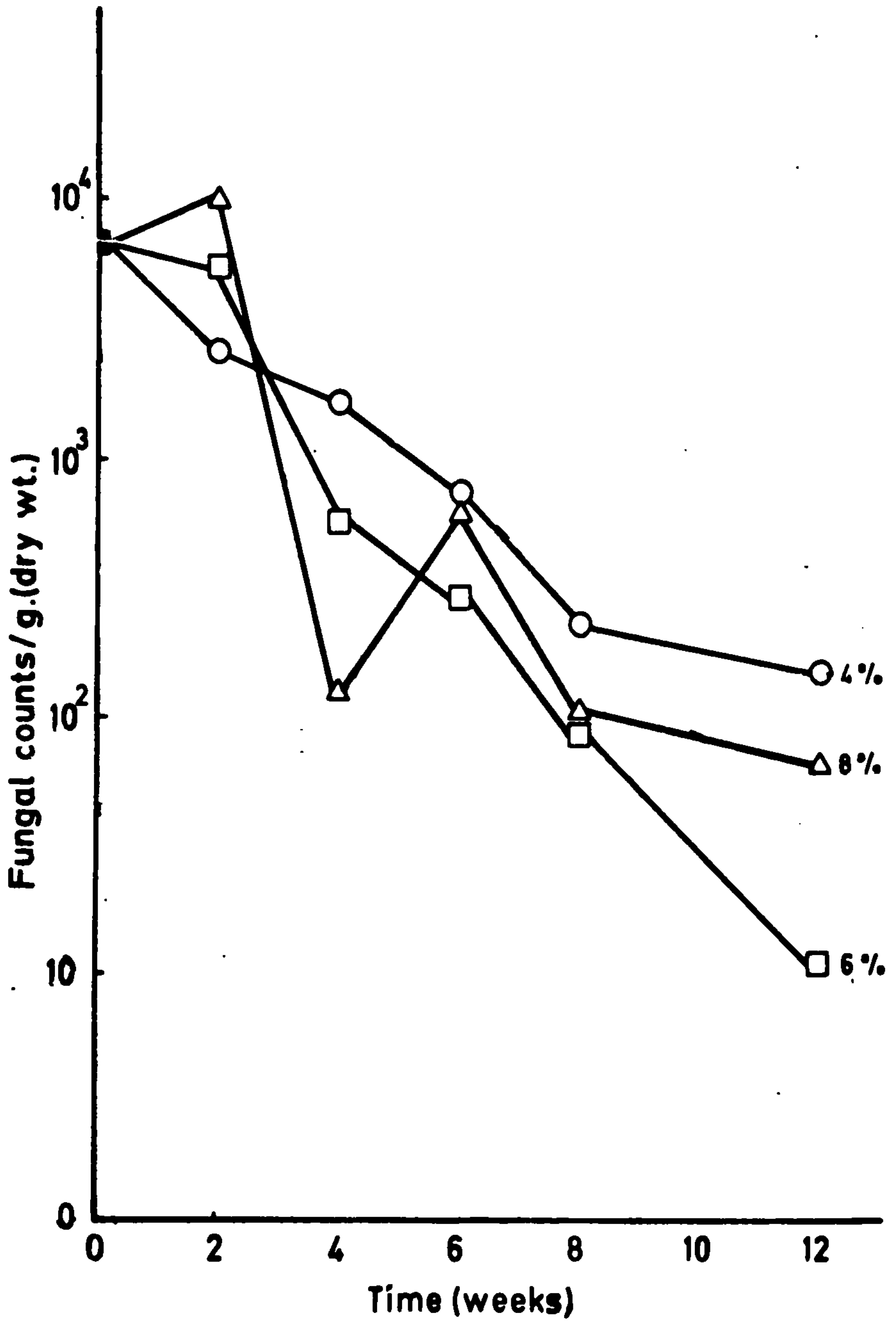


Fig. 5 — Effect of time on fungal counts in samples stored in triple laminated aluminium foil pouches.

Teas stored in miniature chests also showed the same decreasing trend in both the bacterial and fungal counts after about 2-3 months. However when the water activities of the stored teas were compared, the teas in chests showed a very high water activity compared to the teas in pouches (Table 3). This rapid increase in water activity over a short period in teas stored in chests predisposes the tea towards increased growth of micro-organisms. It is known that a water activity of over 0.8 or so promotes the rapid growth of micro-organisms (de Man, 1976).

TABLE 3—*Water activity of stored teas.*

% Moisture	*Mode of Storage*	*Storage period/ mon.*	*Water activity*
4.5	Chest	03	0.60
7.5	,,	03	0.66
11.0	,,	03	0.71
4.0	Pouch	06	0.41
6.0	,,	06	0.49
8.0	,,	06	0.64

Finally, based on the data presented above a strong case for packaging tea in high barrier material as opposed to the conventional chest could be made.

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