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(INDIA)

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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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Research Papers

Research Notes

Studies on the Insecticidal Activity of Garlic Oil. II. Mode of Action of the Oil as a Pesticide in *Musca domestica nebulo* Fabr and *Trogoderma granarium* Everts

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Manuscript Received: 26 November 1973

Mode of action of garlic oil as an insecticide has been investigated usimg larvae and adults of housefly and khapra beetle. The oil neither affects the oxidative phosphorylation nor cytochrome-c-oxidase activity *in vivo* or *in vitro* but affects the level of acetyl cholinesterase (AchE) and/or acetylcholine (Ach). However, the effect of oil varied in the test insects. In housefly adults, inhibition of AchE could not be detected both *in vivo* or *in vitro*, but the changes in the level of Ach were related to the period of exposure to garlic oil. In housefly larvae, activity of AchE was inhibited *in vivo* as well as *in vitro*, and on prolonged exposure to garlic oil, inhibition and recovery of AchE was accompanied by parallel changes in Ach level. Effect of oil on khapra beetle adults was similar to that on housefly larvae but differed markedly from its own larval stage where irreversible inhibition of AchE was not accompanied by changes in Ach level.

Concomitance of symptoms of poisoning with the changes in AchE and Ach level in the two species indicate that this may be one of the primary biochemical lesions responsible for the death of insects treated with garlic oil. The possible reasons for the differences in biochemical response are discussed.

Earlier studies on differential toxicity of garlic oil to housefly and khapra beetle indicated that adults as well as the larvae of both the species respond to the vapours of garlic oil by exhibiting hyper-excitability, ataxia, salivation and excretion[1]. Insecticides, such as pyrethrins, rotenoids, DDT, organophosphates and carbamates, inducing similar symptoms of poisoning in insects,[2] are known to express their lethality by affecting various physiological and biochemical processes including disruption of nerve impulses due to inhibition of acetylcholinesterase and accumulation of acetylcholine, uncoupling of oxidative phosphorylation and inhibition of cytochrome-c-oxidase[3]. We have, therefore, studied the effect of garlic oil on such processes in young adults and mature larvae of housefly *Musca domestica nebulo* Fabr and khapra beetle *Trogoderma granarium* Everts. Observations are presented in this paper, which suggest that garlic oil, like most of the organic insecticides also kills the insects by virtue of its effect on their nervous system.

Materials and Methods

Synthetic garlic oil supplied by Dr S. V. Amonkar of this institute, was a mixutre of diallyl disulphide and diallyl trisulphide. Acetylcholine, cytochrome c, adenosine diphosphate, hexokinase, DLα-glycerophosphate, pyruvate and bovine serum albumin were obtained from Sigma Chemical Company, .S.A.U The other chemicals used were of AR grade either from BDH or Sarabhai-Merck.

Insects : The develppmental stage and age of the insects as well as the procedure of treatment and duration of exposure to garlic oil were similar to those described earlier.[1] Number of insects employed in each experiment and number of replicates will be mentioned at appropriate places in the text.

In vitro *experiments :* To study the effect of oil *in vitro* systems, the oil and Tween 80 in the ratio of 1 : 0.1, were mixed with the reaction medium so as to form an emulsion.

Cytochrome-c-oxidase : Mitochondria were isolated from 1000 to 1500 insects according to the procedure of Rockstein and Bhatnagar[4]. The enzyme was extracted and purified partially[5,6] and assayed by the method of Smith[7]. The reaction mixture consisting of 0.3 ml of 0.01 M potassium phosphate buffer (pH 7.0), 0.2 ml of 1 per cent ferrocytochrome-c (prepared by reducing cytochrome-c with a few mg of potassium ascorbate) and 2.5 ml of distilled water, was taken in a 3 ml cuvette. The reaction was initiated by the addition of 10 μl of enzyme (7 mg protein) after equilibrating the reaction mixture at room temperature (26-27 °C). Blank was prepared by oxidizing cytochrome-c with 0.01 ml of 0.1 M potassium ferricyanide. Oxidation of ferrocytochrome-c was determined by following the decrease in absorbancy of its α-band at 550 mμ in a Hitachi-101 spectrophotometer.

Effect of 1 to 2 per cent of oil on *in vitro* cytochro me-c-oxidase activity was studied by similar procedur e in mitochondria isolated from untreated insects.

Oxidative phosphorylation : Mitochondria were isolated from 200 insects[4], and oxidative phosphorylation was studied by slight modification of Vanderberg and Slater's method[8]. The oxygen consumption was measured in warburg manometer with gas volume of 14.64 ml. The standard reaction mixture consisted of (mM) 15 potassium chloride, 2 ethylene diamine tetra-acetic acid, 2 magnesium chloride, 50 tris, 33 potassium phosphate buffer (pH 7.5), 1 adenosine diphosphate, 30 glucose and 100-140 Cori units of hexokinase. DLα-glycerophosphate (40 mM) and pyruvate (20 mM) were used as the substrate. The total reaction volume was 3 ml, 0.2 ml 10 per cent potassium hydroxide was taken in the central well containing a fluted filter paper. Mitochondrial protein equivalent to 7 mg was added to this reaction mixture. Oxygen consumption was measured at 37°C for half an hour and the reaction stopped by adding 1 ml of 40 per cent trichloroacetic acid.

Phosphorylation was determined by estimating the disappearance of inorganic phosphate in the reaction mixture[9] and protein was estimated by biuret reagent. Each treatment was replicated 5 times.

Acetylcholinesterase (AchE) and acetylcholine (Ach) : Activity of AchE was assayed in whole homogenates of the insects using acetylcholine chloride as the substrate[10]. The insects were homogenised in 2 ml of reaction medium[11] consisting of (mM) 16 veronal buffer pH 8.2, 4.4 magnesium chloride, 0.3 potassium chloride and 1.0 of acetylcholine (Ach). The reaction was carried out for 15 min in case of housefly and 60 min for khapra beetle at 37°C. The unhydrolysed Ach in the reaction medium was estimated by converting it into acetylhydroxamic acid with 2 ml of 7 per cent alkaline hydroxylamine and 6 ml of 0.5 N hydrochloric acid. The tissues were sedimented by centrifugation at 3000 g for 15 min and the clear supernatant (0.5 ml) treated with 10 ml of 1 per cent ferric chloride. The resulting reddish-brown ferric complex was estimated at 540 mμ in Bausch and Lomb spectrophotometer.

Ach in the tissues of insects was estimated by the method of Schallek[12]. Chemical nat re of the Ach extracted from the tissue homogenates was further confirmed by subjecting it to paper partition chromatography[13].

Results

Effect of oil on the level of AchE and Ach in housefly : The level of AchE and Ach have been determined in one to two day old adult houseflies exposed to garlic oil for 15, 30 and 60 min respectively. The activity of AchE was assayed in heads pooled from 10 adults, whereas for Ach 40 heads were pooled together and experiment at each dose level was replicated four times.

TABLE 1. RELATIVE EFFECT OF GARLIC OIL ON IN VITRO ACTIVITY OF CHOLINESTERASE FROM M. DOMESTIC A. NEBULO FABR. AND T. GRANARIUM EVERTS

Oil Concn. %	Acetylcholine hydrolyzed (μ moles) *M. domestica* Adult	*M. domestica* Larva	*T. granarium* Adult	*T. granarium* Larva
0	73.92±3.79	50.55±2.73	27.70±0.87	59.61±0.97
1	73.16±2.71 (99)	27.26±0.85 (53.92)	20.00±0.09 (72.19)	17.97±2.91 (30.15)
2	73.21±2.93 (99.82)	17.28±2.45 (34.18)	13.14±2.35 (47.43)	2.49±3.14 (4.17)
4	74.33±2.03 (100.6)	4.22±3.08 (8.34)	5.18±1.79 (20.97)	—

Percentage of control is given in parenthesis.
—not tested.

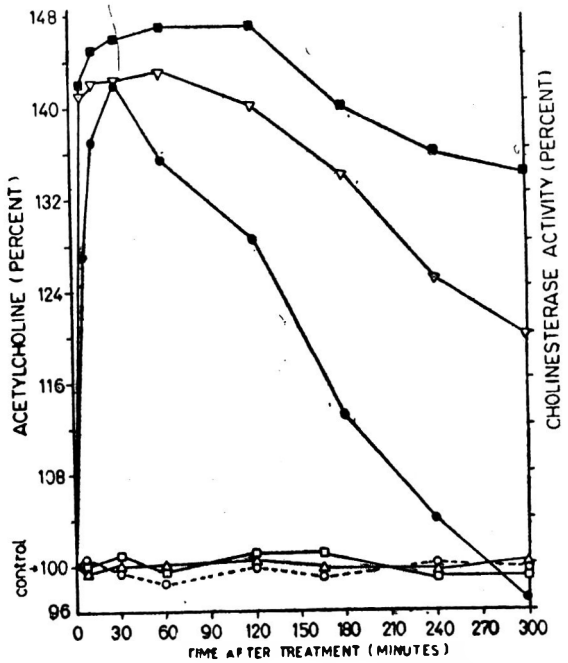

Fig. 1 LEVEL OF ACETYLCHOLINE AND CHOLINESTERASE IN ADULT M. DOMESTICA EXPOSED TO GARLIC OIL
ACETYLCHOLINE : ●15, ▽30, ■60 Minutes exposure
CHOLINESTERASE : ○15, △30, □60 Minutes exposure

In vivo *AchE activity in adult :* From the results presented in Fig 1 it can be seen that exposure of flies to garlic oil vapours for 15 to 60 min did not affect the activity of the enzyme.

In vitro *AchE activity in adult :* Observations on the effect of oil on *in vitro* activity of the enzyme are presented in Table 1. The results indicate that the degree of acetylcholine hydrolyzed by untreated flies (73.92 μ moles/10 fly) was not significantly altered by

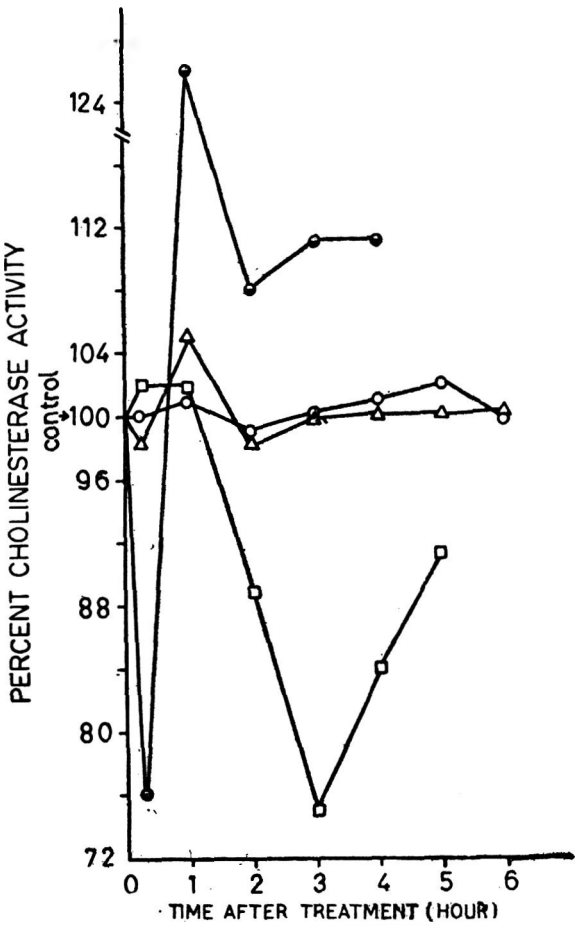

Fig. 2 CHOLINESTERASE ACTIVITY IN LARVA OF M.DOMESTICA EXPOSED TO GARLIC OIL FOR: o 5, Δ 60, □ 180 & • 300 Minutes.

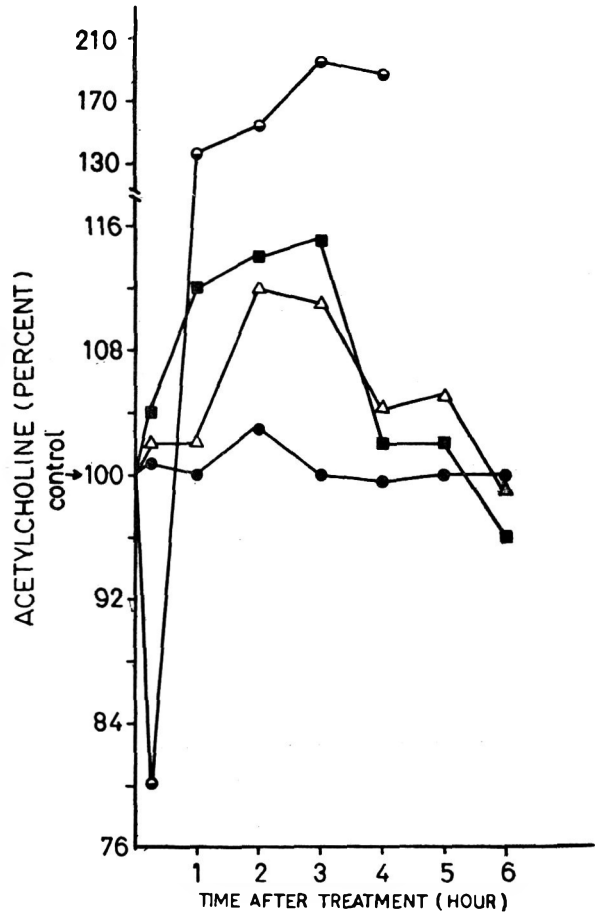

Fig. 3 LEVEL OF ACETYLCHOLINE IN LARVA OF M.DOMESTICA EXPOSED TO GARLIC OIL FOR: • 5, Δ 60, ■ 180 & • 300 Minutes.

addition of 1 to 4 per cent of oil to the fly homogenates indicating that AchE was not inhibited in *in vitro* as well.

Acetylcholine in adult : However, the level of Ach in flies was affected by the toxicant (Fig 1). On 15 min exposure concentraction of Ach increased to its maximum(142 percent)within half-an-hour of treatment. Hourly estimations thereaft·r indicate a gradual decline in Ach level with time and after four to five hour of exposure it was more or less similar to that of the control. Similar observations were made in adults exposed to oil for 30 min (Fig 1), except that increased level of Ach (140 to 143 per cent) persisted for about 2 hr and even after 5 hr of exposure Ach was 20 per cent more than the control. One hour treatment with the toxicant also resulted in similar changes in the concentration of Ach.

In vivo *AchE activity in larva :* From the results in Fig 2 it can be seen that 5 and 60 min exposure did not affect the activity of AchE. However, on 180 min exposure, AchE activity gradually declined to about 76 per cent of the control, followed by 91 per cent recovery within 5 hr of treatment. Exposure of 300 min resulted in an immediate inhibition (24 per cent of the control and recovery of AchE activity and the enzyme showed) increased activity upto 4 hr after treatment.

In vitro *AchE activity in larva :* From the observations presented in Table 1 it can be seen that degree of AchE inhibition increased with increase in concentration of oil from 1 to 4 per cent in larval homogenates. Untreated housefly larvae were able to hydrolyze about 50μ moles of Ach in 15 min this was reduced to 27μ moles on addition of 1 per cent oil to the homogenates. At 4 per cent level of the oil, Ach hydrolyzed was as low as 4μ moles, or 8 per cent of the control.

Acetylcholine in larva : As in the case of housefly adult, the larvae also showed a dosedependent increase in Ach level on exposure to garlic oil. Five min exposure (Fig 3) did not result in any change in Ach concentration. One hour treatment, however, resulted in about 11 to 12 per cent increase by about second hour after exposure, and within next three hours it declined to it, original level. In larvae exposed to oil for 180 mins maximum increase in Ach was recorded within one

hour and the increased level of Ach (12 to 15 per cent of the control) persisted for three hours before it declined to the level of control. At high dose of 300 min the results were quite unexpected, as the increase in Ach (about 93 per cent) was preceeded by about 20 per cent decline in Ach level immediately after exposure.

Effect of oil on AchE and Ach in khapra beetle : Effect of garlic oil exposures for 15, 60 and 180 min on the activity of AchE and level of Ach has been studied in one to two day old khapra beetle females. Experiment at each dose level was replicated four times with 40 females in each replicate.

In vivo *AchE activity in adult :* Observations on the effect of oil on AchE of khapra beetle adults were similar to those on housefly larva. Exposure of 15 and 60 min did not inhibit the enzyme, but on 180 min exposure AchE was inhibited to about 70 per cent of the control. The enzyme fully recovered from inhibition four hours after treatment with the oil.

In vitro *AchE activity in adult :* From Table 1 it can be seen that the activity of AchE was inhibited *in vitro* as well. The amount of Ach hydrolyzed in the presence of 1 per cent oil was 20 μ moles in comparison to about 28 μ moles in the control and AchE activity was reduced to 72 per cent of the control. On addition of 2 to 4 per cent of oil to the adult homogenates, percentage inhibition of AchE in relation to the control was 47 and 21 respectively.

Acetylcholine in adult : Exposure of khapra beetle ladults to the oil for 15 min did not affect the level of Ach, but on 60 min exposure an increase of about 61 per cent was recorded. Observations on the effect of longer exposure of 180 min were similar to those on housefly larva inasmuch as the level of Ach showed a decline to about 70 per cent within one hour of treatment followed by a gradual increase to about 98 per cent of the control.

In vivo *AchE activity in larva :* Effect of garlic oil exposures for 1, 3, 5, 7 and 18 hr on the AchE and Ach has also been studied on 20 to 22 day old khapra beetle arvae. Estimation of enzyme or the endogenous substrate were made four times at various intervals with homogenates of 400 larvae per replicate.

From the observations in Fig 4 it is evident that exposure to garlic oil results in dose related inhibition of the enzyme. Exposure for 1 hr did not affect the AchE, but on 3 to 5 hr exposure, it was reduced to about

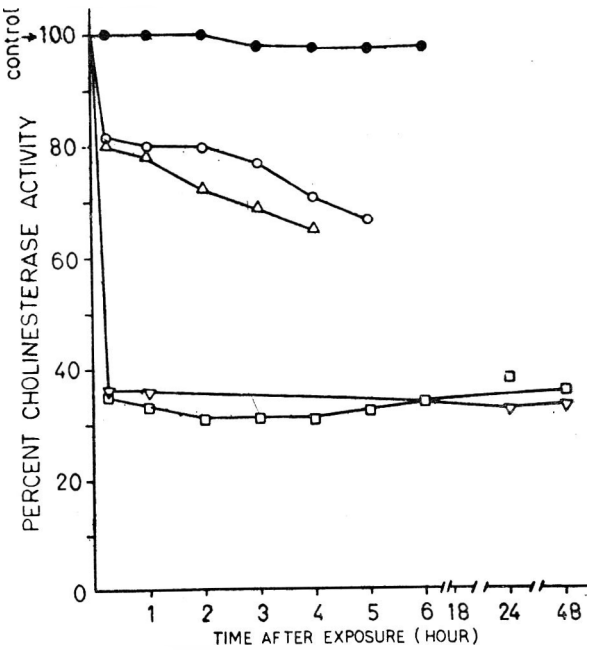

Fig. 4 CHOLINESTERASE ACTIVITY IN LARVA OF T. GRANARIUM EXPOSED TO GARLIC OIL FOR: • 1, o 3, △ 5, ▽ 7, & □ 18 Hours.

80 per cent of the control soon after treatment and further decline of 65 to 67 per cent was recorded after 4 to 5 hr of exposure. With increase in exposure time from 7 to 18 hr, activity of AchE was reduced to about 35 per cent of the control. The enzyme did not recover from inhibition even after 24 to 48 hr of treatment.

In vitro *AchE activity in larva :* In comparison to the adult khapra beetle, AchE from larvae was more susceptible to garlic oil action as even at 1 per cent level of the oil the activity of AchE was reduced to about 30 per cent of the control. Inhibition on addition of 2 per cent oil *in vitro* system was almost 96 per cent when only 2 μ moles of Ach was utilized in comparison to 60 μ moles in the control.

Acetylcholine in larva : The level of Ach (0.04 μ moles/ larva) did not change significantly at any of the test dosages of garlic oil.

Effect of garlic oil on oxidative phosphorylation and cytochrome-c-oxidase : Mitochondrial oxygen uptake of 113 μl and P: O ratio of 2.02 were not affected by garlic oil *in vivo* or *in vitro* experiments with adult flies. Similar results were obtained in case of 20 to 22 day old khapra beetle larvae. These results indicate that, unlike rotenone, garlic oil is not an inhibitor of oxidative phosphorylation.

Garlic oil did not affect the activity of cytochrome-c-oxidase in housefly larva and adults nor in khapra beetle and its larvae, although 1 mM sulfide gave 90 to 96 per cent inhibition under identical conditions indicating that the sulfide moiety in the garlic oil is not responsible for insecticidal action.

Discussion

Our studies indicate that the symptoms of poisoning and eventual death of the insects are not associated with the uncoupling of oxidative phosphorylation or inhibition of cytochrome oxidase, inspite of the toxicant being a sulfide[14]. Concomitance of symptoms of poisoning with that of changes in the level of AchE and/or the nerve transmitter Ach suggests that nervous system may be the chief site for the action of garlic oil in insects.

However, the effect of oil is found to vary even on this system in the two species as well as their developmental stages. In housefly adults we were unable to detect *in vivo* or *in vitro* inhibition of AchE with garlic oil. However, significant correlation was observed between the duration of garlic oil exposure, period for which increased level of Ach persisted after treatment and the recovery and survival of the flies. At sublethal doses the increase in Ach level was for very short period and decline in its concentration to the original level occurred within five hours after treatment, the time at which 66 per cent flies recovered from the toxic effect of the oil.[1] At lethal doses not only the relative increase in the level of Ach was higher but also the time taken for its return to the normal was longer.

More or less similar observations on AchE and Ach in DDT treated houseflies[15] and American cockroach[16] have been interpreted by some workers as side effects and not the primary cause of DDT poisoning, since they occurred very late in poisoning[17]. Such a possibility in case of garlic oil poisoned housefly is ruled out since the changes in Ach level are parallel to the symptoms of poisoning[1]. Failure to detect the inhibition of AchE in adult housefly may be attributed to the labile reactivation factor which is released during homogenization.[18,19] It is, therefore, not surprising that inspite of accumulation of Ach, we failed to demonstrate AchE inhibition experimentally.

That the effect of garlic oil on AchE and Ach is indeed the basis of its biological activity is further supported by our studies on housefly larva where a correlation was observed between increase in Ach and larval mortality reported earlier,[1] though the inhibition of AchE was detected only on prolonged exposure of 180 min. On 5 min exposure, level of AchE and Ach was not affected and 98 per cent larvae developed into fertile adults. On exposure to oil for 180 min, 12 to 15 per cent increase in Ach level was followed by inhibition of AchE and 100 per cent larvae died without pupating. Exposure to oil for 300 min not only resulted in instantaneous inhibition of AchE, but also depression in Ach level by 20 per cent of the control. Inhibition of the enzyme was followed by recovery of its activity as well as increase in Ach level by 33 to 93 per cent. More or less similar observations in case of khapra beetle adults suggest that the changes in the level of Ach may not only be due to the inhibition of AchE but also due to the effect of garlic oil on the mechanism(s) involved in the synthesis and/or release of Ach in the nerve terminals.

Depression in Ach level in insects as a result of insecticide treatment is reported in *Caliphora erythrocephala*[20] in which homogenates prepared 1 hour after treating with DDT, Ach synthesis decreased from 41 to 26 μg/g. The level of Ach rose to 80 μg/g in 4.5 hr of treatment. Depression in evoked Ach release accompanied by increased Ach *in vivo* in the presence of acetylcholinesterase inhibitors like eserine, neostigmine, oxotremorine and organophosphate echothiophate, is not a rare phenomena is further supported by the work of Szerb *et al*[21] and Szerb and Somogyi[22] on rat cerebral cortex.

In khapra beetle larvae, although there appears to be a relationship between inhibition of AchE and larval mortality[1], the enzyme did not show any recovery from the inhibitory effect of garlic oil nor any change was recorded in the level of Ach, *e.g.*, on 1 hr exposure the enzyme was inhibited by 4 per cent, and 96 per cent larvae were able to develop into normal fertile adults. On 7 hr exposure on the other hand, when AchE activity was 25 per cent of the control, 100 per cent larvae died. This together with the observations on the effect of garlic oil on *in vitro* activity of AchE from the two best insects suggest that differences in biochemical response of housefly and khapra beetle to garlic oil may be due to the differences in the characteristics of AchE as well as the endogenous level of Ach.

The endogenous level of Ach is known to be dependent upon the availability of choline. Most of the insects are unable to synthesise choline and even if they do, it is not sufficient to meet their entire metabolic needs. The source of free choline for Ach synthesis is either diet and/or phosphatidyl-choline within the cell.[23] Certain insects, *e.g.*, housefly larvae, have an efficient system for

retaining choline and can grow in its absence[24], but insects like khapra beetle larvae, have been shown to have a specific dietary requirement for choline and fail to grow in its absence[25]. Failure of accumulation of Ach in poisoned khapra beetle in which probably feeding is also affected, may therefore be due to the nonavailability of phosphatidyl-choline as well.

The symptoms of poisoning, *e.g.*, hyperexcitability, salivation and excretion further suggest that besides affecting the synaptic transmission due to inhibition of AchE and/or increase in Ach level, garlic oil may also be affecting the biophysical or physicochemical mechanisms involved in axonic transmission as well as biogenic amines and diuretic hormone which control the salivation and excretion. That the toxic action of garlic is an interaction of hormonal and nerve function needs to be ascertained.

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References

1. Bhatnagar-Thomas, P. L. and Pal, A. K., *J. Fd Sci. Technol.*, 1974, **11**,
2. Narahashi, T. in *Advances in Insect Physiology*, Beament, J. W. L., Treherne, J. E. and Wigglesworth, V. B., Academic Press, Inc., New York, 1971, **8**, 1.
3. O'Brien, R. D., *Insecticides: Action and Metabolism*, Academic Press, Inc., New York, 1967.
4. Rockstein, R. and Bhatnagar, P. L., *J. Insect Physiol.*, 1965, **11**, 481.
5. Fowler, L. R., Richardson, S. H. and Hatefi, Y., *Biochim. Biophys. Acta*, 1962, **64**, 170.
6. Tzagaloff, A. and Maclennam, D. H., *Biochim. Biophys. Acta*, 1965, **99**, 476.
7. Smith, L. in *Methods of Biochemical Analysis* by Glick, D., Interscience Publishers, Inc., New York and London, 1955, **2**, 427.
8. Vandenberg, S. G. and Slater, E. C., *Biochem., J.*, 1962, **82**, 362.
9. Fiske, C. H. and Subbarow, Y., *J. biol. Chem.*, 1925, **66**, 375.
10. Bockendahl, M. and Ammon, R. in *Methods of Enzymatic Analysis*, by Bergmeyer, H. U., 1963, 771.
11. Van Asperen, K., *Nature, Lond.*, 1958, **181**, 355.
12. Schallek, W., *J. Cell. Comp. Physiol.*, 1945, **26**, 15.
13. Augustinsson, K. and Grahn, M., *Acta Chem. Scand.*, 1953, 7, 906.
14. Amonkar, S. V. and Banerji, A., *Science*, 1971, **174**, 1343.
15. Tobias, J. M., Kollros, J. B. and Savit, J., *J. Cell. Comp. Physiol.*, 1946, **28**, 159.
16. Colhoun, E. H., *Can. J. Biochem. Physiol.*, 1959, **37**, 259.
17. Colhoun, E. H., *J. agric. Fd Chem.*, 1960, **8**, 252.
18. Mengle, D. C. and O'Brien, R. D., *Biochem. J.*, 1960, **75**, 201.
19. Sternburg, J. and Hewitt, P., *J. Insect Physiol.*, 1962, **8**, 643.
20. Lewis, S. E., *Nature, Lond.*, 1953, **172**, 1004.
21. Szerb, J. C., Malik, M. and Hunter, E. G., *Can. J. Physiol. Pharmacol.*, 1970, **48**, 780.
22. Szerb, J. C. and Somogyi, G. T., *Nature, New Biology, London*, 1973, **241**, 121.
23. Bridges, R. G. in *Advances in Insect Physiology*, by Treherne, J. E., Barridge, M. J. and Wigglesworth, V. B., 1972, **9**, 51.
24. House, H. L. and Barlow, J. S., *Ann. ent. Soc. Am.*, 1958, **51**, 299.
25. Pant, N. C., *Indian J. Ent.*, 1956, **18**, 259.

Preliminary Studies on the Isolation of Whey Proteins from Buffalo Milk

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A comparative study was undertaken to evaluate the suitability of sodium hexametaphosphate and ferric chloride for isolation of proteins from three different types of whey derived from buffalo milk. The components of the precipitated proteins were studied for any changes in their electrophoretic behaviour resulting from the ionic precipitation using the discontinuous polyacrylamide gel plate electrophoresis. The study revealed striking differences in the beta-lactoglobulin and alpha-lactalbumin fractions of buffalo and cow milk. Both polyvalent ions could be effectively used for the isolation of most of the components of whey proteins. A comparison of the protein fractions isolated by ionic precipitation from raw milk acid whey and cheese whey with the corresponding whey protein fractions of raw milk showed very close resemblance in their electrophoretic mobilities. The protein fractions isolated from *Paneer* whey, however, showed a different electrophoretic pattern.

In the recent years, there has been a considerable emphasis on the isolation of the whey proteins employing cold precipitation techniques. This makes it possible to isolate the protein in a soluble form, suitable for further processing and incorporation into food products. Methods have been developed abroad for isolating whey proteins of cow milk using polyvalent ions. The use of the polyphosphate ions in the acidified whey for the precipitation of whey proteins has been reported in a process patented by Garden[1]. In another process

patented by Weimer [2], ferric ions have been used for the precipitation of whey proteins. Jones *et al*[3] have reported the use of 'Ferripolyphosphate'— a liquid complex of ferric ions with long chain polyphosphate for reclaiming proteins from whey.

This investigation was undertaken to study the efficacy of hexametaphosphate and ferric ions for the precipitation and isolation of various whey protein fractions of buffalo milk using the cold precipitation technique.

Materials and Methods

Preparation of the samples : Samples of buffalo milk were taken from the pooled milk of a herd of Murrah buffaloes and cows maintained at NDRI, Karnal. Raw milk whey was prepared by acidification of raw milk with dilute hydrochloric acid (1:4) at pH 4.6. Cheese whey was obtained during cheddar cheese manufacture using pasteurised milk. *Paneer* whey was obtained by citric acid precipitation of milk heated to 80°C, according to the method of Bhattacharya *et al.* [4]

Isolation of whey proteins : For the precipitation of whey proteins, sodium hexametaphosphate was added at the rate of 0.5 per cent in the whey, and pH was adjusted to 2.5 using dilute hydrochloric acid (1 : 4). The flocculated whey proteins were then centrifuged out at 5000 **g** for 15 min in a Jenetzski T-24 centrifuge. The precipitate was titrated with 1/10 whey volume of 0.25 per cent calcium hydroxide. Then pH was adjusted to 8.9 with ammonium hydroxide solution (1:4). Precipitate of calcium sulphate was then centrifuged out at 5000 **g**, and pH adjusted again to 5.0 to cause flocculation of whey proteins, which were finally centrifuged out.

When ferric chloride was used for the isolation of proteins at 0.8 per cent level at pH 8.9, a gelatinous precipitate was formed. This was centrifuged out at 5,000 rpm for 15 min as described earlier.

Discontinuous acrylamide gel plate electrophoresis: For studying the fractions of the whey proteins which could be isolated by the cold precipitation technique as well as comparison of electrophoretic behaviour with their native state, raw skim milk was used, as suggested by Bailey and Lemon. [5] Samples of the whey protein isolates were suspended to give about 5 per cent protein Aronsson's buffer (pH 8.9) and dialysed against 0.05 M sodium chloride at 4°C for 48 hr with four changes of dialysing solution.

Stacking gel with 5 per cent Cynogum-41(pH 6.4) and running gel with 7.5 per cent Cynogum-41 (pH 8.9) were prepared and set in plates of size 18 cm ×9 cm ×0.4 cm, as suggested by Holmes [6]. Samples were applied on 1cm × 0.4 cm pieces of Whatman 3 mm paper, and inserted into the slots in the stacking gel, 1 cm away from the running gel. Ten samples were placed in this manner length wise. Both electrodes were filled with 0.16 M sodium borate (pH 8.6), and contact made with the gel using two folds of Whatman 3 mm paper. Electrophoresis was carried out at 160V, 60mA at 4°C (Filter paper inserts were removed after 15 min) till the brown 'borate' boundary had moved about 7 cm past insertion line (about 4 hr). Staining was done according to Crambach *et al*[7] with Coomassie Blue (Colab Laboratories Inc. Chicago Heights, 111. 60411, U.S.A.). Densitometeric plots were taken after 48 hr of intensification in 10 per cent trichloro acetic acid on a Carl Zeiss Jena Densitometer (Model 32326) with 5 × 1 mm slit opening using Farb filter (colour filter) No. 3, and Bereich (amplitude) adjustment at 0.8.

For purpose of identification of the whey protein fractions samples of beta-lactoglobulin, bovine serum albumin (Miles Laboratories, U.S.A.) and alpha-lactalbumin (Nutritional Biochemicals Corp., Cleavland, Ohio, U.S.A.) were obtained. Further purification of beta-lactoglobulin and alpha-lactalbumin was done on a 2.6 cm×80 cm column of Sephadex G-75 as suggested by Armstrong *et al*[8]. These fractions were dialysed against distilled water for 48 hr (with four changes) and freeze dried. For application on gel these samples were dissolved in Aronssons buffer (pH 6.4) to give about 5 per cent protein and electrophoresis carried out along with the milk samples as described earlier.

Results and Discussion

On the basis of the electrophoretic technique certain interesting differences were discernable in the beta-lactoglobulin and alpha-lactalbumin fractions of buffalo and cow milks. The electrophoretic patterns of the proteins isolated indicate that the technique of cold precipitation employing sodium hexametaphosphate and ferric chloride can be effectively used to recover most of the fraction of proteins from the three types of wheys used in this investigation. However, the fast moving component of the beta-lactoglobulin of buffalo milk was not present in any of the proteins isolated by the ionic precipitation technique.

Whey protein fractions of buffalo and cow milks : As may be observed from the densitometric plots, the pooled raw milk of both buffalo and cow exhibit three fractions in the beta-lactoglobulin regions (Fig 1, B-1 and C-1). While two of the slower moving beta-lactoglobulin fractions of the buffalo milk and electrophoretic mobilities close to the beta-lactoglobulin A and C fractions of the cow milk, the third component of buffalo milk exhibited faster mobility than all fractions of the whey protein fractions of both types of milk. The relative mobilities of the whey protein fractions of both buffalo and cow milk were therefore, calculated relative to this fastest moving component of the buffalo milk. The values of relative mobilities of all the fractions studied are given in the Tables 1 and 2. Earlier, Sen and Sinha[9] (using the horizontal strip paper electrophoresis) reported no variants in the beta-lactoglobulin of buffalo milk and faster mobility of the alpha-lactalbumin fractions of buffalo milk compared to cow milk.

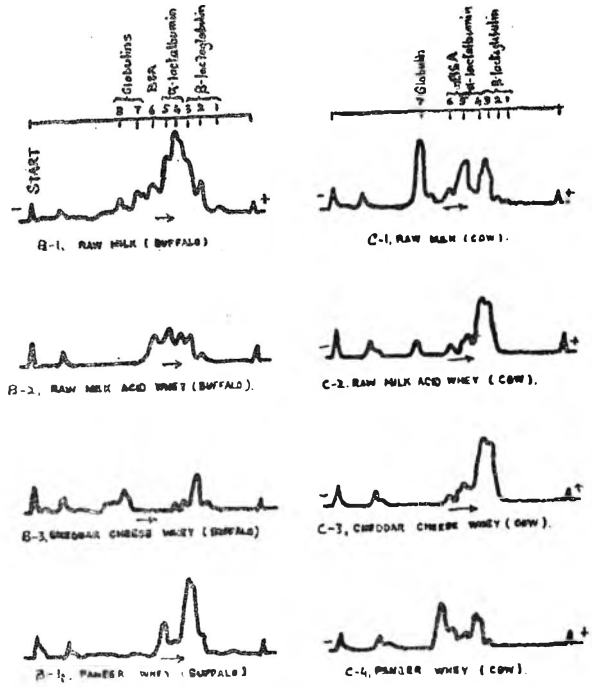

FIG 1. Densitometer plots of the proteins from differently prepared whey using sodium hexametaphosphate.

Protein fractions isolated from raw milk acid whey : When sodium hexametaphosphate was used for the

TABLE 1. RELATIVE ELECTROPHORETIC MOBILITY OF THE FRACTIONS OF WHEY PROTEINS ISOLATED BY SODIUM HEXAMETAPHOSPHATE ON DISCONTINUOUS POLYACRYLAMIDE GEL PLATE ELECTROPHORESIS

		Baffalo				Cow			
		Raw milk	Raw milk whey	Cheese whey	Paneer whey	Raw milk	Raw milk whey	Cheese whey	Paneer whey
β-lactoglobulin	Fast ..	1.00	—	—	—	—	—	—	—
β-lactoglobulin	A ..	0.93	0.91	0.94	0.90	0.94	0.94	0.94	0.94
β-lactoglobulin	B ..	—	—	0.88	0.88	0.88	0.88	0.91	0.91
β-lactoglobulin	C ..	0.85	0.85	—	—	0.84	0.84	0.87	0.89
α-lactoglobulin	A ..	0.80	0.80	0.81	0.82	0.80	0.77	0.82	0.80
α-lactoglobulin	B ..	0.74	0.74	0.76	0.75	0.74	0.74	0.74	0.75
Serum albumin	..	0.67	0.67	—	0.68	0.67	0.67	0.67	0.68
Serum protein	..	0.58	—	—	—	—	0.51	0.51	0.62
Serum protein	..	0.48	—	0.48 0.39	0.50	0.48	0.49	0.49	—

TABLE 2. RELATIVE-ELECTROPHORETIC MOBILITY OF THE FRACTIONS OF WHEY PROTEINS ISOLATED BY FERRIC CHLORIDE ON DISCONTINUOUS ACRYLAMIDE GEL PLATE ELECTROPHORESIS

		Buffalo				Cow			
		Raw milk	Raw milk whey	Cheese whey	Paneer whey	Raw milk	Raw milk whey	Cheese whey	Paneer whey
β-lactoglobulin	Fast ..	1.00	—	—	—	—	—	—	—
β-lactoglobulin	A ..	0.93	0.91	—	0.85	0.94	—	0.93	0.92
β-lactoglobulin	B ..	—	—	—	—	0.88	0.88	—	—
β-lactoglobulin	C ..	0.85	0.85	0.85	0.82	0.84	0.84	0.84	0.82
α-lactoglobulin	A ..	0.80	0.82	0.80	0.76	0.80	—	0.80	0.79
α-lactoglobulin	B ..	0.74	0.74	0.74	0.70 0.68	0.74	0.72	0.70	0.72
Serum albumin	..	0.67	0.67	—	0.64	0.67	0.67	0.69	0.67
Serum protein	..	0.58	—	0.59	0.59	—	0.62	0.55	0.59
Serum protein	..	0.48	—	0.45	0.46	0.48	—	—	0.40 0.36

isolation of proteins from the acid whey of raw buffalo milk, two fractions each of beta-lactoglobulin and alpha-lactalbumin, and one of serum albumin could be recovered (Fig 1, B-2). The protein fractions isolated from cow milk under similar conditions exhibited three fractions of beta-lactoglobulin, and two of alpha-lactalbumin and one of serum albumin (Fig 1, C-2). The electrophoretic mobilities of all the components isolated were observed to be very close to that of their native state, as may be seen from Table 1.

In the case of protein fractions isolated using ferric chloride from the acid whey of raw buffalo milk, again two fractions each of beta-lactoglobulin and alpha-lactalbumin and one of serum albumin were present. The proteins isolated from cow milk under similar conditions exhibited two fractions in the beta-lactoglobulin region, one in alpha-lactalbumin and in the serum albumin region. Here again the electrophoretic mobilities were observed to be very close to that of their native state. The values of the relative mobilities of the various protein fractions are given in Table 2.

Protein fractions isolated from cheese whey : The proteins isolated by use of sodium hexametaphosphate from the cheese whey derived from buffalo milk, exhibited two fractions each of beta-lactoglobulin and alpha-lactalbumin, and comparatively richer fraction of serum albumin. Under similar conditions of isolation of proteins from cow milk, three fractions were observed in beta-lactaglobulin, one of serum albumin, and two of alpha-lactalbumin (Fig 1, B-3 and C-3).

The use of ferric chloride for the isolation of proteins from cheese whey gave almost similar response in case of buffalo milk (except that the mobility of one of the components was slower, B-3 of Fig 2). Under similar conditions of isolation of proteins from cow milk, the isolated protein exhibited two fractions in each of beta-lactoglobulin, alpha-lactalbumin and one in serum albumin region (Fig 1, C-3).

The relative mobilities of all these components were again observed to be very close to that of their native state (Table 2).

Protein fractions isolated from paneer *whey :* In the case of proteins isolated from *Paneer* whey of buffalo milk employing sodium hexametaphosphate for the precipitation, two fractions of each of beta-lactoglobulin, alpha-lactalbumin and one of serum albumin

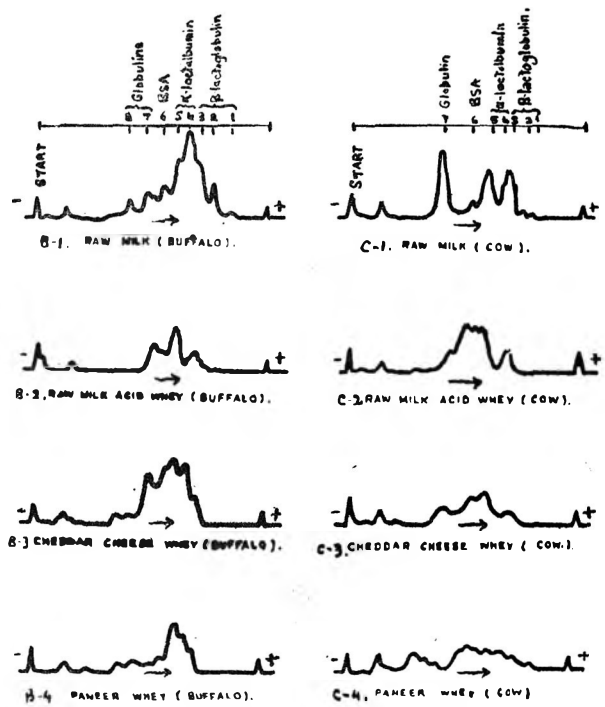

FIG 2. Densitometer plots of the proteins from differently prepared whey using ferric chloride.

were observed. However the concentration of a component in the serum albumin region was observed to be relatively higher. Likewise in the proteins isolated from the cow milk under similar conditions, three fractions were present in the beta-lactoglobulin and two in each of alpha-lactalbumin and serum albumin regions (Fig 1, B-4 and C-4).

Further, when ferric chloride was employed for the isolation of proteins, from the *Paneer* whey of buffalo milk two fractions each of beta-lactoglobulin, alpha-actalbumin and serum albumin were isolated (Fig 2, B-4). In the case of cow milk under similar conditions, the isolated protein exhibited two fractions in the beta-lactoglobulin, and alpha-lactalbumin regions and four other components in the albumin regions.

As may be observed from their relative mobilities given in Tables 1 and 2, the isolated protein fractions of *Paneer* whey exhibited slightly slower mobilities compared to their native state.

Our studies show that both sodium hexametaphosphate and ferric chloride can be effectively employed for the cold precipitation of the proteins from most of the raw milk acid whey as well as cheese whey derived from buffalo and cow milks. The electrophoretic behaviour of the proteins isolated resemble very closely

with the native state. Also these two polyvalent ions can be utilised for isolation of proteins from *Paneer* whey of both buffalo and cow milk, however, the electrophoretic behaviour of the fractions isolated is different from their native state, probably due to the heat induced changes occurring during the manufacture of *Paneer*.

References

1. Gordon, W. G., *U.S. Pat.* 2,377,624 (1945).
2. Weimer, A. C., *U.S. Pat.* 1,381,608 (1955).
3. Jones, Susan B., Kalan, E. B., Jone, T. C. and Hazel, *J. Agric. Fd Chem.*, 1972, **20**, 229.
4. Bhattacharya, D. C., Mathur, O. N., Srinivsan, M. R. and Samlik, O., *J. Fd Sci. Technol.*, 1971, **8**, 117.
5. Bailey, L. F. and Lemon, M., *J. Reprod. Fert.*, 1966, **11**, 473.
6. Holmes, R. *Biochem. Biophys. Acta*, 1967, **133**, 174.
7. Crambach, A., Reisfield, R. A., Wejckoff, M. and Zacceari, J., *Anal. Biochem.*, 1967, **20**, 150.
8. Armstrong, J. McD., Hopper, K. E. and McKenzie, H. A., *Biochem. Biophys. Acta*, 1970, **214**, 419.
9. Sen, A. E. and Sinha, N. K., *Nature, Lond.*, 1961, **190**, 343

Some Studies on the Preparation of Intermediate Moisture Guava

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Intermediate moisture guava was prepared by the immersion-equilibration procedure using a soak solution containing glycerol, sucrose, water and potassium sorbate. The product had 36 per cent moisture; 38.45 per cent glycerol; 16.1 per cent sucrose; 0.196 per cent potassium sorbate and a water activity (aw) of 0.75. Samples packed in cans maintained their acceptability for more than 6 months at 0°C, upto 6 months at room temperature (25-30°C) with slight browning and weakening of flavour beyond 4 months and upto 3 months at 37°C. Non-enzymatic browning showed a slight increase in room-temperature samples and significant increase in samples stored at 37°C between 4-6 months. The product was resistant to bacterial, yeast and mould growth and was microbiologically sound for direct consumption. Reuse of soak solution did not affect the quality of the product.

Intermediate moisture (IM) foods which are partially dehydrated foods with moisture content in the intermediate range, *i.e.*, 20 to 50 per cent, stabilised by using suitable additives so as to keep water activity low at safe levels (0.6–0.85) from the angle of microbiological spoilage, have attracted the attention of many workers recently. The principle behind the development of such foods is that one need not dehydrate foods to the 5-10 per cent moisture level dictated by microbiological stability. There will be substantial reduction in drying and reconstitution time and better retention of original flavour and texture compared to conventional hot air dried or heat processed (canned) foods if the food is dehydrated to an intermediate moisture level. The IM foods will have the additional advantage of possessing sufficient moisture to provide the necessary plastic mouth feel to enable the food to be eaten without any further preparation and will keep lonʌ without refrigeration or thermal processing in ang hermetically sealed container.

Preparation of some meat and vegetable dishes baed on intermediate moisture components is describeds by Hollis *et al*[1] who found their limited acceptability over 3 months. Stability of IM foods in appropriate systems with respect to lipid oxidation[2] and microbial growth[3] has been reported.

The potential advantage of IM foods for special military situations have been discussed by Brockmann[4] who considers these as eminently more suited for combat rations as compared to fully dehydrated or canned foods.

Investigations on the application of this techniqLe to the development of varieties of fruits for use in combat rations were undertaken in this laboratory. Results of experiments on the preparation of intermediate moisture guava are reported in this paper.

Materials and Methods

Raw material: Fully ripe guavas (white fleshed variety) procured from the local market were used.

Preparation of fruit: The fruits were washed, soaked in 0.1 per cent potassium permanganate solution

for 15 min, washed thoroughly free of permanganate and trimmed to remove the stem and blossom ends. The fruits were then halved, seeds removed by coring and cut into slices of about 3 cm length and 1 cm thickness.

Preparation of intermediate moisture guava: The IM product was made by the immersion-equilibration procedure wherein the raw fruit slices were blanched and equilibrated in a solution having a composition similar to the one used by Hollis *et al*[1] for apple slices and composed of glycerol, 42.33; sucrose, 42.33; water, 14.84; potassium sorbate, 0.45; and potassium metabisulphite, 0.05 per cent.

The method consisted in adding the fruit slices to the soak solution (prepared fruit: soak solution, 1 : 2.4) previously heated to 95 °C, keeping the fruit immersed in the solution at 90 °C for 3 min and then immediately cooling the mixture to room temperature. The well immersed fruit was allowed to equilibrate in the solution overnight in a refrigerator and then drained thoroughly over a stainless steel wire mesh. The drained fruit slices were packed as such in 301 × 206 (8 oz) SR lacquered cans.

Analytical methods: The proximate composition of the IM guava was determined by the AOAC methods[5]. Free sulphur dioxide was determined by the ISI method.[6]

Sucrose was determined in both IM guava and the equilibrated solution by the Lane and Eynon method[5] and potassium sorbate by the method of Nusy and Bolin.[7] Acidity was estimated by titration with N/100 sodium hydroxide solution. The glycerol content of the finished product and soak solution after equilibration were calculated by difference since available methods (AOAC) are time consuming and cumbersome applicable only to alcoholic drinks, vinegar, etc. and did not give good results when applied to IM fruit.

Water activity of the product was determined by measuring the equilibrium relative humidity (ERH) by a modification of the graphical interpolation method of Landock and Proctor[8]. Five gram lots of the material were exposed to different R. H's ranging from 63 to 96 per cent at room temperature (25 °C) in desiccators containing saturated solutions of different salts having definite R. H. for 24 hr and the gain or loss in weight of each sample determined. The values were plotted with reference to the relative humidities, the gains in weight being shown above and losses in weight below a horizontally drawn zero base line representing no change in weight. A smooth curve was drawn through the plotted points and the ERH interpolated at the point where the curve intersected the zero base line.

Storage studies : The IM guava slices packed in cans (about 200 g per can) as above were stored at 0 °C, room temperature (25–30 °C) and 37 °C, and examined periodically by a taste panel for colour, flavour and texture.

Non-enzymatic browning was measured by a modification of the method of Hendel *et al*[9] by extracting 5 g of the sample with 100 ml of 66 per cent alcohol and measuring the optical density of the clear extract in a Klett Summerson photoelectric colorimeter at 420 mμ. The results were expressed as $E^{5\%}_{1cm}$ 420 mμ. Browning was also followed by measuring the colour directly using Lovibond Tintometer and the colour expressed in red, yellow and blue units.

Microbiological status of the products was evaluated by measuring the total plate count, *Staphylococcus*, coliforms, yeasts and moulds by the methods recommended by the American Public Health Association[10].

Reuse of soak solution : With a view to standardise the procedure for reuse of equilibrated soak solution two methods were tried :

(i) Removal of about two-thirds of the water from the filtered solution by evaporation in a steam jacketed kettle and adjustment of the sugar, water, glycerol and potassium sorbate contents back to the same level as in the original soak solution. The adjustment was done based on estimation of the water, sucrose and sorbate in the used solution after concentration by methods described above and of glycerol content by difference.

(ii) Using a proportion of the equilibrated solution to make fresh solution through additiona ingredients based on analysis as above[7] Quantity of equilibrated solution that could. be reused by this method was limited by its water content.

IM guava was made reusing the equilibrated soak solution and its composition and shelf life studied simultaneously along with the sample prepared by first soak with a view to find out any changes that might be caused due to reuse of the solution.

TABLE 1. DATA ON THE PREPARATION OF INTERMEDIATE MOISTURE GUAVA

Particulars	Prepared fresh fruit	IM product	Soak sol. after equilibration	Equilibrated soak sol. after concentration
Moisture (%)	85.9	36.0	33.8	13.2
pH	3.9	4.4	4.9	—
Acidity (as % citric)	0.3	0.18	0.16	—
Total sugar (%)				
Dextrose	5.2	16.9	34.1	44.3
Sucrose	4.9	16.1	32.3	42.1
Sugar/acid ratio	16.5	89.4	201.8	—
Potassium sorbate (%)	—	0.196	0.243	0.28
Glycerol (by dif. %)	—	38.45	33.90	44.70
E.R.H. (%)	—	75	—	—
Yield (%)				
Raw wt. basis	65	30	—	—
Prepared fruit wt. basis	—	50	—	—

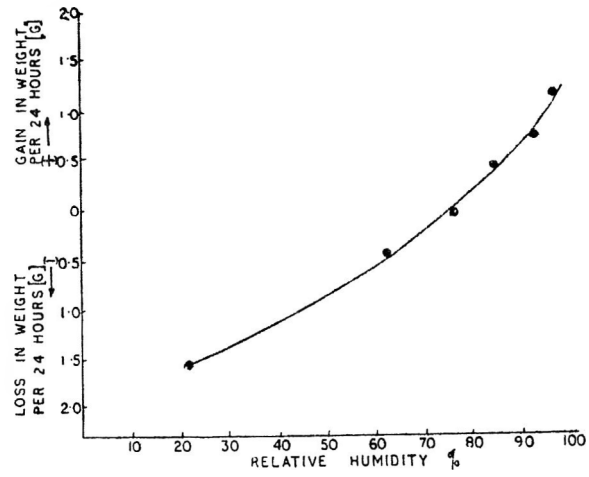

FIG 1. ERH curve for IM guava at room temperature (25°C)

Results and Discussion

Data on the preparation of IM guava are given in Table 1. An IM product of excellent flavour and good texture and colour comparable to the fresh fruit was obtained. It was moist and soft enough to be eaten as such without any further rehydration.

Proximate composition of the product was: moisture, 36.0; total sugars (as sucrose), 16.1; ether extractives, 0.1; protein (N × 6.25), 0.27; crude fibre, 8.5; total ash, 0.58; glycerol and other carbohydrates (by difference), 38.45 per cent. The calculated calorific value was 235 per 100 g (assuming 4.3 calories per gram for glycero[5]). Sulphur dioxide content was 112 ppm.

The ERH curve determined by the graphical interpolation method is given in Fig 1 and it shows that the product had an ERH of 75 per cent. The water activity achieved (0.75) was low enough to prevent the growth of bacteria as such and that of yeasts and moulds in the presence of potassium sorbate (0.2 per cent).

Data on the storage aspects of the product at 0°C, room temperature and 37°C upto 6 months storage are given in Table 2. Samples stored at 0°C were unchanged in organoleptic quality and found to be highly acceptable even after 6 months. Samples kept at room temperature were found to be acceptable upto

TABLE 2. CHRACTERISTICS OF INTERMEDIATE MOISTURE GUAVA DURING STORAGE

Storage temp. °C	Period (months)	Non-enzymatic browning Extract ($E^{5\%}_{1.0\ cm}$ 420mμ)	Direct (Lovibond Tintometer units) Y	R	B	Organoleptic quality
0 ..	2	0.090	7.0	4.0	3.0	Good colour, texture; excellent flavour.
	4	0.086	7.0	4.0	3.0	,,
	6	0.090	7.0	4.0	3.0	,,
Room temp. ..	2	0.090	7.4	4.1	3.0	Comparable to control in colour, texture, flavour.
	4	0.112	8.8	5.0	3.0	Slightly brown, softer and weaker in flavour than control; acceptable.
	6	0.228	8.3	5.2	4.5	Light brown; softer and weaker in flavour than control; just acceptable.
37 ..	2	0.122	8.1	4.7	3.0	Light brown; comparable to control in texture and flavour; acceptable.
	4	0.154	9.8	6.5	4.9	Brown; softer and weaker in flavour than control; just acceptable.
	6	0.306	9.0	7.0	5.9	Dark brown; softer than control; very weak in flavour; unacceptable.

6 months although there was slight browning and weakening of flavour beyond 4 months. There was no rancidity or off-flavour. Samples kept at 37 °C were acceptable only upto 3 months beyond which there was considerable browning and weakening of flavour. There was a slight softening of the tissue at room temperature and 37 °C compared to 0 °C sample but this improved their acceptability. The cans were unaffected internally in all the samples.

Non-enzymatic browning measured both in terms of Lovibond Tintometer units and as optical density of the 5 per cent alcoholic extract showed significant increase in brown colour of samples stored at 37 °C and a slight increase in room temperature in samples between 4–6 months.

Although the experiments were carried out only in cans, the product could be preserved equally well in kraft paper (60 B.C.) aluminium foil (0.02 mm) polythene (150 gauge) laminate pouches.

Microbiological analysis of the product after 6 months storage at the three temperatures showed that while at 0 °C the total plate count was 220–290 colonies per gram it was negligible at room temperature and at 37 °C. *Staphylococcus*, coliforms, yeasts and moulds were negligible at all the temperatures. From the data, it can be concluded that the IM guava was resistant to bacterial, yeast and mould growth and was microbiologically sound for direct consumption.

The slightly more total plate count observed at 0 °C compared to negligible counts at room temperature and 37 °C is possibly due to the organisms present in the equilibrated material just prior to packing which had either survived the blanching process or came through subsequent contamination while handling at the time of packing. This is because no bacteria can grow in the product subsequent to packing since the water activity (aw) of the product (0.75) is much lower than the minimum 0.86 required for any bacterial growth and as such is unfavourable for growth. Our observation further confirms the earli erfindings by Hollis *et al.*[1] who made initial counts and counts after one month and 4 months storage at 38 °C for bacteria, mould and yeast for nine IM items and found the plate count to decrease considerably and the mould and yeast count to be negligible throughout. By inoculation studies using pathogenic organisms in the same IM systems, these authors showed that the product not only prevented the growth of pathogens but caused their reduction to negligible levels.

No data are available in the literature on the feasibility and mode of reuse of the equilibrated soak solution. This is very important factor influencing the cost of the product particularly in view of the high cost of glycerol. Conditions were therefore worked out for the reuse of the equilibrated soak solution. Of the two methods tried, removal of water by concentration in a kettle followed by adjustments based on analysis was preferred since this facilitates reuse of the entire solution. There was practically no change in the ERH, composition and the organoleptic quality of the product which was observed to have a shelf life very similar to that of the material prepared by first soak.

Acknowledgement

The authors express their grateful thanks to Dr (Mrs) Rugmini Sankaran for her help in the microbiological analysis of the product.

References

1. Hollis, F., Kaplow, M., Halick, J. and Nordstrom, H., *Parameters for Moisture Content for Stabilisation of Food Products* (Phase 2), U.S. Army Natick Labs, Contract DAAG 17-67-C-0089, 1969.
2. Labuza, T. P., McNally, L., Gallagher, D., Hawkes, J. and Hurtado, F., *J. Fd Sci.*, 1972, **37**, 154.
3. Labuza, T. P., Cassil, S. and Sinskey, A. J., *J. Fd Sci.*, 1972, **37**, 160.
4. Brockmann, M. C., *Fd Technol, Champaign*, 1970, **24**, 896.
5. *Official Methods of Analysis*, Association of Official Agricultural Chemists, Washintgon, D.C., 9th Ed., 1960.
6. *Indian Standard Specification for Dehydrated Cabbage*, Indian Standards Institution, New Delhi. IS 4627, 1968.
7. Nusy, P. S. and Bolin, H. R., *J. Fd Sci.*, 1962, **27**, 370.
8. Landrock, A. H. and Proctor, B. E., *Mod. Packg.*, 1951, **24**, 123.
9. Hendel, E. C., Bailey, G. F. and Taylor, D. H., *Fd Technol., Champaign*, 1950, **4**, 344.
10. *Recommended Methods for the Microbiological Examination of Foods*, American Public Health Associatin, N.Y., 1966

Development of Long-keeping Bread

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Bread with the addition of ascorbic acid 100 ppm, α-amylase 200 ppm, lactic acid 0.5 per cent V/W, black pepper 3 per cent and CMC 2 per cent + glycerol 1 per cent + sorbitol 1 per cent (all on flour wt.) can be preserved upto two months in flexible packs when packed by sorbic acid treated wrapping paper technique combined with heat treatment. The most effective method of controlling staling during storage is vacuum or nitrogen packing in sanitory cans.

Bread and bread products are the desired items in the service rations. Normally the breads procured from the trade or service bakeries deteriorate in texture and flavour after 48 to 72 hr of storage at ambient temperature. To meet the requirements of Defence Services specially the Navy, work was undertaken in this laboratory on the development of long-keeping bread with a shelf-life of three months. Besides keeping the bread mould free, the problem faced in long term storage of bread is, the staling phenomena characterised by change in taste and aroma, and increased hardness and crumbliness of crumb. The various methods adopted to solve these problems and results achieved are reported in this communication.

Materials and Methods

All raw materials like wheat flour, oil hydro, sugar, salt, etc., used in the experiment were procured from trade.

Bread making : The breads were made with *maida* (wheat flour of 70 per cent extract). The per cent basic ingredients used in the formulation of bread on flour basis were : dried yeast, 1; salt, 2; sugar, 6; hydrogenated oil, 5; non-fat milk solids, 2; and water as needed. An optimum mixing time was employed and straight dough procedure with 2.5–3 hr fermentation time at 25–30 °C adopted. Proofing time was 60 min at 40 °C and baking was done for 30 min at 220 °C.

Starch film coating on bread : Starch slurry was made with corn starch and water by boiling to gelatinize the starch. Sorbic acid was added after cooling to 60–65 °C and then stirred vigorously. Bread was coated with slurry manually and dried in a cabinet drier for nearly 2 hr at 60–70 °C to get a dry starch film coating on bread. The breads were then packed in 60-g kraft paper/150-g polythene pouches and subsequently heat treated to 95 °C for 30 min.

Spraying alcohol on bread : Alcohol and alcoholic solution of sorbic acid was sprayed on breads (by manually operated atomiser sprayer), immediately wrapped in butter paper and subsequently sealed in 300 g polythene pouches or immediately packed in paper/polythene laminate pouches.

Packaging with sorbic acid treated crepe paper : The solution prepared for paper treatment consisted of alcohol (90 per cent), 100 ml; sorbic acid, 3.0 g; Embanox-6, 0.08 ml. Crepe paper was treated with this solution so as to have an uptake of 2.0 g sorbic acid/sq. meter of paper, and was air dried. Bread was wrapped with this paper, heat sealed in 60 g/kraft paper/150 g polythene laminate pouches and finally heat treated at 95 °C/30min.

Canning of bread : For canning $2\frac{1}{2}$ size cans were used. Dough (200 g) with the composition stated earlier was put in the can. Proofing (45 min/40 °C) and baking (220 °C/30 min) were done in the can itself. The cans were hermetically sealed and processed at 15 lb/30 min and 10 lb/30 min. The cans were then evacuated to 24 in vacuum for high vacuum packing and flushed with nitrogen for inert gas packing.

Preparation of bread with fatty esters/antistaling agents : Calcium/sodium steoroyl lactylate and polyoxy ethylene sorbiton mono-stearate were added (0.2 to 0.5 g/100 g flour) in molten fat, whereas GMS, propylene glycol mono-stearate, sorbtion mono-stearate and distilled mono-glycerides were added (0.2 to 0.5 g/100 g flour) in the form of 15 per cent gel and 25 per cent emulsion with pH adjustment of 6.8 and 7.3 respectively. Ascorbic acid was added at 50–300 ppm level.

Analytical techniques : Moisture, fat, protein, ash, alcohol contents were determined as per AOAC[1] methods. Sorbic acid was estimated as per the method of Schmidt.[2]

TABLE 1. EFFECT OF SORBIC ACID IN DOUGH

Sorbic acid added %	Mouldy breads %	Days of observation	Sorbic acid retention %
0.00	100	10	—
0.10	100	15	—
0.15	54.2	15	—
0.20	41.6	15	—
0.25	29.1	30	0.112
0.275	16.6	30	0.137
0.30	10.7	30	0.175

Storage temp: 24–32 °C.
Addition of sorbic acid beyond 0.30% (on flour wt. basis) severely interfered with yeast activity in dough.

Sorbic acid in dough : Table 1 shows the effect of sorbic acid on bread preservation. It is to be noted that 0.3 per cent of sorbic acid on flour weight basis was found to be optimum for preservation. Beyond this level it severely interfered with yeast activity in dough. Below this level spoilage was heavy.

Sorbic acid like any other mould inhibitors such as propionates, benzoates, parabens (p-hydroxy benzoic acid) seriously interferes with the yeast activity during dough fermentation resulting in reduced volume and closed texture of bread. Hence it was necessary to suitably modify the process of bread preparation. It is evident from Table 2 that if yeast is increased from 1 to 1.5 per cent and proofing time from 1 to 1.5 hr it is possible to obtain bread with not much significant volume loss provided sorbic acid is added in the dough just before proofing stage.

TABLE 2. DETERMINATION OF OPTIMUM CONDITIONS OF ADDITION OF SORBIC ACID IN DOUGH

Yeast added %	Proofiing time hr	Decresae in bread volume %
1.0	1.0	Control
1.5	1.0	15-20
2.0	1.0	,,
2.5	1.0	,,
1.5	1.0	,,
1.5	1.5	< 5
1.5	2.0	,,
1.5	2.5	,,

Bread formulation with 0.3% sorbic acid (on flour wt. basis) packed in paper/poly laminate pouches.
With the addition of 0.3% sorbic acid in dough, yeast 1 5% (on flour wt) and proofiing time, 1.5 hr was found to be optimum.

TABLE 3. EFFECT OF STARCH FILM COATING CONTAINING SORBIC ACID ON PRESERVATION OF BREAD

Starch slurry composition	Slurry in bread (g/100 g)	Sorbic acid in bread (g/100 g
Corn starch—100 parts Sorbic acid—0.5 parts Water: To give 5% slurry	18.1	0.0029
Corn starch—100 parts Sorbic acid—0.45 parts Water: To give 6% slurry	19.3	0.0031
Corn starch—100 parts Sorbic acid—0.45 parts Water: To give 7% slurry	21.3	0.0040
Corn starch—100 parts Sorbic acid—0.30 parts Water: To give 8% slurry	23.6	0.0035

Packaging: Sealed in 60 g kraft paper/100 g polythene laminate pouches, heat treated to 95 °C for 30 min.

Results and Discussion

Sorbic acid treated starch film coating : Table 3 shows the effect of sorbic acid incorporated starch film coating on preservation of bread. It had been observed that in order to stabilise the bread against mould attack the dry starch film coating (containing sorbic acid) should be continuous and unbroken. Wherever the film was broken or disjointed, there were pockets of mould growth on storage of bread. Seven per cent starch slurry was found to be optimum for application on bread which also gave optimum thickness of starch film on drying. In 5 and 6 per cent starch slurry batches there were stray cases of discontinuous starch film resulting into pockets of mould growth in stored bread. The overall concentration of sorbic acid in the bread is as low as 0.0029 to 0.0040 per cent (bread wt. basis). These breads when packed in 60 gauge kraft paper/ 150 gauge polythene pouches and subsequently heat treated at 95 °C for 30 min remained mould free for three months. However, after 8 wk of storage the breads became hard and crumbly due to staling.

Sorbic acid in wrapping paper : The preservation characteristics of commercially available breads by wrapping with sorbic acid impregnated wrapping paper has already been reported[3]. The commercial breads were pre-wrapped with crepe paper impregnated with sorbic acid and sealed in paper/poly laminate pouches. They were subsequently heat treated at 95–100 °C for 30 min. It was observed that with certain optimum level of sorbic acid and an antioxidant in crepe paper

the commercial breads could be kept mould free upto 6 months. But unsliced breads were found organoleptically acceptable upto 4 weeks storage and upto 8 weeks with the application of butter and jam or after toasting.

Preservation of bread based on alcohol : Spraying of 400 g breads with atomiser sprayer (hand operated) using alcohol concentration of 3 to 6 per cent and packing in polythene laminate pouches showed (Table 4) that such breads stored upto one month with about 20 per cent spoilage in alcohol concentration of less than 5 per cent (V/W) and 10 per cent spoilage with those sprayed with 5–6 per cent (V/W) alcohol. However, the concentration of alcohol in breads was not uniform due to the use of hand operated sprayer, the values ranged from 1.7 to 4.1 per cent (V/W) in breads sprayed with alcohol concentration of 3 to 6 per cent (V/W). Combination of 2.5 to 3.75 per cent (V/W) alcohol and 0.3 to 0.45 per cent sorbic acid as a spray solution and packing in inner wrap of butter paper followed by outer wrap of 300 g polythene pouches kept these breads mould free for three months but they developed positive bitterness and marked offensive odour. It was found easier to preserve slices/sandwiches by alcohol spray method[4]. Consistently mould free sandwiches (20 g fruit jam applied in 20 g each of two bread slices) sprayed with 95 per cent alcohol and incorporating 3 g alcohol per sandwich were obtained even after a storage of six months under ambient temperature of 24–30 °C when pre-wrapped into butter paper and sealed in 60 g kraft paper/.02 mm aluminium foil/150 g polythene laminate pouches. It will appear that if efficient uptake of alcohol is ensured in whole bread as in sandwiches, it may be possible to prepare uniformly consistent mould free breads with shelf life of three months.

Control of staling in stored bread : Staling is defined as decreasing consumer acceptance of baking products by changes in the crumb other than those resulting from the action of spoilage organisms. Change in taste and aroma, increased hardness of crumb, increased capacity of crumb, increased starch crystallinity of crumb, decreased susceptibility of crumb to attack by α amylase are some of the most important characteristics of staling phenomena[5]. The protein molecules in wheat gluten occur in native state in aggregated coiled structures are considerably straightened and partially broken down into smaller and more linear units[6]. These units then subsequently join together by disulphide linkages into three dimensional matrix by disulfidesulphydryl exchange reactions. During the stage of baking the three dimensional network of gluten sets to give more rigid form and gelatinized starch molecules are embeded in it. Entrapped gas in the dough system expands leading to cell wall expansion and finally rupture of cell walls giving ultimately the cellular structure of bread crumb. The cellular structure gives rise to large surface within the crumb which may be exposed to direct air and vapour movements. The bread system as such is a complex system, a very unstable physically and chemically.

TABLE 4. EFFECT OF ALCOHOL/ALCOHOLIC SOLUTION OF SORBIC ACID ON PRESERVATION OF BREAD

Alcohol (%) sprayed on bread	Sorbic acid in bread %	Alcohol retention %
3.0	—	1.70
4.0 ..	—	2.36
5.0 ..	—	3.12
6.0 ..	—	4.10
2.5 ..	0.30	1.41
3.75 ..	0.45	2.11

When bread is allowed to age the gelatinized starch molecules retrograde with the release of water molecules and with the formation of new H-bond between the hydroxyl groups of adjacent chains.[7] This association of starch molecules together with loss of water gives rise to crumb hardness. Hellman *et al.* were able to relate starch gel crystallinity changes during ageing to crumb firmness of bread suggesting crystallinity might be a significant factor in staling.[8] Also in this process the original starch imbeded structure of gluten network is disrupted since starch shrinks away from gluten network giving rise to crumbliness in stale bread.[9] The water released by starch may be absorbed by protein matrix and also by the crust of the bread which affects its crispness which is another negative factor in stale bread.

Further, progressive interactions also take place between starch, sugar, other carbohydrates, lipids, proteins, vitamins, etc., in a system which containes about one-third as moisture. The delightful aroma of freshly baked bread is transient and subtle and the compounds responsible may be broadly classified as acids, alcohols, esters and carbonyl compounds, each group having its own inherent stability and properties of reactivity on storage. The result of this, together with starch retrogradation, liquid oxidation and interactions in other components will introduce off aroma and off-flavour in stored bread.

Control of staling by moisture mimetic agents : Various attempts to control staling in bread and aiming at a

storage life of 3 months were made. Table 5 indicates the results obtained by use of various moisture mimetic agents in bread formulations. These moisture mimetic agents with their preponderance of hydroxyl groups were expected to retain more moisture in the bread. It will appear if initially the bread contains little more moisture, the crumb hardness on storage may be reduced to some extent. It was observed that additional initial moisture intake in bread samples by virtue of presence of moisture mimetic agents was marginal, the maximum being 2.1 per cent (Table 5). Also there was practically no difference between the moisture retention capacity of experimental bread samples incorporating these agents and control samples on one month's storage of bread. Nevertheless, when examined organoleptically the stored bread samples containing some of these agents like CMC or CMC+ sorbitol + glycerol were found softer compared to control samples. Apart from difference in texture of crumb, there was difference in physical sensations emanating from chewing the two types of breads. Certainly, for overall staling the moisture is not a very significant index for stalness in bread as described in preceeding paragraphs. Pectin, sodium alginate, glycerol and sorbitol did not have any significant effect. Guar gum gave offensive odour to stored bread samples. The combination of carboxy-methyl cellulose (2 per cent), glycerol (1 per cent) and sorbitol (1 per cent) (on flour wt. basis) was most effective.

Control of staling by chemicals : Some of the chemicals like mono glyceroides are used in bread preparation as fat emulsifying agent for better distribution of fat in gluten and starch components of dough. Various types of fatty esters are now commercially available which are claimed to be anti-staling agents for bread. One such widely used chemical is calcium/sodium stearoyl-2 lactylate.[10-13] It is known to act as dough conditioner and emulsifier, permitting the production of high quality baked products over a wide range of processing and ingredient variation retard the retrogradation of starch resulting in longer shelf life and freshness. Polyoxyethylene sorbiton mono-stearate has also been studied as an anti-staling agent and dough conditioner in yeast raised baked foods[14]. The effect of distilled mono-glycerides, propylene-glycol mono-stearate, tartrated ester mono-glycerides, stearoyl fumarate, lecithin in bread dough and anti-firming agents have also been studied[15]. It is presently held that the surfactant, being absorbed on the surface of the starch granule, interferes with bonding between the protein fibirls which surround the starch granule. In addition by complexing with starch, surfactants retard the development of more rigid cross-lined amylose chains which reduce the compressibility of these protein network to which they are attached[15]. Recently Krog and Nybojensen have confirmed the formation of insoluble complex with amylose by saturated mono-glycerides in Danish white bread.[16]

In the present study, calcium and sodium stearoyl lactylate when added with dough at the level of 0.5 per cent (on flour wt.) preblended with the fat, gave somewhat uniform crumb texture initially but on long storage by sorbic acid treated paper there was practically no difference in crumb hardness between experimental and control samples when examined organoleptically. Long term storage contributed extraneous flavour and slight bitterness in stored bread. Polyoxyethylene sorbiton mono-stearate at a level of

TABLE 5. CONTROL OF STALING OF BREAD BY MOISTURE MIMETIC AGENTS

Moisture mimetic agents	Quantity per 100 g flour g	Additional initial moisture intake %	One month after storage at amb. condition: Moisture loss % Expt. samples	Control samples	Organoleptic quality comp. to control
Pectin	.. 1.0	1.6	2.9	2.1	No difference
Glycerol	.. 2.0	Nil	3.2	2.5	,,
Sorbitol	.. 5.0	Nil	2.5	2.8	,,
Sodium alginate	.. 1.0	1.5	3.4	3.0	,,
Carboxy methyl cellulose	.. 2.0	1.9	2.1	2.7	Softer
Guar gum	.. 2.0	1.9	3.2	3.5	Offensive odour
Pectin+sorbitol	.. 0.5+2.0	1.8	1.9	2.5	No difference
Sorbitol+glycerol	.. 2.0+2.0	1.1	2.7	3.5	,,
Carboxymethyl cellulose+glycerol+sorbitol	.. 2.0+1.0+1.0	2.1	2.6	2.2	Softer

Prewrapped with sorbic acid treated crepe paper, sealed in paper/poly laminate pouches. Heat treated to 95°C/30 min.

0.25 per cent on flour weight did not retard the crumb hardness but imparted undesirable flavour on long term storage. When 0.3 per cent GMS was mixed with 0.2 per cent polyoxyethylene sorbiton mono-stearate, no difference was observed in reducing crumb hardness in stored bread. Commercial glycerol mono-stearate, sorbiton mono-stearate, propylene glycol mono-stearate did not contribute to reduction of crumb hardness on long term storage. Distilled mono-glycerides containing 90 per cent mono-glyceride also did not contribute towards reduction in crumb hardness of bread on long term storage. Besides they contributed slight bitterness in stored bread.

The improving mechanism of ascorbic acid in dough has been observed due to oxidation of -SH groups in dough by dehydroascorbic acid, an oxidation product of ascorbic acid.[17] Johnston *et al*[18] observed the breaking of H-bond in protein aggregate by ascorbic acid in mechanical dough development process.

In the present study it was observed that the bread treated with ascorbic acid at a level of 100 ppm preserved by sorbic acid bread wrapping paper technique showed less crumb hardness compared to control bread when evaluated organoleptically after one month.

Control of staling of bread by enzyme : Enzyme system from malt has been proved to be useful in dough development because of its action on damaged flour starch to provide fermentable sugar needed by the yeast for active fermentation. Heat resistant amylase has been suggested to be useful ingredient for controlling staling in bread. To oppose the staling trend in bread, some breakdown of starch during oven baking is desired[5]. Schultz *et al*[19] observed that addition of small quantities of α-amylase to bread baked on commercial scale resulted in an improved product which staled at approximately at decreased rate. But the storage study was restricted upto 108 hr after baking. Malt extract at the level of 0.1 to 0.2 per cent on flour weight and heat stable α-amylase at the level of 50–300 ppm on flour weight basis was used in the present study. It was observed that when α-amylase was added in the bread formulation at the level of 200 ppm on flour weight basis and the bread was preserved for one month by sorbic acid treated wrapping paper technique the experimental breads had lesser crumb hardness compared to control bread when evaluated organoleptically. Use of malt extract did not show any perceptible advantage.

Control of off-aroma in stored bread by spices, condiments and synthetic flavours : The stored bread developes off-aroma; flavour enhancement of breads by addition of condiments/spices had also been attempted by earlier workers[20]. The findings of the use of spices/flavours based on organoleptic evaluation may be summarised as follows : Black pepper (0.5 to 3.0 g/100 g flour) was stable enhanced the acceptability of the bread and served the objective appreciably for which it had been used. Cumin seed, green chillies and ajwan (0.5 to 3.0 g/100 g flour) were relatively stable and acceptable in stored bread. Mace, cardamom and cinnamon at the above levels were unstable. Garlic and ginger (0.5 to 3.0 g/100 g flour) had undergone considerable flavour modifications. As regards synthetic flavours, we observed pineapple flavour to be the only stable flavour; at 0.5 to 1.0 g/100 g flour level; rest of the flavours like lemon, mango, vanilla, mint, ginger used at the level of 0.5 to 1.0 g/100 g flour were observed to be either unstable or undergoing flavour modifications. Roasted groundnut seed (5.0 g/100 g flour) though enhanced the acceptability of fresh bread, on long term storage contributed to rancidity in stored bread. Roasted sesame seed, at the above level and mango pulp and tomato puree at 10 to 20 g/100 g flour though enhanced the acceptability of fresh bread, did not make any appreciable difference in acceptability in stored bread. Fruit pieces like pineapple alone (osmatic dehydrated) at 5 percent level in combination with guava (accelerated freeze dried at 3 per cent) in bread formulations enhanced the acceptability of fresh bread but on long term storage, fermented odour was perceptible in and around the fruit pieces in the bread crumb. Black pepper added at the level of 3 per cent on flour weight in bread formulation gave the best results.

Control of staling of bread by canning under high vacuum and inert gas : It may be observed that staling of bread could be controlled considerably by canning and the breads were acceptable on three months storage under ambient condition. There was practically no difference in acceptability between high vacuum and nitrogen packed breads. As regards processing conditions both the conditions 15 lb/30 min and 10 lb/30 min were observed to be effective for preservation. Since the former one caused more browning in bread, the latter was preferred. Sliced breads deep fat fried in oil hydro and canned similarly had smoother texture on three months storage.

User trial : Two types of breads (pepper bread and sweet bread) when put to users evaluation trial by submarine crews were liked by the subjects.

Proximate composition of bread : The bread contained 34.6, 2.8, 5.8 and 1.8 per cent moisture, crude fat, protein and ash respectively.

References

1. *Official Methods of Analysis*, Association of Official Agricultural Chemists, Washington D.C., 10th Edn, 1965.
2. Schimdt, H., *Z. Anal Chemie.*, 1960, **178,** 173.
3. Gosh, K. G., Srivatsava, A. N., Nirmala, N. and Sharma, T. R., *J. Fd Sci. Technol.*, 1973, **10,** 105.
4. Chakrabarty, T. K., Dwarakanath, K. R. and Bhatia, B. S., *Indian Fd Pckr*, 1971, **25,** 31..
5. Karl, O. Hertz, *Fd Technol., Champaign*, 1965, **19,** 90.
6. Donald D. Kasarda, *Baker's Dig.*, 1970, **44,** 20.
7. Kent Jons, D. W. and Amos, A. J., *Modern Cereal Chemistry*, Food Trade Press, London, 1967, 295.
8. Hellman, N. N., Barbara Farichild and Sehti, F. R., *Cereal Chem.*, 1954, **31,** 495.
9. France, W. J. and Wragg, B. H., *Upto Date Bread Making*, Maclaren and Sons Ltd., London, 1968, 133.
10. Tenney, R. J. and Schmidt, D. M., *Baker's Dig.*, 1968, **42,** 38.
11. Tsen, C. C. and Hoover, W. J., *Baker's Dig.*, 1971, **45,** 38.
12. Tsen, C. C., Hoover, W. J. and Phillips, D., *Baker's Dig.*, 1971, **45,** 20.
13. Fran Belshow, *Fd Process*, 1971, **32,** 24.
14. Longhand, R. K. and Thalheimer, W. G., *Cereal Chem.*, 1971, **48,** 283.
15. Marston, P. E. and Short, A. L., *Fd Technol. Austr.*, 1969, **21,** 154.
16. Krog, N. and Nybojensen, B., *J. Fd Technol.*, 1970, **5,** 77.
17. Tsen, C. C., *Cereal Chem.*, 1965, **42,** 86.
18. Johnston, W. R. and Manseth, R. E., *Baker's Dig.*, 1972, **46,** 20.
19. Schultz, A. S., Schoonover, F. H., Fisher, R. A. and Jackal, S. S., *Cereal Chem.*, 1952, **29,** 200.
20. Jackal, S. S., *Bakery Prod. Mkt.*, 1969, **4,** 51.

Studies on the Production and Storage Behaviour of Spray Dried Mango Milk Powder

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Studies were carried out on the processing conditions and keeping quality of mango milk powder—a spray dried product comprising of fresh mango solids, milk solids and sugar. Changes in the physical and chemical quality attributes of the product were studied during a storage period of one year at room temperature and at a controlled temperature of 30 ±1°C. There was no perceptible change in colour, flavour and reconstitution of the product but there was a progressive increase in the free fat content, volatile free fatty acids, acidity and TBA values. However the over all quality of the powder remained acceptable at the end of one year at either of the temperatures of storage.

Ready-to-reconstitute food products are becoming increasingly popular the world over. Mango milk powder is an interesting addition to the list and is prepared from mango fruit solids, milk solids and sugar in a spray dried form. It can be readily reconstituted into pleasant fruit milk drink or as an ingredient in ice cream, puddings, etc.

Breen *et al*[1] observed that the shelf life of spray dried mixtures ranged from 2–4 weeks for carrot/pumpkin, tomato, skim milk powder and to one year or more for dried skim milk with apple, bananas, prune and maize.

Hedrick *et al*[2] manufactured flavoured drinks, and other dairy products using carboxy-methyl-cellulose, pectin and various buffering salts and stabilizers. The reconstituted foam spray dried cherry milk samples were comparable in flavour to reconstituted freeze-dried samples.

Preliminary laboratory trials carried out indicated that a beverage with 20.0 per cent total solids consisting of 4.0 per cent mango solids, 10.0 per cent MSNF and 6.0 per cent sugar had the maximum acceptability. Based on these results, it was proposed to standardise the technique for the production on a pilot plant scale spray dried mango milk powder, which when reconstituted with water will give a beverage of the above composition. The raw materials, the manufacturing procedures, etc., for the process are given below.

Materials and Methods

The raw materials used for making the mix consisted of (i) fresh concentrated buffalo skim milk; (ii) sweet buffalo milk cream or concentrated standardised milk with the desired fat, SNF ratio; (iii) fresh ripe mango fruits. Mangoes were washed, peeled and cut into small pieces before making into pulp in a high speed mixer. 'Chausa' variety of mangoes were used for the study; (iv) refined cane sugar of ISI grade; and (v) glycerol mono-stearate as emulsifier and sodium alginate as a stabiliser.

Preparation of the mix : Based on the preliminary trials each 100 kg of the mix is made to contain 36.0 kg fruits (20 per cent solids) 10.8 kg sugar, 52.6 kg concentrated milk (30 per cent solids), 14.0 kg cream (40 per cent fat) and 400 g stabiliser. The concentrated skim milk cream and sugar were well mixed and preheated to 50°C followed by the addition of glycerol mono-stearate and sodium alginate in a ratio of 1:1 at the rate of 1 per cent of the total solids of the mix with vigorous mixing for complete incorporation.

Homogenisation and pasteurisation : The heated mix was filtered and homogenised at a temperature of 65–70°C in a single stage Rannie homogeniser under a pressure of 170 kg/cm^2 for first stage and 35 kg/cm^2 for the second stage. The homogenised mix was pasteurised at 65.5°C for 30 min in a hot water tank, followed by cooling to 10°C.

Mixing of concentrated milk and mango pulp : The pulp was mixed with the cooled concentrated milk. The fruit milk mix was then filtered through a muslin cloth keeping losses of the solids to the minimum. The viscous mix clogs pores of the cloth and needs frequent washing. The filter cloth is dipped in 5 ppm chlorine water before reuse.

Drying : The anhydro spray drier Type II was used to dry the fruit mix at an air inlet temperature of 170–175°C and outlet temperature of 98–100°C to give a product with a moisture content of 2.5 per cent. The overall composition of mango milk powder was maintained at milk fat, 14.0; milk SNF, 40.0; sugar, 27.0 mango solids, 18.0; and stabiliser, 1.0 per cent.

Collection and packaging : The mango milk powder was collected in polythene bags and contact with air was avoided as far as possible. It was allowed to cool and about 150g was filled in each presterilised tin plated cans hermetically sealed and gassed twice with nitrogen at interval of 24 hr.

Storage : Forty tins from each trial were kept at room temperature (8–40°C) and an equal number in an incubator maintained at 30±1°C to study the keeping quality.

Analysis of the raw materials : The product immediately after manufacture and during storage was analysed according to the following standard procedures:

Total solids of the concentrated skim milk determined at 20°C by Bausch and Lomb refractometer, pH of the concentrated milk at 20°C by Metrohm single electrode pH meter; acidity of the concentrated milk by titrating 10 g of milk against N/10 NaOH using 0.5 per cent phenolphthalien as indicator; fat in cream was tested by Gerber method as per ISI method. The SNF of cream was calculated using the following formula :

$$\% \text{ SNF in cream} = \frac{\%\text{SNF in milk}(100 - \%\text{ fat in cream})}{(100 - \%\text{ fat in milk})}$$

Flavour of dried mango milk : The flavour was judged by a panel of judges after reconstitution (10 g of the powder dissolved in 45 ml of water in a mixy).

Colour of mango milk powder : Based on the colour standards suggested by Kashmiri Lal[3] for dry milk, following colour standards were developed. The basic mixture for the colour standard was prepared by adding 10 g of sodium chloride, 0.1 g of potassium chromate and 0.02 g of potassium dichromate. The base was mixed with the following amount of potassium chromate to make the working standards.

Standard No.	Pot. dichromate mixed g
1	0.03
2	0.04
3	0.05
4	0.06
5	0.07
6	0.08
7	0.09

Fat and moisture in the powder were tested by using the Majonnier equipment,[4] acidity and PH as per ISI method[5]. Mango milk powder (13 g) was reconstituted with warm (35°C) water (100 ml) before testing the pH and acidity. Average particle density, bulk density and per cent volume occupied by powder particles were determined by the method described by Beckett *et al*[6].

Solubility index : Solubility by Howat *et al*[7] method, and average particle size by the method described by janzen *et al*[8].

Dispersibility : The method adopted was as follows : Fifty-two grams of the mango milk powder was mixed with 400 ml of water at 24°C for 20 min using a mixer. The liquid was allowed to stand for 5 min, and kept in beakers of 100 ml capacity in refrigerator. The rate of sedimentation was estimated by determining the total solids of supernatant fluid at 6 hr intervals. The dispersibility is inversely related to the sedimentation

TABLE 1. ANALYSIS OF FRESH MANGO MILK POWDER

Test	Batch No. 1	2	3	4
Organoleptic	Acceptable	Acceptable	Acceptable	Acceptable
Colour	Creamy yellow	Creamy yellow	Creamy yellow	Creamy yellow
Colour standard	1	1	1	1
Fat (%)	14. 06	14. 04	14. 01	13. 98
Moisture (%)	3. 40	3. 52	3. 48	3. 46
Acidity (% lactic acid)	0.085	0.085	0. 09	0. 09
pH	6. 50	6. 50	6. 55	6. 55
Solubility index (ml)	0. 50	0. 80	0. 60	0. 50
Solubility (%)	99. 00	98. 4	98. 6	99. 0
Bulk density	0. 60	0. 62	0. 60	0. 63
Dispersibility (%)	100	100	100	100
Particle size (μ)	39	42	40	41
Free fat (g/100 g fat)	7. 00	7. 60	7. 72	7. 82
V.F.A. *	2. 5	3. 0	3. 0	2. 5
Peroxide value	Nil	Nil	Nil	Nil
TBA value (optical den.)	0.992	0.046	0.060	0.074

* VFA: Volatile fatty acid (N/10 NaOH/100g of powder)

and was expressed in terms of the sedimentation of per cent solids after 24 hr.

Free fat of the powder was determined by the method of Hall and Hedrick[9]; peroxide value by the method described by Smith[10]. Oxidative rancidity[11] and steam volatile free fatty acids[12] indicating the degradation of milk fat during storage were also determined as per the methods.

Results and Discussion

The results of the chemical analysis are given in Table 1. The data on quality changes during storage are given in Table 2.

Organoleptic quality : The powder was acceptable organoleptically during one year of storage. There was no objectionable flavour.

Colour : It was found that colour of the powder increased from initial value of 1 to 4 at room temperature and it was still darker at 30 ± 1 °C where the increase was from 1 to 6 during the storage period of one year. The change in the colour may be due to the interaction of reducing sugar, lactose and fructose (from fruits) with NH_2 groups of the proteins and rate of this reaction is faster at higher temperature of storage. This is evident from the colour changes. However, the colour change was not objectionable at the end of one year of storage.

Solubility : During a storage period of one year there was a slight decrease in the solubility of the powder (98.5 to 98.0 per cent); temperature of storage did not have any noticeable effect on the solubility.

Dispersibility : No change in the dispersibility of the powder at either of the temperature of storage was recorded and this may be attributed to the low milk solids content as compared to milk powders and the presence of highly soluble cane sugar in the powder. There was a progressive (though slight) decrease in the pH of the powder with the period of storage (6.5 to 6.3) during one year of storage; temperature did not influence the rate of change in pH (Table 2). The

TABLE 2. CHANGES IN THE QUALITY ATTRIBUTES OF MANGO MILK POWDER DURING ONE YEAR OF STORAGE AT ROOM TEMPERATURE (8-40 °C) AND AT 30 ± 1 °C

Parameters	Period of storage (in months) 3 months		6 months		9 months		One year	
	Room temp.	30 °C	Room temp.	30 °C	Room temp.	30 °C	Room temp.	30 °C
Colour	1	2	2	3	3	4	4	6
pH	6.4	6.4	6.3	6.3	6.3	6.3	6.3	6.3
Acidity	.08	.085	.085	.085	.09	.09	.09	.09
Solubility %	99.0	99.0	98.5	98.5	98.0	98.5	98.0	98.5
VFA	4.1	4.3	4.8	5.0	5.5	6.0	6.7	7.2
Free fat (g/100g fat)	7.4	7.8	8.5	8.6	9.9	9.7	11.0	11.6
TBA value (optical density)	0.068	0.072	0.084	0.088	0.100	0.107	0.115	0.123

TABLE 3. MEAN AND STANDARD ERROR OF DIFFERENT CHARACTERISTICS OF MANGO MILK POWDER DURIN STORAGE AT ROOM TEMPERATURE (8-40°C) AND AT 30±1°C

Parameters	Mean		Standard error	
	Room temp.	30±1°C	Room temp.	30±1°C
Colour ..	2.500	3. 75	±0.599	± .740
pH ..	6.325	6. 32	±0.022	±0.022
Acidity ..	0.086	0. 086	±0.011	±0.011
Solubility (%) ..	98.37	98. 62	±0.207	±0.103
V.F.A. ..	5.27	5. 62	±0.480	±0.546
Free fat (g/100 g fat) ..	9.20	9. 425	±0.683	±0.713
TBA value (optical density) ..	0.0955	0.0975	±0.010	±0.009

maximum increase in the acidity was 0.01 per cent at both the temperatures of storage. This increase could be due to the breakdown of lactose to lactic acid and also due to the increase in free fatty acids produced by the degradation of milk fat.

The changes in the free fat content recorded over a period of one year at the two temperatures showed a regular increase. The increase was more at the higher temperature of storage; the values being 7.0 to 11.0 per cent at room temperature and 7.2 to 11.6 per cent at 30±1°C. These results indicate that with the increase in storage period more and more coalescence of the fat took place which resulted in the liberation of the fat in free form. The change was more rapid at elevated temperature of storage (30°C) which caused a greater increase in the free fat content.

The changes in the volatile free fatty acid content recorded at the regular intervals of one month indicated a progressive increase. At room temperature the increase was 3.5 to 6.7 ml of N/10 NaOH per 100 g of powder at 30±1°C it was 3.5 to 7.2 ml of N/10 NaOH per 100 g of powder. The increase in the VFA, content is attributable to the degradation of milk fat of the powder and liberation of free fatty acids during the storage. The change as could be expected was more rapid at the higher temperature of storage. The test for peroxide value was negative and this was so because of packaging of the product under nitrogen. There was a regular increase in the TBA values from 0.060 to 0.115 at room temperature of storage and 0.065 to 0.123 at 30±1°C over a period of one year of storage. T.B.A. value is a measure of the oxidation of fat and the browning of the milk powder. Since the peroxide value was recorded as nil, the increase in TBA values showed that the interaction between milk proteins and reducing sugars, lactose, glucose and fructose took place to form a colcured complex compound. These measurements sufficiently substantiated the qualitative observations revealed on colour.

References

1. Breene, M. and Coulter, S. T., *J. Dairy Sci.*, 1967, **49,** 762.
2. Hedrick, T. I., Schamdrel, S. H. and Hunter, W. B., *Dairy Sci. Abstr.*, 1968. **30,** 370.
3. Kashmiri, Lal, *Studies on Some of the Factors Influencing the Solubility of Roller Dried Buffalo Milk (Whole and Skimmed)*, 1968. M.Sc. dissertation, Punjab University.
4. *Laboratory Manual: Methods of Analysis of Milk and Its Products*, Milk Industry Foundation, Washington, 1949, 207.
5. *Determination of Fat in Whole Milk, Evaporated (Unsweetened) Milk, Separated Milk, Skim Milk, Butter Milk and Cream by the Gerber Method*, IS:1224-1958, Indian Standards Institution, New Delhi.
6. Beckett, D. C., Emmons, D. B. and Elliot, J. A., *The Determination of Bulk Density, Particle Density and Particle Size Distribution of Skim Milk Powder, B. 913*, XVI International Dairy Congress, Copenhagen, Denmark, 1962.
7. Howat, G. R. and Smith, J. A. B., *J. Dairy Sci.*, 1939, **10,** 498.
8. Janzen, J. J., Swanson, A. M. and Macntire, J. N., *J. Dairy Sci.*, 1963, **36,** 305.
9. Hall, C. W. and Hedrick, T. I., *Drying of Milk and Milk Products*, AVI Publishing Co., Westport, Connecticut, 1966.
10. Smith, J. A. B., *J. Dairy Res.*, 1939, **10,** 294.
11. Kenney, M. and Bassette, R. , *J. Dairy Sci.*, 1959, **42,** 945.
12. Hempenius, W. L. and Liska, B. J., *J. Dairy Sci.*, 1968, **51,** 221.
13. Buchanan, R. A., *J. Soc. Diary Technol.*, 1968, **21,** 3.
14. Staurt Patton and George W. Kurtz, *J. Dairy Sci.*, 1951, **34,** 669.

A Comparative Study on the Efficiency of Different Adsorbent Columns for the Clean-up of Pesticide Residue Extracts

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The performance of different adsorbent columns for cleanup of the pesticide residue extracts have been compared. Activated charcoal was found to be more efficient for the removal of pigments and fats wherein the recoveries of both chlorinated and phosphatic pesticide residues were 100 per cent with 1:1 petroleum ether and ethyl ether mixture as eluant.

Analysis of samples for the determination of pesticide residues involves three stages—extraction, clean up and quantitative estimation. During extraction, large amount of co-exrtactives always appear which sometimes interfere with the desired results. So, specific clean up is absolutely necessary which should be efficient enough to eliminate all interfering materials like fats and pigments. A number of workers have reported work on the column clean up of residue extracts. The most commonly used adsorbents for the cleanup of pesticide residue extracts are florisil,[1,2] alumina[3,4], silica gel[5], cellulose[6], charcoal[7], silicagel-celite[8], acid-celite[9], etc. Lakshminarayana *et al.*[10] used charcoal in TLC as thin layer multiband plate for clean up. Florisil has been claimed to be the most excellent adsorbent for colouring matter and it has been included in the AOAC official method. But it being a proprietory product, the problem lies in its availability and cost. Further, its chromatographic characteristics differ with the activation status.

In the present investigation the efficiency of the columns of alumina, silica gel, silica gel-celite, magnesium trisilicate, magnesium hydroxide and charcoal have been compared. Magnesium trisilicate has earlier been processed at this laboratory with some success for stimulating the properties of florisil in column clean up[11] and magnesium hydroxide with some treatments has been used in TLC in the separation of the pesticide residues.[13-14]

Materials and Methods

Eleven glass columns (dia. 14 cm) were filled each with 2 g of the adsorbents. The extraction procedure has been as in a previous publication.[14] The extracted solution (5 ml) containing known amount of pesticides was applied to each of them. In each case volume of the eluting solvent used was 75 ml. The extract was concentrated to a known volume. It was then applied to the thin layer plate of silica gel G(0.25 mm thickness, activated at 100°C for 1 hr). The quantities of pesticides in the different eluates were calculated by comparing the intensity of colour of the spots of the samples with those of the standards. For chlorinated pesticides, the mobile solvent used was n-hexane and the chromogenic agent was 2 per cent diphenylamine in acetone. The sprayed plate was developed by keeping under uv for 30 min.[13] For organophosphorous pesticides, the mobile solvent was acetic acid (9+1) and the chromogenic agent was 0.5 per cent palladium chloride in 8 per cent HCl.

Results and Discussion

It is seen from the Table 1 that alumina whether inactivated or activated at the temperatures of 100 and 550°C though showed the complete removal of fat and a partial removal of pigments, gave 100 per cent recovery of chlorinated pesticides whereas it was poor in case of malathion. This has also been observed by Egan *et al.*[12] Secondly, it could not retain the natural colour of wheat flour and the colour of butter yellow, whereas, it removed the colour of extracts obtained from fruits, vegetables and other cereals. It was further observ d that alumina could retain the colour of wheat flour extract provided the sample solution has already undergone acetonitrile partitioning treatment. For fatty foods such as milk, ghee, butter, etc., the eluate was found to contain little amount of fat but it was not sufficient enough to interfere in the estimation by TLC method.

Silica gel, slica gel-celite columns were not satisfactory due to the presence of both fat and pigments in the eluate. Silica gel activated at 100°C for 3 hr retained a major portion of both chlorinated and organophosphorous pesticides. However, the incor-

TABLE 1. RECOVERY OF DDT, INDANE AND MALATHION IN THE PRESENCE OF PIGMENTS AND FAT USING DIFFERENT ADSORBENTS AND ELUTING SOLVENTS

No.	Adsorbent	Eluting solvent	Elution rate	Pigments in eluate	Fat in eluate	Recovery* %	Malathion recovery %
1A	Alumina unactivated	Hexane	Moderate	—ve[3]	Trace[1]	100	Poor
B	Alumina activated at 100°C—3 hr	,,	,,	,,	,,	,,	,,
C	Alumina activated at 100°C—7 hr	,,	,,	,,	,,	,,	,,
D	Alumina activated at 550°C—3 hr	,,	,,	,,	,,	,,	,,
2	Silica gel activated at 100°C—3 hr	,,	,,	+ve	+ve[2]	35-40	20
3	Silica gel+celite (1+1)	,,	,,	Slight	+ve	70,85	30
4A	Mg trisilicate activated at 100°C—3 hr	,,	V. slow	+ve	+ve	35-40	<20
B	,,	6% ethyl ether in pet. ether	,,	—ve	+ve	50,70	50
5	$Mg(OH)_2$ unactivated	Hexane	Quickest	+ve	+ve[1]	40,100	50
6A	Activated charcoal	Hexane	Quick	—ve	—ve	50-60	50-60
B	,,	Pet. ether+ethyl ether (1+1)	,,	—ve	—ve	100	100

* Single Nos. indicate DDT and lindane both; first no. DDT and Second no. Lindane.
[1] Fat do not interfere in TLC.
[2] Fat interferes in TLC.
[3] Butter yellow and colour of wheat flour.

poration of celite with silica gel as 1:1 mixture relieved 70-85 per cent of Lindane and DDT in the eluate but was not effective for malathion.

Though magnesium trisilicate activated at 100°C for 3 hr retained pigments, the elution speed was too slow and complete recovery of pesticides could not be achieved. But on passing 200 ml each of 6 per cent ethyl ether in petroleum ether, 15 per cent ethyl ether in petroleum ether, and 50 per cent ethyl ether in petroleum ether, the result was satisfactory. However, in this case also, the elution speed was too ow.

Unautoclaved magnesium hydroxide gave poor recoIveries of DDT and malathion with the presence of pigments though the elution was faster for the said recoveries. The column containing activated charcoal showed excellent adsorptions of pigments and fat from the residue extracts. It retained not only the natural colours of cereals and vegetables, but also, the artificial oil soluble colour like butter yellow. The 1:1 mixture of petroleum ether and ethyl ether was more efficient in eluting 100 per cent of DDT, Lindane and malathion residues from the column as compared to 50-60 per cent by hexane. The elution rate was also good.

So, the activated charcoal can successfully be used for the clean up of cereals, vegetables and fatty foods by the column procedure. The solvent partitioning step prior to column clean up, can be omitted. Thus, we can eliminate the use of costly solvents like acetonitrile dimethyl formamide, etc., which are essential in the solvent partitioning step. This is particularly important in routine work involving multiple analysis.

References

1. Mills, P. A., *J. Ass. off. agric. Chem.*, 1959, **42**, 734.
2. Moats, W. A., *J. Ass. off. agric. Chem.*, 1963, **46**, 172.
3. De Faubert Maunder, M. J., Egan, H. Godly, E. W., Hammond, E. W., Roburn, J. and Thompson, J., *Analyst*, 1964, **89**, 168.
4. Lichtenstein, E. P. and Schulz, K. R., *J. econ. Ent.*, 1959, **52**, 124.
5. Moats, W. A., *J. Ass. off. agric. Chem.*, 1962, **45**, 354.
6. Onley, J. H. and Mills, P., *J. Ass. off. agric. Chem.*, 1962, **45**, 983.
7. Moats, W. A., *J. Ass. off. agric. Chem.*, 1964, **47**, 587.
8. Samuel, B. L., *J. Ass. off. agric. Chem.*, 1966, **49**, 346.
9. Davidow, B. J., *J. Ass. off. agric. Chem.*, 1950, **33**, 130.
10. Laisluminarayana, V. and Krishna Menon, P., *J. Fd Sci. Technol.*, 1969, **6**, 272.
11. Central Food Laboratory, unpublished results.
12. Egan, H., Hammond, E. W. and Thompson, J., *Analyst*, 1964, **89**, 175.
13. Mukherjee, G., Mathew, T. V., Mukherjee, A. K. and Mitra, S. N., *J. Fd Sci. Technol.*, 1971, **8**, 152.
14. Mukherjee, G., Mathew, T. V. and Mitra, S. N., *Res. Ind.*, 1971, **16**, 281.
15. Banerjee, T. S., Mukherjee, G. (Smt.), Mukherjee, A. K. and Mathew, T. V., *Res. Ind.*, 1973, **18**, 150.

Microbiological Quality of Infant Milk Foods*

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The total plate counts of most of the infant milk food samples were below 50,000 per gram as specified by the Indian Standards Institution. Mesophilic bacteria comprised the largest group of organisms, followed by thermoduric and thermophilic organisms. Flat-sour and thermophilic aerobic spore-formers were extremely low. Significantly none of the samples showed the presence of putrefactive anaerobes. Proteolytic and lipolytic organisms represented on an average 46 and 25 per cent of the total plate count flora. Yeasts were present in extremely small numbers, while no moulds could be detected in any of the infant milk food samples. Coliform bacteria were low in numbers, generally, 2 to 8 per gram while *E. coli* was absent in all the samples examined. The need to re-examine some of the procedures recommended by the Indian Standards Institution for the evaluation of microbiological quality of infant milk foods has been discussed.

The development of the dairy industry supported by sound cattle improvement programmes and animal husbandry practices is being given high priority in national planning[1]. At present there are several units in India, manufacturing milk powder and infant milk foods with licenced capacities of 34,618 tonnes and 34,887 tonnes respectively[2]. The present day demand for milk powder is estimated at 40,000 tonnes which is being met by massive imports to the tune of 22,000 tonnes valued at Rs 10 crores based on 1972 prices[3]. The demand for milk powder during the fifth plan period is estimated to rise to 70,000 tonnes while that for infant foods is assessed to be around 60,000 tonnes[2]. There is thus a tremendous potential in our country for the growth of the infant food industry in the next decade.

Since infant foods are designed for consumption by a very vulnerable group of the population the need to exercise great care at every stage in processing cannot be underestimated. To safeguard foods against microbial hazards, microbiological standards have been proposed to serve as indices of quality assurance[4,5]. We report in this paper regarding the general microbiological profile of some of the leading brands of infant milk foods.

Materials and Methods

Preparation of samples: Samples of infant milk foods (Glaxo, Amul and Lever's Baby Food) were obtained from the local retailers. The tins were opened aseptically and 10 g of the sample was weighed into sterile Erlenmeyer flasks containing glass beads and reconstituted in 90 ml sterile 1.25 per cent sodium citrate solution at 45°C, stirred to give a homogeneous mixture and serially diluted.

Medium 1 recommended by the Indian Standards Institution (ISI) was routinely employed for determination of total plate counts. The medium contained (per cent) glucose, 0.1; peptone, 0.5; yeast extract, 0.25 agar, 2.0 adjusted to pH 7.0 and will henceforth be referred to as Total Plate Count (TPC) agar[4,5]. M 2 contained (per cent) yeast, 0.3; peptone, 0.5; agar, 2.0; and fresh milk 1.0 (v/v), pH 7.0—7.2[4,5] and was employed only for standardization purposes (Table I). Beef lactose agar was prepared according to Sherman's procedure[2,6]. Standard methods agar and beef infusion agar were prepared essentially according to routine procedures[6]. Plates were generally incubated at 32°C for 24-48 hr. When spreaders were encountered the method suggested by the APHA was followed[7].

Direct microscopic count was determined after staining 0.01 ml of the serially diluted sample with Norths aniline oil methylene blue stain[8].

Thermoduric counts were obtained by subjecting the reconstituted milk food sample to 62.8°C for 30 min., cooling, plating on TPC agar and incubating at 32°C for 24-48 hr. Thermophilic counts were determined by incubating the plates at 55°C for 48 hr.

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Mesophilic aerobic spore-bearers were enumerated after heating reconstituted sample at 80°C for 10 min. Samples were cooled, plated on starch milk agar[9] and incubated at 37°C for 48-72 hr. Mesophilic anaerobic spore-bearers were detected by pouring 10 ml of the reconstituted milk (10^{-1} dilution) into sterile test tubes and heating at 80°C for 10 min. Samples were cooled, overlayed with 2 ml molten agar, incubated at 37°C for 3 days and observed for "stormy" fermentation. Thermophilic spore-formers including the counts of total aerobic spores, flat-sour, T.A. spoilage and sulphide stinker types were determined according to standard methods[6].

Proteolytic organisms were detected by using standard methods agar containing 10 per cent sterile skim milk[7] and Standard methods caseinate agar of Martley *et al*[10]. Serial dilutions were made in phosphate buffered distilled water[7] in order to avoid the possible inhibition of caseolytic activity of lactic *Streptococci* by sodium citrate[11]. Plates were incubated at 32°C for 48-72 hr and flooded with 1.5 per cent acidified mercuric chloride solution and only those colonies retaining the zone of clearance were considered as proteolytic.

Lipolytic bacteria were detected by their ability to hydrolyze fat stained with nile blue sulphate[12] according to the procedure described by Skerman[13].

Lactic acid bacteria were enumerated employing the selective medium recommended by Emard and Vaughn[14].

Yeast and mould counts were carried out by plating on potato-dextrose agar and incubating the plates at 25°C for 3-5 days[6].

Indicator organisms: The most probable number (MPN) method was adopted for the enumeration of coliforms and *Escherichia coli* employing Brilliant green lactose bile broth[6,7]. Loopfulls from tubes showing positive gas formation were streaked on Endo agar. Typical red colonies, at least 0.5 mm in diameter exhibiting a metallic sheen were picked up and confirmed by the IMViC tests.

Results and Discussion

Total plate counts have been generally employed to assess the sanitary quality, organoleptic acceptability, safety and utility of foods[15,16]. Initially attempts were made to standardize the methodology for the microbiological quality evaluation of infant milk foods. The total plate counts have been reported to vary considerably with the methods employed for reconstitution, nature of dilution blank, holding time of the reconstituted sample and the temperature of incubation[17-21]. The effect of various reconstituting media on the total counts of infant milk foods is given in Table 1. Consistently higher total counts were obtained with 1.25 per cent sodium citrate as the reconstituting medium. Addition of Tween-80 did not improve the recoverability significantly. Peptone water which has been found to be a suitable diluent for a number of products[22,23] and phosphate buffered distilled dilution water[7] were found to be less satisfactory than sodium citrate solution. Ghodekar and Nambudiripad[24] have reported the superiority of glass distilled water and Ringer's solution over sodium citrate for the determination of total plate counts in skim milk powders. However, in our present study, because of the high fat content of the infant milk foods, phosphate buffered distilled water was not suitable as separation of fat was appreciable. No bactericidal effect has been reported at the levelof sodium citrate employed[19].

The effect of temperature of incubation on total plate count is reported in Table 1. Higher counts

TABLE 1. EFFECT OF RECONSTITUTING MEDIA, TEMPERATURE OF INCUBATION AND PLATING MEDIA ON TOTAL PLATE COUNTS OF INFANT MILK FOODS

	TPC /g
Reconstituting medium (4)	
Saline ..	15,500
Buffered distilled water[b] ..	15,900
0.1% Peptone-water ..	16,260
1.25% Sodium citrate ..	16,633
1.25% Sodium citrate+0.1% Tween-80 ..	16,300
Temperature of incubation[c] (4)	
32°C ..	21,620
37°C ..	18,740
Plating medium[d] (5)	
TPC Agar No. 1 ..	19,480
TPC Agar No. 2 ..	19,060
Standard methods agar ..	18,860
Beef lactose agar ..	16,860
Beef infusion agar ..	16,390

Figures in parenthesis indicate the number of samples examined.

(*a*) Average of at least triplicate plates.

(*b*) Prepared by diluting 1.25 ml stock phosphate buffer solution (34 g KH_2PO_4 per litre, pH 7.2) to 1 litre with distilled water.

(*c*) TPC agar No. 1 was employed and 1.25% sodium citrate used for reconstitution.

(*d*) 1.25% sodium citrate employed for reconstitution. Plates were incubated at 32°C for 24-48 hr.

were observed at 32°C than at 37°C. The American Public Health Association recommends the use of 32°C for dry milks and most foods [6,7] while the ISI recommends incubation of the plates at 37°C for 48hr[4,5]. Several workers have reported lower total plate counts as temperature of incubation is raised above 32°C [6,24,25]. In view of these findings, the incubation temperatures as recommended by the ISI need to be revised.

The suitability of several media such as the ISI specified agar,[4,5] standard methods agar[6], beef-infusion agar[6] and beef lactose agar[26] for the determination of total plate count was examined (Table 1). Beef-infusion agar had been reported to be satisfactory for microbiological examination of dairy products as it permitted recovery of sub-lethally damaged cells,[27] especially the thermoduric lactic streptococci[28]. Sherman[26] reported that beef lactose agar increased the number and size of colonies from milk samples. However, in the present study, the recovery of micro-organisms from infant milk foods was not significantly higher in these media in comparison with the ISI specified medium 1, which in fact, gave higher total counts than the standard methods agar.

The total plate counts and direct microscopic counts of a number of infant milk foods is reported in Table 2. Four out of the ten samples examined averaged total counts in excess of 29,000 per gram, while the remaining exhibited low counts. No sign of organoleptic degradation was noticeable in these products. Our data confirms that the ISI specified maximum limit of 50,000 per gram is adequate and safe to protect the consumer. Most samples of infant milk foods showed a decrease in total counts on storage at 25°C confirming earlier reports[29].

An attempt was made to study whether determination of direct microscopic count (DMC) could throw more light on the history of the infant milk foods as it has been successfully employed in the dairy industry to aid in quality control[30]. In most of the infant milk foods, the DMC was more than 71,000 per gram and generally less than 612,000 per gram. Two samples showed exceptionally high counts of 3.06×10^6 and 14.0×10^6 per gram respectively suggesting the very poor quality of milk employed. Some correlation was noticeable between total plate count and direct microscopic count in most samples except sample 9. Judging by the DMC, most infant milk foods appear to have been manufactured from good quality milk.

Thermoduric and thermophilic bacteria including the spore-formers survive pasteurization and the drying operations. Their numbers in relation to total mesophilic counts are given in Table 3. Thermoduric bacteria were lower in numbers than mesophilic types and the thermophilic counts were the lowest. The thermodurics typified by *Streptococcus thermophilus* and *S. durans* may arise from milk or from improperly sanitized process equipment[31].

Flat-sour and thermophilic aerobic spore-formers were detected in most of the samples although their

TABLE 2. TOTAL PLATE COUNT AND DIRECT MICROSCOPIC COUNT OF MARKET SAMPLES OF INFANT MILK FOODS

Sample No.	Total plate count/g	Microscopic count/g
1 ..	1,500	173,000
2 ..	29,000	510,000
3 ..	1,000	71,400
4 ..	2,700	102,000
5 ..	33,000	3,060,000
6 ..	1,900	408,000
7 ..	1,000	340,000
8 ..	50,000	14,000,000
9 ..	790	500,000
10 ..	34,000	612,000

TABLE 3. MICROBIOLOGICAL PROFILE OF INFANT MILK FOODS*

Organisms	Average	Range
	(per g.)	
Mesophilic bacteria ..	12,000	790—33,800
Thermoduric ,, ..	6,200	350—31,000
Thermophilic ,, ..	1,066	380—1,900
Mesophilic spores (aerobic) ..	123	10—300
,, (anaerobic) ..	nil	—
Thermophilic spores ..	77	13—134
Flat-sour ..	14.5	7—35
T.A. spoilage ..	nil	—
Sulphide stinker ..	nil	—
Proteolytic bacteria (% of total count)		
(i) Standard methods agar ..	46.5	23.0 — 69.0
(ii) ,, ,, caseinate agar	56.1	34.1 — 76.3
Lipolytic bacteria ..	25.0	2.2 — 78.1
Mesophilic lactic acid bacteria[a] ..	790	60 — 4,070
Thermophilic[b] ,, ,, ..	24	15 — 45
Yeasts[b] ..	4	—
Moulds ..	nil	—
	(MPN per g)	
Coliforms[b]	5	2—46
E.coli ..	nil	—

a = No. of samples positive, 7 out of 10.
b = No. of samples positive, 5 out of 10.
* 10 samples were examined.

numbers were fairly low ranging from 7 to 35 and 13 to 134 per gram respectively. The mesophilic aerobic spore formers ranged from 10 to 300 per gram, averaging 123 per gram (Table 3). These spore-formers could arise from the milk, ingredients used in the manufacture of infant foods, improperly sanitized equipment or aerial contamination prior to packaging. Curiously, none of the samples showed the presence of putrefactive T.A. and sulphide stinker types of spoilage organisms.

Proteolytic organisms were detected both by standard methods agar[7] (SMA) containing 10 per cent sterile skim milk and standard methods caseinate agar (SMCA) of Marteley *et al*[10] which is known to permit detection of even weakly proteolytic species. On an average, 46 to 56 per cent of colonies appearing on total plate count agar were found to be proteolytic. Higher recovery of proteolytic organisms was possible on SMCA than on SMA medium (Table 3). During long periods of storage, these proteolytic organisms could be expected to produce pronounced loss of acceptability[32-34]. The lipolytic bacteria constituted a smaller percentage of total count and averaged to about 25 per cent of the total count. One sample was significantly different in that 78 per cent of the colonies on total plate count agar exhibited lipolysis (Table 3). Subsequent to lipolysis the liberated free fatty acids can lead to the development of off-flavour and rancidity which vary considerably with the type of free fatty acids liberated[35].

Mesophilic and thermophilic lactic acid bacteria were generally low and averaged 790 and 24 per gram and were detected in 70 and 50 per cent of the samples respectively. Yeasts were present in only 50 per cent of the samples and averaged to the extremely low level of 4 per gram. None of the infant milk food samples showed the presence of moulds (Table 3).

Coliforms were present in half of the samples examined. Two of the samples showed coliform count of 32 and 46 per gram respectively while the remaining positive samples averaged 2 to 8 per gram (Table 3). The maximum limit for coliforms in infant milk foods is 10 per gram[5]. Thus, the majority of the samples of infant milk foods conform to the ISI specifications regarding coliforms. None of the samples were positive for *E. coli*.

For the detection and enumeration of coliforms, particularly, when present in low numbers, the MPN method has been found to be more reliable[36] while direct plating on violet-red bile (VRB) agar can be followed when the counts are high[37]. Yet, in spite of the low limits for coliform organisms specified in milk powder and infant milk foods, the ISI recommends direct plating on VRB agar. Further, *Enterococci* are likely to survive the pasteurization treatments given to milk[38] and these organisms were in fact observed to grow on VRB agar producing dark red colonies. Similarly, enterococci in baby foods have been shown to produce red colonies on deoxycholate agar[39]. Thus, in presence of *Enterococci* detection and enumeration of coliforms by direct plating is rendered difficult. The levels of faecal *Streptococci* in infant foods, their identification and studies on their heat stability will be reported in later papers. In view of these difficulties it may perhaps be advisable to specify the use of the MPN technique instead of direct plating methods as recommended by the ISI[4,5].

The use of coliforms and *E. coli* as indices of sanitary quality and public health safety has been criticised by several workers[40,41] since, coliforms and *E. coli* are known to be destroyed by processing operations given to foods[42] while pathogenic bacteria could be more resistant to adverse environmental conditions than *E. coli*[43]. The failure to recover coliforms or *E. coli* in some samples suggests that either the contamination is extremely low or, as is more likely, that the processing, operations effectively destroy these organisms. Hence, the use of coliform index as a guideline for the sanitary quality of such products which have been subjected to a lethal heat treatment needs to be re-examined. Since enterococci survive the several processing operations given to foods they could, perhaps, serve as a better index of sanitary quality in infant foods also[44-47].

References

1. *Report of the Group on Food Panel on Consumer Industries*, National Committee on Science and Technology, New Delhi, 1973, 22.
2. *Report of the Working Group on Milk and Dairy Products and Animal Husbandry Including Meat*, N.C.S.T. Panel on Consumer Industries, Government of India, Ministry of Agriculture, New Delhi, May 1973, 2.
3. Ramaswamy, S., Directorate General of Technical Development, Government of India (personal communication).
4. *Specifications for Milk Powder (Whole and Skim)*, *I.S.* 1165-3 1967. Indian Standards Institution, New Delhi.
5. *Specifications for Infant Milk Foods*, *I.S.* 1547-1968, Indian Standards Institution, New Delhi.
6. Sharf, J. M., *Recommended Methods for the Microbiological Examination of Foods*, American Public Health Association, Inc., New York, 2nd Edn., 1966.
7. *Standard Methods for the Examination of Dairy Products*, American Public Health Association, Inc., New York, 12th Edn., 1957.
8. North, W. R. Jr., *J. Ass. off. agric. Chem.*, 1945, **28**, 424.

9. Grinsted, E. and Clogg, L. F. L., *J. Dairy Sci.*, 1595, **22,** 178.
10. Martley, F. G., Jayashankar, S. R. and Lawrence, P. C., *J. appl. Bact.*, 1970, **33,** 363.
11. McDonald, J., *Can. J. Microbiol.*, 1957, **3,** 411.
12. Knaysi, G., *J. Bact.*, 1941, **42,** 587.
13. Skerman, V. B. D., *A Guide to the Identification of the Genera of Bacteria,* William and Wilkins Pook Co., Baltimore, 1959.
14. Emard, L. O. and Vaughn, R. H., *J. Bact.*, 1952, **68,** 487.
15. Silliker, J. H., in *Microbiological Quality of Foods*, Edited by Slanetz, L. W., Chichester, C. O., Gaufin, A. R. and Ordal, Z. J., Academic Press, New York, 1963, 102.
16. Angelotti, R., *Publication No.* 1142, U. S. Public Health Service, Washington, D.C., 1964.
17. Higginbottom, C., *J. Dairy Res.*, 1945, **14,** 184.
18. Hiscox, E. R., *J. Dairy Res.*, 1945, **14,** 175.
19. Garrison, E. R., *J. Bact.*, 1946, **52,** 711.
20. White, A. H., *Sci. Agric.*, 1947, **27,** 405.
21. Galesloot, T. E. and Stadhounders, J., *Neth. Milk Dairy J.*, 1968, **22,** 158.
22. Hall, H. E., *J. Milk Fd Technol.*, 1970, **33,** 311.
23. King, W. L. and Hurst, A., *J. appl. Bact.*, 1963, **26,** 504.
24. Ghodekar, D. R. and Namburdipad, V. K. N., *Indian J. Dairy Sci.*, 1973, **26,** 56.
25. Samagh, B. S. and Dudani, A. T., *Indian J. Diary Sci.*, 1966, **19,** 106.
26. Sherman, J. M., *J. Bact.*, 1916, **1,** 481.
27. Hammer, B. W., *Dairy Bacteriology*, John Wiley & Sons, New York, 2nd Ed., 1946.
28. Nelson, F. E., *J. Bact.*, 1943, **45,** 395.
29. Hunzikar, O. F., *Condensed Milk and Milk Products*, La Grange, Illinois, 5th Edn., 1935.
30. Forest, H. L. and Small, E., *Proc. XV Internat. Dairy Congr.*, 1959, **3,** 1883.
31. Williams, W. L., *J. Milk Technol.*, 1943, **6,** 133.
32. Jay, J. M., *Appl. Microbiol.*, 1967, **15,** 943.
33. Levine, R. E., *Appl. Microbiol.*, 1968, **16,** 1734.
34. Herbert, R., Hendric, M., Gibson, D. and Shewan, J. M., *J. appl. Bact.*, 1971, **34,** 41.
35. Alford, J. A., Smith, J. L. and Lilly, H. D., *J. appl. Bact.*, 1971, **34,** 133.
36. *Standard Methods for the Examination of Water and Waste Water*, American Public Health Association, Inc., New York, 12th Ed., 1965.
37. Hartman, P. A., *Fd Res.*, 1958, **23,** 532.
38. Thomas, N. and Laxminarayana, H., *Indian J. Dairy Sci.*, 1972, **27,** 52.
39. Silliker, J. H. and Greenberg, R. A., in *Food-borne Infections and Intoxications*, edited by Riemann, H., Academic Press, New York, 1969.
40. Raj, H. and Liston, J., *Appl. Microbiol.*, 1961, **9,** 171.
41. Buttiaux, R., *J. appl. Bact.*, 1959, **22,** 153.
42. Raj, H. and Liston, J., *Fd Technol., Champaign*, 1961, **15,** 429.
43. Mossel, D. A. A., *J. appl. Bact.*, 1962, **25,** 20.
44. Raj, H., Wiebe, W. J. and Liston, J., *Appl. Microbiol.*, 1961, **9,** 295.
45. Zaborowski, H., Huber, D. A. and Rayman, M. M., *Appl. Microbiol.*, 1958, **6,** 97.
46. Raj, H. and Liston, J., *Fd Technol., Champaign*, 1963, **17,** 1295.
47. Larkin, E. P., Litsky, W. and Fuller, J. E., *Am. J. Publ. Hlth*, 1956, **46,** 464.

Compressed Ready-to-eat Fruited Cereals

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Dehydrated fruits and fruit powders were prepared by osmotic drying, foam-mat drying and spray-drying, and used in suitable combinations along with puffed cereals in the formulation of recipes for compressed ready-to-eat fruited cereals. Banana, mango, pineapple and tomato gave highly acceptable products. When packed in an inner wrap of cellophane and then in an outer pouch of paper-aluminium foil-polythene laminate, the formulations were acceptable upto 6 months at 37°C and for one year under ambient conditions (20-30°C) except those with osmotic dried fruits wherein hardening of the fruits due to moisture transfer was noticed beyond 6 months under ambient conditions. Microbiological tests showed that the ingredients and formulations were within safe limits for direct consumption.

Results of studies on the preparation of some ready-to-eat compressed food formulations in complete meal form incorporating cereals, pulses, vegetables and meat were reported earlier[1]. In subsequent user trials they were reported to produce a sensation of dryness in the mouth, thus rendering them difficult to swallow. Consequent to this there was apparently a tendency to drink more water and the formulations were therefore reported to be thirst provoking.

Since the primary aim of the experimental work was to develop compressed food bars for direct consumption under conditions precluding preparation, with secondary emphasis on rehydration, an alternate approach of mitigating the sensation of dryness and thirst was therefore tried by the incorporation of certain classes of substances known as moisture mimetic agent like polyhydric alcohols such as glycerol and sorbito sugars, fruits, fruit acids and fats and oils. Experi-

mental work on the use of dehydrated fruit pieces and powders in combination with sugar, skim milk powder and puffed cereals in the development of compressed ready-to-eat formulations is reported here.

Materials and Methods

Puffed rice and wheat : Oil expanded rice was obtained from paddy (long coarse grain variety) and oil expanded broken bulgar or wheat flakes obtained by the methods described earlier.[1]

Fruits : Fruits from the local market were dehydrated by osmotic dehydration using sugar syrup as per the method described by Ponting *et al*[2] with some slight modifications. Banana (*Pachabale*), mango (*Badami*), pineapple, apple (*Kesari*) and guava were used in the studies.

The prepared fruit (slices/chunks) was heated in an equal weight of 70° Brix sugar syrup containing 0.1 per cent potassium metabisulphite at 90°C for 3 min cooled to room temperature quickly, and allowed to soak over-night ensuring complete immersion of the fruit pieces by the use of a stainless steel wire mesh placed on top of the fruit slices.

The fruit was then drained thoroughly and soaked again overnight in the same syrup after concentrating the syrup to 70°C Brix in a steam jacketed kettle and cooling.

The fruit was finally drained thoroughly, spread in a single layer on aluminium trays coated with a thin layer of glycerin and dried in a cross flow hot air drier initially at 80°C for 1 hr and subsequently at 65–70°C for about 6 to 8 hr to a total soluble solid content of about 85° Brix.

Fruit juice powders : Powders were obtained from the following fruits by the foam-mat drying technique after giving suitable pre-treatment as follows :

Preparation of fruit puree/juice : Apples were peeled, cored, sliced, ground in a Waring Blendor into puree along with potassium metabisulphite (0.05 per cent) and water (250 ml/kg of sliced fruit) and then sieved through 30 mesh. Mangoes were peeled and pulped using a 30 mesh sieve. Bananas were peeled and pulped using 30 mesh along with potassium metabisulphite (0.05 per cent) and water (100 ml/kg of peeled fruit). Tomatoes were cored, cut into pieces, cold pressed using screw type juice extractor and sieved through 30 mesh. Pineapples were peeled, sliced, juice extracted using a screw type juice extractor and sieved through 30 mesh. Guavas were sliced, heated to 90°C with water (250 ml/kg of fruit) and pulped using a 30 mesh sieve.

Treatment of the puree/juice : The fruit puree-juice obtained as above with the exception of guava was heated to 90°C and cooled. Potassium metabisulphite was added at 0.05 per cent level on pulp weight basis in the case of mango, tomato, pineapple and guava. Except tomato and pineapple, the purees of other fruits were used as such for foam-mat drying.

In the case of tomato, 90 per cent of the fresh juice of 5° Brix was concentrated to 20° Brix in a steam jacketted kettle or plate evaporator (APV Junior) and cut back with the remaining fresh juice to get a puree of about 15° Brix.

In the case of pineapple, 50 per cent of the fresh juice of 15° Brix was concentrated to 30° Brix and cut back with the remaining fresh juice to get a concentrate of about 20° Brix.

Foaming and dehydration : The fruit puree was heated to 65°C, mixed thoroughly with distilled mono glyceride (Riken Type—S manufactured by M/s. Riken Vitamin Oil Co., Tokyo, Japan) dispersed in a little water at 65°C at a level of 1.0 per cent for tomato, 1.5 per cent for pineapple and 2 per cent for mango, apple, banana and guava on pulp weight basis and whipped using a laboratory stirrer with a whipping attachment for 10 min for mango and banana, 15 min for pineapple and guava and 20 min for tomato and apple. The foam was spread in plain aluminium trays in the form of thin sheet at the rate of 0.25 kg/tray of size 40 cm×80 cm and dried in a cross flow hot air drier initially at 80°C for 30 min and subsequently at 65–70°C for 30–90 min. The dried foam was scraped after cooling the trays to room temperature and the powder collected in a dry atmosphere to prevent its caking. The powder was further dried to a moisture content of below 3 per cent using fused calcium chloride as in-pack desiccant.

Fruit juice powders, spray dried : The puree/juice from mango, banana, tomato and pineapple was prepared as described above.

Two-thirds of fresh tomato juice of 5° Brix was concentrated in a steam jacketed kettle or plate evaporator to 20° Brix and cut back with remaining fresh juice to get a concentrate of 10° Brix. Salt (sodium chloride) was added at a level of 4 percent on total soluble solid content of the tomato concentrate.

The fruit pulp/juice was cooled to 5°C, mixed with skimmed milk powder equal in quantity to the soluble solid content of the concentrate and homogenised. Where necessary additional water was added to get a sprayable consistency in the pulp (about 400–500 ml for banana and 100–150 ml for mango per kg of pulp and none for tomato and pineapple). The mixture was again cooled to 5°C and dried in spray drier (Portable Universal Laboratory Model—Zahn-Ravo-Rapid, W. Germany, with an evaporation capacity of 1–3 litres/hr of water)with an inlet temperature of 140–150° for tomato, mango and pineapple and 150–160°C for banana and product temperature of 70–80°C, using a nozle spray and compressed air at a pressure of 6–8 kg/sq cm. The material was fed at a rate of 2.5 to 3.5 kg/hour.

Recipes were formulated as per Table 2 based on organoleptic tests. Powdered sugar and skimmed milk powder were added to suit taste. They were mixed together as such in a dry mixer except in the case of osmotic dried fruits where the fruit pieces were first coated with the powdered sugar prior to mixing with the other ingredients to prevent their coalescing together and forming lumps.

Compressing and packaging : The formulations were compressed at ambient temperature into blocks (4 cm×4 cm) each weighing 100 g using laboratory model 12 ton Carver Hydraulic Press and iron moulds and packed as reported earlier.[1] A pressure of 3000 psig and dwell period of 10 sec was found optimum.

Proximate composition and microbiological analysis : Proximate composition of the compressed ready-to-eat fruited cereals were determined by the AOAC methods.[3] Some of the formulations and their ingredients were also analysed for total plate count, *Staphylococcus*, coliforms, yeasts, moulds and thermophiles according to the methods recommended by APHA.[4]

Results and Discussion

The characteristics of dehydrated fruits and fruit powders prepared by osmotic dehydration, foam-mat drying and spray drying are given in Table 1. An analytical data (proximate composition) on the various compressed ready-to-eat fruited cereals are presented in Table 3.

Studies on the shelf life of the compressed fruited cereals under accelerated (37°C) and ambient (20–30°C) conditions showed that all the formulations were acceptable for one year under ambient conditions and for 6 months under accelerated storage as judged by organoleptic evaluation by a panel of judges excep

TABLE 1. CHARACTERISTICS OF DEHYDRATED FRUITS/FRUIT POWDERS PREPARED BY OSMOTIC DRYING, FOAM-MAT DRYING AND SPRAY DRYING

Product	Soluble solids* (%)	Total solids (%)	pH	Acidity‡	Brix/acid ratio	Yield†
Osmotic dried fruits						
Banana ..	85	85.5	5.1	0.29	293	20
Mango ..	81	82.3	3.8	0.54	150	20
Pineapple ..	84	86.0	3.7	0.89	95	12
Apple ..	85	89.3	3.8	0.23	370	46
Guava ..	85	86.5	4.3	0.29	293	30
Foam-mat dried fruit powders						
Banana ..	85	96.6	4.9	1.44	59	11
Mango ..	80	94.0	4.0	3.34	24	10
Pineapple ..	85	98.6	3.8	5.74	15	9
Apple ..	65	94.8	3.7	2.37	27	10
Guava ..	60	96.9	3.9	2.81	21	9
Tomato ..	90	97.6	4.3	6.94	13	3
Spray-dried fruit-skim milk powder (SMP) mix						
Banana-SMP ..	95	98.1	5.9	1.25	76	19
Mango-SMP ..	90	97.5	5.2	2.26	40	16
Tomato-SMP ..	85	96.8	4.7	4.86	17.5	6

* By refractometer; † On fresh weight basis; ‡ As anhydro citric.

TABLE 2. RECIPES FOR COMPRESSED READY-TO-EAT FRUITED CEREALS (PERCENTAGE BY WEIGHT)

Ingredients	Type of composition						
	1	2	3	4	5	6*	7*
Rice, oil expanded or Wheat, oil expanded bulgar or oil puffed flakes	50	50	50	60	60	78	68
Fruit chunks, osmotic dried	40	20	20	—	—	—	—
Fruit powder, foam-mat dried	—	20	—	25	—	20	—
Fruit pulp-SMP mix, spray dried	—	—	25	—	30	—	30
Sugar powder	5	5	5	10	10	—	—
Skim milk powder	5	5	—	5	—	—	—
Salt powder	—	—	—	—	—	1.5	1.5
Pepper powder	—	—	—	—	—	0.5	0.5

* Tomato only.

TABLE 3. PROXIMATE COMPOSITION AND CALORIFIC VALUE OF COMPRESSED READY-TO-EAT FRUITED CEREALS

Fruited cereal	Composition*	Composition (g/100 g)						Calories 100 g
		Moisture	Carbohydrate	Protein	Fat	Crude fibre	Ash	
Banana-Rice ..	1	7.2	72.2	6.1	12.1	1.4	1.0	421
	2	5.0	72.7	6.7	13.1	1.1	1.4	435
	3	4.6	72.3	8.1	12.1	1.2	1.8	430
	4	2.5	72.3	7.3	15.8	0.8	1.3	460
	5	2.1	71.5	9.3	14.5	0.8	1.8	453
Mango-Rice ..	1	8.5	72.0	5.7	12.2	0.9	0.7	420
	2	6.2	71.0	6.4	14.5	0.8	1.1	440
	3	5.4	71.6	8.3	12.2	0.9	1.6	429
	4	3.2	70.5	7.1	17.4	0.7	1.1	467
	5	2.2	70.7	9.8	14.6	0.9	1.8	453
Pineapple-Rice ..	1	7.0	73.7	5.7	12.1	0.8	0.7	426
	2	4.5	74.1	6.0	13.3	0.7	1.4	440
	4	2.0	73.1	6.7	16.0	0.6	1.6	463
Apple-Rice ..	1	5.7	75.3	5.5	12.1	0.7	0.7	432
	2	4.6	73.4	5.6	14.3	1.2	0.9	445
	4	3.0	71.1	6.3	17.3	1.3	1.0	465
Guava-Rice ..	1	6.8	71.4	5.7	12.1	3.3	0.7	417
	2	4.7	68.6	5.9	14.7	4.8	1.3	430
	4	2.4	67.7	6.6	17.7	4.2	1.4	456
Tomato-Rice ..	6	2.5	64.8	7.0	20.1	1.8	3.8	468
	7	2.7	62.7	12.3	16.6	1.0	4.7	449

* *See* Table 2.

in the case of formulations containing osmotic dried fruits alone or in combination with fruit powders wherein hardening of the osmotic dried fruit piece was noticed beyond 6 months storage under ambient conditions due to moisture transfer from the fruits to the low moisture puffed cereal during storage. This problem did not, however, exist where the formulation was based entirely on juice powder only or where accelerated freeze dried (AFD) fruit pieces were used instead of osmotic dried fruits in view of their very low moisture content.

Further foam-mat dried or spray dried juice powders imparted uniform fruit flavour throughout the bar since

TABLE 4. MICROBIOLOGICAL DATA (COLONIES/G) ON SOME OF THE COMPRESSED FRUITED-CEREAL FORMULATIONS AND THEIR INGREDIENTS

Ingredient/Formulation	Total plate count	*Staphylococcus* †	Coliforms	Yeasts (Y) Moulds (M)	Thermophiles
Osmotic dried fruits					
Banana ..	290-300	—ve	—ve	—ve	—ve
Pineapple	30-40	60-120	—ve	Y: Nil M: <10	—ve
Apple ..	210-290	<10	<10	Y: Nil M: 30	0-50
Guava ..	190-250	90-100	—ve	Y: Nil M: <10	—ve
Foam-mat dried fruit powders					
Banana ..	150-200	—ve	—ve	Y: 80 M: 80	30-60
Mango ..	140-190	0-50	—ve	Y: 110-250* M: 190-200*	0-10
Pineapple ..	23-31	14-20	—ve	Y: <10 M: <10	—ve
Apple ..	80-360	—ve	—ve	Y: 40-100** M: 50**	30-40
Guava ..	22-40	0-20	—ve	Y: <10 M: <10	—ve
Spray dried powder					
Tomato-SMP ..	50-70	80-180	0-25	Y: 10-30 M: 40-120	—ve
Compressed formulations					
Banana-Rice (Type 2) ‡ ..	200-300	70-180	35-60	<10	Negligible
Pineapple-Rice (Type 2) ‡ ..	100-250	—ve	<10	<10	20-60
To mato-Rice (Type 6) ‡ ..	200-400	0-40	—ve	10-30	10-30

* *Yeasts* (Y): Mostly *Saccharomyces*; *Moulds:* Mostly *Mucor*, *Rhizopus* and *Penicillium*.
** *Yeasts:* Mostly *Saccharomyces;* *Moulds:* Mostly *Trichoderma* Sp. and *Aspergillus niger*.
† Staphylococci wherever noticed were noncoagulase positive.
‡ *See* Table 1.

they could be mixed easily with the cereals prior to compression while in the case of osmotic dried fruit pieces, besides the fruit being weaker in flavour, uniform mixing and distribution were difficult to achieve as a result of which the fruit flavour was confined to the fruit pieces. Formulations containing foam-mat dried or spray dried fruit powders are therefore considered ideal to achieve the full flavour of fruit and extended shelf life.

Of the various fruits tried, banana, mango, pineapple and tomato gave highly acceptable products. Formulations based entirely on fruit powder were superior to those based on a combination of powder and osmotic dried fruits which in turn were superior to those based entirely on osmotic dried fruits. Microbiological data on some of the compressed fruited cereals and their ingredients presented in Table 4 show that they are within microbiologically safe limits for consumption as such.

Limited tests with the Army personnel on the fruited cereals have indicated the acceptability of formulations based on fruit juice powders.

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References

1. Jayaraman, K. S., Anthony Das, S., Ramanathan, L. A. and Bhatia, B. S., *J. Fd Sci. Technol.*, 1970, **7**, 200.
2. Ponting, J. D., Waters, G. G., Forrey, R. R., Jackson, R. and Stanley, W. L., *Fd Technol., Champaign*, 1966, **20**, 1365.
3. *Official Methods of Analysis*, Association of Official Agricultural Chemists, Washington, D.C., 9th Edn., 1960.
4. *Recommended Methods for the Microbiological Examination of Foods*, American Public Health Association, N.Y., 1966.

Package Design for Moisture Sensitive Product—Vermicelli

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Studies carried out to design a suitable consumer package for vermicelli have revealed that moisture contents of higher than 13.2 per cent and lower than 9.5 per cent are critical with respect to mould growth and fragility of the product respectively. The product with an initial moisture content of 11.9 per cent, when packed in units of 250 g in 200 gauge high density polyethylene pouches or 450-g units in 300 gauge low density polytheylene flat pouches of the size 100 × 300 mm will have 83 days shelf lives at 38 °C and 92 per cent R.H. which corresponds to a shelf-life of about 290 days under ambient conditions of 25-30 °C and 70-80 per cent R.H. Additional use of duplex board carton with window and over-wrapped with cellophane results in a longer shelf-life for the product.

Vermicelli, chiefly a starchy product is generally prepared out of processed blends of cereal flours and common salt has an important place in the sweet and savoury preparations in the dietary. The product is generally obtained in thin long strands of about 2 mm thickness and 200 mm length and is commonly made on home-scale. In recent years, considerable quantities of the product are being produced in small scale industries and the trend in increased production and sales pattern shows that the product has good market in the country as well as great potential for export.

Currently, vermicelli, with a proximate composition[1] of moisture, 11.7; carbohydrates, 78.3; proteins, 8.7; fat, 0.4; and crude fibre, 0.2 per cent is offered for sale in lots of 250-g and 450-g wrapped in kraft paper, which does not provide protection from moisture ingress and breakage during transit. The present study was undertaken to design a consumer-pack for the product to give a shelf-life of about 6 to 8 months under normal conditions of storage prevailing in the country.

The product has two levels of critical moisture content, the upper limit corresponds to its susceptibility to fungal spoilage and softness and the lower limit at which it becomes brittle and fragile. As such, a study of moisture sorption characteristics of the product under different humidities is important for designing a suitable package to withstand storage hazards during both inland and export marketing.

Materials and Methods

Vermicelli and its moisture sorption characteristics : Freshly made vermicelli, obtained from a local firm, with an initial moisture content of 11.9 per cent (on dry weight basis) was used in the present studies.

Humidity moisture relationship of the product was carried out at 27°C by exposing weighed quantities of the sample in petri dishes to RH ranging from 11 to 92 per cent built up in desiccators using appropriate saturated salt solutions.[2] The samples were periodically weighed to study gain or loss in moisture in the product and the weighings were continued till the samples attained a constant weight or till the onset of visible fungal growth, whichever was earlier. Results are presented in Table 1.

Packaging materials : (*a*) Low density polyethylene (LDPE) 200 gauge; (*b*) LDPE 300 gauge; (*c*) high density polyethylene (HDPE) 200 gauge; (*d*) Duplex board for carton; and (*e*) 400 MXXT cellophane as overwrap for cartons.

Pack size : For consumer packs flexible pouches of size 100 × 300 mm with an effective area of 100 × 250

TABLE 1. HUMIDITY AND MOISTURE SORPTION RELATIONSHIP OF VERMICELLI AT 27°C

RH (%)	Equilibrium moisture content (%)
11 ..	6.70*
22 ..	7.34†
32 ..	9.55†
44 ..	10.56
52 ..	11.61
64 ..	12.71
73 ..	13.54‡
86 ..	19.32§
92 ..	22.37§

Initial moisture content, 11.91%
* Very brittle † Brittle
‡ Slightly soft § Mould growth within 15 days

between the seals were used. Both 250-g and 450-g quantities of vermicelli were used in such pouches. For export pack a rigid carton with an approximate surface area of 700 cm^2 was used for packing 450 g. The carton was overwrapped with cellophane.

Theoritical deduction of accelerated storage life at 38°C and 92 per cent RH : From the required storage life of 6–8 months under ambient conditions (25–30°C) and 70–80 per cent RH, accelerated storage life at 38°C and92 per cent RH was derived by using the following emperical equation :

$$T_2 = T_1 \left[\frac{P_1}{P_2}\right]^K \left\{\frac{R_1 - \frac{R_o + R_c}{2}}{R_1 - \frac{R_o + R_c}{2}}\right\}$$

Where T_2 and T_1 are the shelf-lives of the product at RH conditions of R_2 and R_1 and at temperatures t_2 and t_1 respectively. R_o and R_c are the initial and critical RHs of the product. P_1 and P_2 are the saturated water vapour pressures at temperatures t_1 and t_2. 'K' is a constant equal to 1.0 for polyethylene and 1.6 for cellophane. The accelerated storage life was worked out to be $1\frac{1}{2}$ to 2 months.

Theoretical deduction of permeability requirements of packs : The water vapour transmission rates (WVTR) required of the packs under accelerated conditions were theoretically derived on the basis of the accelerated storage conditions, the storage life under these conditions as derived above, an assumption of average RH of 60 per cent inside the unit packs during the storage period, total effective surface area of the packs and the permissible moisture uptake of 1.3 per cent. The values thus derived are given in Table 2.

Selection of packaging materials for storage studies: Based on the permeability requirements of the packs derived as above the following packaging materials were selected for storage trials:

TABLE 2. REQUIRED WVTR VALUES OF PACKAGING MATERIALS

Sample	WVTR, $g/m^2.d$
250-g Flexible pouch ..	3-4
450-g ,, ..	7-8
250-g Rigid container ...	2-3
450-g ,, ...	4-5

(1) 200 gauge LDPE film witha WVTR of 6.40 g/m^2/day at 38°C and 92 per cent RH for packing 250 g of the product.
(2) 300 gauge LDPE film with a WVTR of 5.50 g/m^2/day at the same above conditions for packing 250 and 450 g of the product.
(3) 200 gauge HDPE film with a WVTR of 3.87 g/m^2/day at the same above conditions to pack 250 g of the product.
(4) Duplex board cartons with an overwrap of 400 MXXT cellophane with a WVTR of 2.68 g/m^2/day at the same above conditions for packing 450-g of the product.

The WVTR were determined by standard ISI method.[4]

Storage trials: Storage trials of the selected packs were carried out at 38°C and 92 per cent RH. The individual packs were periodically weighed to follow the moi sture pick-up and examined for on set of fungal growth.

Results and Discussions

Moisture sorption studies: It can be observed from Table 1, that vermicelli with an initial moisture content of 11.9 per cent (dry weight basis) equilibriates to an RH of 52 per cent. In general, processed food products are prone to fungal spoilage at 60 per cent RH and above[5], in which region the moisture pick-up is too rapid. It can be inferred from the sorption results that equilibrium moisture content of 13.2 per cent at 70 per cent RH is critical for the product to protect against mould growth and fungal spoilage. Further, it was observed that the product equilibrated to 73 per cent RH with an equilibrium moisture content (EMC) of 13.54 per cent had become slightly soft where as that equilibrated to a RH of 32 per cent had a moisture content of 9.5 per cent and had become brittle and crumby. Thus, the lower moisture content of 9.5 per cent is critical to the product.

The sorption data presented in Table 1 indicates that the sorption curve for vermicelli would be sigmoid in shape akin to that of pure starch and other highly starchy products such as corn, rice and cereal flours. The calculated monolayer moisture value which defines the stable region for most of the dehydrated and processed starchy goods happens to be approximately 6 per cent (ERH-15 per cent RH)[6]. For vermicelli, the mono-layer value was found to be 6.5 per cent. The advantage of extending the shelf-life of the product by reducing the initial moisture content upto the mono-layer value of 6.5 per cent however, is not feasible, as below 9.5 per cent, the product becomes brittle.

TABLE 3. MOISTURE PICK-UP BY UNIT PACKS STORED AT 92 PER CENT R.H. AND 38°C

Packaging material	Quantity	Moisture pick-up (%) at indicated storage time (days)							
		11	22	29	42	54	70	83	102
200 gauge LDPE pouch ..	250	0.44	0.90	1.14	1.50	1.83	2.22	2.53	2.98
400 gauge LDPE pouch ..	250	0.39	0.78	0.99	1.31	1.64	1.96	2.22	2.59
200 gauge HDPE pouch ..	250	—	0.49	0.57	0.77	0.97	1.17	1.35	1.62
300 gauge LDPE pouch ..	450	0.21	0.44	0.57	0 79	0.99	1.23	1.40	1.68
Duplex-board window cartons overwrapped with MXXT cellophane ..	450	0.80	1.54	1.91	2.44	2.83	3.32	..	..

Package design: For predominantly moisture sensitive product such as vermicelli requiring protection against ingress of moisture it is necessary to select packaging materials with required permeability characteristics. The choice of a suitable packaging material could be made by an equation given by Paine.[3] Translation of storage life at accelerated conditions of 38°C and 92 per cent RH to that at ambient conditions is often difficult, but in the present studies this deduction was done by using an emperical equation which is an extension of Paine's equation[3].

Since a moisture content of 13.2 per cent is the higher critical limit, vermicelli with an initial moisture content of about 11.9 per cent has a permissible uptake of moisture of about 1.3 per cent during storage. This value was taken into consideration in deriving the theoretical WVTR of the packs (Table 2).

Storage trials: The purpose of the storage studies under accelerated conditions was to verify the correctness of selection of packaging materials on theoretical basis. The results of this trial are given in Table 3. It can be seen from this that the permissible moisture pick-up of 1.3 per cent has been exceeded in 250-g unit packs of 200 and 300 gauge LDPE and 200 gauge HDPE in 35, 42 and 83 days respectively. Though no visible fungal spoilage was observed the product had developed an unpleasant, musty odour and acidic taste thereby rendering it unacceptable. Under similar conditions of storage, 450-g unit pack in 300 gauge LDPE had moisture pick-up of 1.4 per cent at the end of 83 days. Thus, the shelf-life obtained by packing 250 g of the product in 200 guage HDPE pouch and 450 g of the product in 300 gauge LDPE pouch are slightly more than the required shelf-life of about 70 days at accelerated conditions, which corresponds to about 290 days at ambient conditions. From these observations it may be seen that the shelf-life of vermicelli could be doubled by packing nearly twice the quantity in almost an identical pouch. The permissible moisture pick up of 1.3 per cent was attained within 22 days storage in packs of duplex board cartons overwrapped with 400 MXXT cellophane which is contrary to expectations. The failure of seals of the overwraps at high humidity conditions within a few days of storage may be responsible for such high moisture pick-ups. Thus the results suggest that overwrapping with only MXXT cellophane does not provide the desired self-life for the product. So, it is desirable to pack the product in a primary pouch of 300 gauge LDPE film before inserting into cartons.

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References

1. Gopalan, C., Rama Sastry, B. V., and Balasubramanian, S. C., *Nutritive Value of Indian Foods*, National Institute of Nutrition, India, 1971, 162.
2. Funk, W. A., *Mod. Pack.*, 1946-47, 20, 135.
3. Paine, F. A., *Fundamentals of Packaging*, Blakie & Sons Ltd., 1962, 290.
4. *Methods of Sampling and Test for Paper and Allied Products, I.S.*—1060, Part II, Indian Standards Institution, New Delhi, 1960.
5. Christian, J. H. B., *Recent Advances in Food Science*, Butterworths Publication, London, 1963, 3, 248.
6. Salwin, H., *Freeze Drying of Foods*, National Academy of Sciences, National Research Council, Washington, D.C, U.S.A., 1962, 68.

EFFECT OF FAT AND SUGAR VARIATION ON THE ACCEPTABILITY OF *SHRIKHAND* PREPARATION

Shrikhand resulting from the combination of 3 per cent fat (of milk) and 36 per cent sugar (by weight of _Chakka_) is palatable and acceptable and hence it may be recommended for adoption by the trade.

Shrikhand is a milk product common in Maharashtra, Gujarat and Karnataka. It is prepared from curd by addition of sugar and flavouring agents like saffron, camphor and dried fruits. Considering its popularity an attempt was made to standardise the procedure of its preparation.

For the purpose of the experiment, different starter culture strains were obtained from NDRI, Karnal- *Streptococcus lactis* (H), *Leuconostoc citrovorum* (543) and *Lactobacillus bulgaricus* (LBW) were maintained by carrying out transfers on every third day in a sterilised skimmed milk. It was found out from the preliminary trials that a mixture of above three in equal proportions incubated at 34°C for 15 hr resulted in a *Dahi* (curd) of desirable texture and pleasing aroma. This was employed as a starter culture.

Throughout the experimental period buffalo milk was used. Fat content, being the major factor governing the cost and acceptability of the product was varied to arrive at an optimum level. Since buffalo milk is employed commonly for the preparation of *Shrikhand*, a level of 7 per cent fat (F_3) was introduced as a control in the experiment. Two different levels of fat, *viz.*, 3 per cent as F_1 and 5 per cent as F_2 were taken for comparative study. Milk was accordingly standardised at the above fat levels before processing. For each batch 1½ litres of milk was used.

The milk was boiled for 10 min and cooled to 34°C. This was inoculated with the mixed culture at the rate of 2 per cent. The same was incubated at 34°C and *Dahi* was formed after 16 hr. This *Dahi* was tied in a muslin cloth and hung for 6 hr to drain out water. The whey resulted in the formation of *Chakka* which is the *Shrikhand* base.

The experiment was laid out in a 3^2 Factorial design. Two factors, namely, fat and sugar and 3 levels of fat (as stated above), and 3 levels of sugar for palatability were tried. In all there were 9 treatment combinations. These were F_1S_1, F_1S_2, F_1S_3, F_2S_1, F_2S_2, F_2S_3, F_3S_1, F_3S_2 and F_3S_3 corresponding to the following:

	Fat %		*Sugar %*
F_1	3	S_1	36
F_2	5	S_2	42
F_3	7	S_3	48

Sugar percentage was on the basis of weight of *Chakka*. The *Chakka* obtained was mixed with adequate amount of sugar (as per treatment). This was mixed properly by working it through the *Shrikhand patra*, *i.e.*, hand operated *Shrikhand* machine. Condiments and spices were added at the following levels (for 250 g of *Chakka*):

Saffron colour	..	5 c.c. of 0.6% solution
Nutmeg	..	0.50 g
Cardamom	..	0.40 g
Charoli	..	2.00 g

The product as obtained was subjected to evaluation by trained judges for texture, flavour and sweetness. Standard score cards were provided to all. In all 8 replications were taken and everytime the product was judged by more than 10 persons.

The scores offered by the judges were subjected to statistical analysis and average percentages were transformed to degrees (by angular transformation). These were subjected to analysis of variance. This showed that the treatments were non-significant, and implied that the treatments did not differ from each other.

To find out the effect of the interaction of fat × sugar on scoring of *Shrikhand* the treatment sum of squares (TSS) was partitioned into three compartments that due to fat, sugar and fat × sugar.

It is clear from Table 1 that the results were not significant for fat, sugar and interaction. It can be inferred that the combinations that were considered for scoring did not differ from each other in the opinion of judges. Finally from the marketability point of view any combination that has been studied is accept-

Source	df	SS	MSS	F	F 5%
Replication ..	7	740.5	105.8	18.70**	218
Fat (F) ..	2	23.0	11.5	2.03 NS	3.18
Sugar (S) ..	2	14.9	7.5	1.32 NS	3.18
Interaction(FXS)..	4	13.2	3.3	0.58 NS	2.54
Error ..	56	316.6	5.6		
Total ..	71	1108.3			

** Highly significant NS Not-significant

able though from the economic point of view minimum fat and sugar combinations are preferable.

Shrikhand resulting from the combination of 3 per cent fat (of milk) and 36 per cent sugar (by weight of *Chakka*) is palatable and acceptable and hence it may be recommended for adoption by the trade.

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PROXIMATE ANALYSIS AND ESSENTIAL AMINO ACID COMPOSITION OF IMPROVED VARIETIES OF OATS

Three new varieties of oats namely *NP*-101, *Rapida* and *Kent* and a commercial sample have been evaluated for their chemical and essential amino acid compositions. *Rapida* variety has maximum protein content of 14.2 per cent as compared to 7.8 per cent of the commercial sample. The results indicate a possible inverse relationship between the protein content and lysine content for these varieties.

Oats (*Avena sativa*) rank next to wheat and barley in importance of food crop of the temprate region and is cultivated in most of the Western countries. It is also one of the most nutritious cereals used for human consumption.[1] Oats, mainly cultivated in Punjab and Uttar Pradesh are primarily used as a fodder crop in India. In recent years, oats is gaining importance for its use in breakfast food items like rolled oats, oat meal, etc. New varieties of improved agronomical characters are being developed by Indian Agricultural Research Institute (IARI). Little published data are available regarding Indian varieties of oats, their chemical composition and nutritive value. Three new varieties namely *NP*-101, *Rapida* and *Kent* developed by IARI along with a commercial sample used in food industry have been studied in this investigation.

The samples were finely powdered in a grinder to pass through 45 mesh sieve. Moisture, fat (as ether extractives), crude fibre and total ash contents were determined by AOAC methods[2]. The essential amino acid composition was determined using microbiological assay technique[3]. Lysine, histidine, methionine, phenyl-alanine and cystine were determined using *Leuconostoc mesenteroides P*-60, as test organism. Arginine and threonine were estimated using *Streptococcus faecalis R*, while *Lactobacillus arabinosus* was used for estimating leucine, isoleucine, valine and tryptophan. For estimating tryptophan, alkaline hydrolysis was carried out using 5 N NaOH[4].

The proximate analysis and amino acid composition of oat samples are given in Tables 1 and 2.

The data in Table 1 indicate that the protein content of new varieties of oat is considerably higher than that of commercial sample. *Rapida* variety has the highest protein content (14.2 per cent), nearly double that of commercial sample (7.8 per cent), while, varieties *NP*-101 and *Kent* (11.6—11.8 per cent) are nearly 50 per cent more in protein content than that of commercial sample.

Ether extractives in commercial sample were slightly higher than in new varieties. However, no significant variation was observed in the ether extractives of new varieties. In contrast, the total ash content of new varieties is considerably higher than that of commercial sample.

It is interesting to note that the *Rapida* variety with a protein content, nearly double that of commercial sample has comparatively a low lysine content (2.3 per cent)

TABLE 1. PROXIMATE COMPOSITION (g/100 g) OF OAT VARIETIES

Constituent	Varieties			
	NP 101	*Rapida*	*Kent*	Commercial
Moisture ..	8.0	7.7	7.4	7.7
Protein (N×6.25) ..	11.8	14.2	11.6	7.8
Fat ..	4.8	4.4	4.7	5.7
Total ash ..	3.3	3.9	3.7	2.3
Crude fibre ..	9.0	9.8	9.3	8.5
Carbohydrate (by diff.)	63.1	60.0	63.3	68.0

TABLE 2. ESSENTIAL AMINO ACID COMPOSITION (g/16 g N) OF OAT VARIETIES

Amino acids	Varieties			
	NP 101	Rapida	Kent	Commercial
Lysine ..	3.4	2.6	3.1	3.6
Methionine ..	1.3	1.2	1.2	1.6
Histidine ..	1.9	1.6	1.6	1.9
Phenylalanine ..	4.0	3.7	4.2	3.9
Cystine ..	1.5	1.2	1.5	1.8
Leucine ..	5.5	5.2	6.8	7.6
Isoleucine ..	4.4	3.4	4.6	4.4
Valine ..	5.3	4.3	4.6	5.2
Threonine ..	3.3	2.8	3.4	3.6
Arginine ..	7.0	6.0	7.7	7.0
Tryptophan ..	1.3	1.5	1.4	1.2

The lysine content of 3.4 per cent for variety *NP*-101 compares favourably with that of commercial sample and other reported values[5] (3.1–3.6 per cent). The methionine content (1.2–1.3 per cent) as well as the total sulphur-amino acids (2.4–2.8 per cent) are slightly lower than that of commercial sample and other reported values[5] of 1.2–1.6 per cent and 3.0–3.4 per cent respectively. With regard to threonine, except for the low value of 2.8 per cent in case of *Rapida* variety, the other two varieties are comparable to commercial sample and reported values in their threonine contents (2.4–3.6 per cent). All the new varieties have a tryptophan content comparable to that (1.4 per cent) of FAO reference protein pattern.

The data on the protein contents and amino acid make-up given in Tables 1 and 2, perhaps indicate a negative correlation between protein and lysine contents. This is corroborated by similar reports for hybrid oats, wheat and rice varieties with high protein content[6-10].

Animal experiments[6] reported earlier have shown that the values obtained by multiplying per cent lysine content by per cent protein content in rice had a better correlation with overall growth promoting values than the lysine content alone. These values calculated for all the new varieties of oats are higher (36.0–40.1) than that (28.1) of the commercial sample *NP*-101 has the maximum value of 40.1.

The new varieties developed at IARI, and *Rapida* variety in particular, appear to be promising in view of their high protein content, and in combination with lysine-rich legumes can form good blends of protein achieved through mutual amino acid supplementation. Alternately, in the manufacture of breakfast foods with lysine fortification, these new varieties offer a better source of raw materials as compared to the samples available today, as they are low in protein value.

The observation that, higher the protein content, lower is the lysine content of oat varieties, is of prime importance in developing new varieties of cereals which are generally low in proteins and primarily deficient in lysine.

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References

1. Pomeranz, Y., *CRC Critical Rev. Fd Technol*, 1973, **4**, 377.
2. *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, D.C., 11th Ed., 1970.
3. Barton-Wright, E. C., *Microbiological Assay of Vitamin B-Complex and Amino Acids*, Sir Isaac Pitman Publication, London, 1952.
4. Barton-Wright, E. C. and Curtis, N. , *Analyst, Lond.*, 1948, **73**, 330.
5. Kuppuswamy, S., Srinivasan, M. and Subrahmanyan, V., *Proteins in Foods*, Indian Council of Medical Research, Special Report Series No. 33, New Delhi, 1958.
6. Srinivasan, K. S., Shamanthaka Sastry, M. C., Indira, K. and Desikachar, H. S. R., *J. Fd Sci. Technol.*, 1970, **1**, 95.
7. Clark, W. L. and Potter, G. C. in *Symposium; Seed Proteins*, edited by Inglett, G. E., AVI Publishing Company, Inc., 1972, 171.
8. Gupta, Y. P., *Sci. Cult.*, 1970, **36**, 652.
9. Hepburn, F. N. and Bradley, W. B., *Cereal Chem.*, 1965, **62**, 140.
10. Yadav, S. P., Lal, B. M. and Gupta, Y. P., *Indian Miller*, 1973, **4**, 27.

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LARGE SCALE TRIALS FOR STABILIAZTION OF RICE BRAN WITH STEAM

Large scale trials for stabilization of rice bran were carried out with conventional steaming equipment such as tempering and preconditioner units of oil expeller and steaming tanks used in parboiling. Bran treated in this equipment could be stored up to 25 days without appreciable rise in free fatty acids.

Steaming of either paddy or bran had been found earlier to effect inactivation of lipase in bran.[1] It was of interest to evolve simple, cheap and practical methods of inactivation of lipase in bran using conventional steaming equipment. The following methods were tried in one of the commercial rice mills in Madhya Pradesh. Fresh bran, within 10 to 12 hr after milling, was treated in the following equipment.

Tempering unit: This unit, being used for preconditioning oil bearing materials,consists of a jacketed cylindrical drum with facilities for open and jacket steam. Paddle type conveyors are provided which convey and mix the material. Bran could be continuously treated in this equipment. The capacity was about 1 tonne of bran per hour. The retention time was about 15 min.

TABLE 1. FREE FATTY ACIDS IN BRAN SAMPLES FROM RAW RICE TREATED IN TEMPERING UNIT, PRECONDITIONERS OF LARGE AND SMALL OIL EXPELLER AND STEAMING TANK

Treatments	Moisture (%)		FFA (%) after storage for indicated days							
	After treatment	Storage	0	2	4	6	10	15	25	40
Tempering unit										
Untreated ..	—	6.2	7.4	9.8	12.0	—	33.1	46.2	60.0	—
Treated ..	8.2	6.0	—	7.4	7.9	8.1	8.4	9.3	11.9	20.2
Large expeller										
Untreated ..	—	6.5	9.8	—	15.1	21.1	32.5	48.5	—	—
Treated ..	8.7	5.8	—	9.8	10.1	—	10.9	12.5	13.0	20.1
Small expeller										
Untreated ..	—	6.8	9.5	—	15.1	21.1	32.5	48.5	—	—
Treated ..	8.9	5.2	—	9.7	9.8	9.9	9.8	—	12.0	15.0
Steaming tank (Parboiling)										
Untreated ..	—	6.7	6.3	15.1	21.1	—	—	41.0	56.5	—
Steamed 15 min. ..	8.5	6.5	—	9.6	10.1	10.4	10.6	11.3	16.5	33.4
Steamed 20 min. ..	8.8	6.8	—	6.3	6.5	6.8	7.0	8.0	10.8	31.1
Steamed 30 min. ..	9.5	6.4	—	6.8	6.8	7.0	7.1	—	11.3	28.8

Preconditioning units of oil expeller: These consist of semi-cylindrical drums provided with paddle type mixing and conveying action. In these units also both jacketed and open steam heating has been provided. Bran could be treated continuously. Two such preconditioners, each having a capacity of 1 tonne and of 0.25 tonne bran per hour were used. The retention time was about 15 and 8 min respectively.

Parboiling steaming kettles: These are vertical cylindrical tanks with conical bottom having opening at bottom with a shutter and provided with perforated vertical pipe for passing steam. The tank could take 0.25 tonne bran per batch. Two such adjacent tanks were used.

About 2 tonnes, one tonne, and half-tonne fresh bran were treated in tempering unit, preconditioners of large and small oil expellers respectively. The temperature of outcoming bran was 95 to 97°C in all the cases. The heated bran was shade dried overnight, mixed thoroughly and filled in jute bags and stored under ambient conditions along with an untreated sample. Representative samples were drawn from each bag periodically for estimation of free fatty acids (FFA)[2]. Moisture was determined in the initial bran, immediately after steaming and after shade drying. Moisture pick up during steaming was 2 to 3 per cent but this was lost during cooling of the steamed bran.

Data in Table 1 indicate that there was no appreciable increase in FFA upto 25 days storage period in samples treated in tempering unit, large and small oil expeller preconditioning units.

In case of bran treated in steaming tanks 20 min steaming time seems to be essential to get inactivation of lipase and such treated bran could be stored upto 52 days without appreciable increase in FFA.

FFA increased thereafter in all samples due to insect infestation as reported earlier.[1] FFA in untreated bran was more than 45 per cent after 15 days storage period.

These experiments have indicated that such simple steaming equipment could be used for stabilization of bran using steam as heating media. The resultant bran could remain stable for about 25 days. Adequate protection against insect infestation may further enhance the storage period of such stabilized bran. Bulk quantities of bran (0.25–1 tonne per hour) could be treated by these methods. Such equipment are already available, their cost is also relatively low (about Rs. 5,000 each preconditioner except the tempering unit which is estimated to cost Rs. 10,000). Tempering unit seems to be the best among the units tried.

Further continuous commercial trials are planned in the next paddy season to examine their suitability to produce low FFA oil on commercial scale.

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References

1. Viraktamath, C. S. and Desikachar, H. S. R., *J. Fd Sci Technol.*, 1971, 8, 70.

2. *Official Methods of Analysis*, Association of Official Agricultural Chemists, Washington, D. C., 9th Edition, 1960.

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INFLUENCE OF ADDED ASCORBIC ACID ON INTERNAL CORROSION OF TIN-PLATE IN CANNED MANGO NECTAR

Ascorbic acid and its degradation product, furfural have been shown to act as accelerators of corrosion when added to mango nectar.

Mango pulp contains 5 to 100 mg/100 g ascorbic acid which varies with the variety[1-4]. Ascorbic acid is known to be an efficient antioxidant and its addition to canned apple halves[5] controlled browing, reduced head-space oxygen and protected the container from corrosion. Suzuki[6] and Hernandez[7] reported the acceleration effect of ascorbic acid and the degradation products such as dehydroascorbic acid and 2. 3 diketo gulonic acid on corrosion of tinplate. Therefore experiments were undertaken to study the effect of added ascorbic acid to inhibit corrosion of the tinplate by reducing the headspace oxygen in canned mango nectar.

Ascorbic acid was estimated by the method of Robinson and Stodtz.[8] Tin content was determined by the volumetric method described by Mackenzie[9]. Furfural in the samples was determined by the colorimetric method.[10]

Different concentrations (50,100 and 200 mg/100 g) of ascrobic acid were added to *Badami* variety mango nectar containing 20 per cent pulp, 15° Brix and 0.3 per cent acidity (as anhydrous citric acid) and canned in 1 lb jam size [301×309 cans (E 100)]. Canned products were stored at 37°C and examined periodically for 12 months. During cut out analysis vacuum, ascorbic acid, tin and furfural were determined and appearance of the can interior was noted.

Tin content (Table 1) and extent of corrosion increased with increase in the concentration of ascorbic acid. With increase in the extent of corrosion ascorbic acid content decreased significantly. pH was 4.2 in control as well as treated and there was no variation during storage.

These results indicate that addition of ascorbic acid will not have beneficial effect in reducing corrosion. On the other hand, it accelerated the process of corrosion which agreed with the results of Suzuki[6] and Hernandaz.[7]

In these studies furfural which is one of the degradation products of ascorbic acid[11,12] increased with increase in the concentration of ascorbic acid (2. 3, 2.8, 3.7, 7.7 mg per cent in control and in presence of 50,100 and 200 mg per cent ascorbic acid respectively). Hence, the influence of furfural on corrosion of tinplate has been studied.

Tinplate was cut into strips of 8×2 cm and placed in test tubes, covered with sugar syrup containing different concentrations (2.5, 5 and 10 mg/100 g) of distilled furfural which was heated to 85°C. These tubes were sealed, processed in boiling water and cooled in cold water.

These samples were stored at 37°C, for 12 months and corrosion rate was estimated by determining the percentage loss in weight of the tinplate strips.

There was no appreciable difference in corrosion rate between treated and control samples upto 6 months. After 12 months at the level of 2.5 and 5 g per cent added furfural, corrosion rate as slightly higher (4.5 and 4.75 per cent, than the control (4.2 per cent) but

TABLE 1. INFLUENCE OF ASCORBIC ACID ON CORROSION OF TIN PLATE DURING STORAGE AT 37°C

Storage period (months)	Tin content (ppm) after adding ascorbic acid at indicated levels			
	Mango nectar	50 mg/100 g	100 mg/100 g	200 mg/100 g
Initial ..	38	40	39	38
3 ..	75	90	122	145
6 ..	146	169	183	229
9 ..	262	293	320	380
12 ..	318	363	382	496

at 10 mg per cent level corrosion rate was more (6.0 per cent as compared to the rates at lower levels, indicating that furfural acts as a mild accelerator of corrosion.

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References

1. Jain, N. L., *Rev. Fd Technol., India*, 1961, 3, 132.
2. Teotia, S. S. and Awasthi, R. K., *Indian Fd Pckr*, 1966, 20 (3), 10.
3. Nanjundaswamy, A. M., Rahim, A. and Siddappa, G. S., *Indian Fd Pckr*, 1966, 20 (3), 15.
4. Suryaprakash Rao, P .V., Prasad, P. S. R. K., Girdhar, N. and Nageswara Rao, G., *Indian Fd Pckr*, 1968, 22 (3), 11.
5. Hope, G. W., *Fd Technol., Champaign*, 1961, 15, 548.
6. Suzuki, K., *Canners J. (Kanzume Jiho)*, 1971, 50, 104. (*Fd Sci. Technol. Abstr.*, 1973, 5, 5F212.)
7. Hernandez, H. H., *Fd Technol., Champaign*, 1961, 15, 543.
8. Robinson, W. B. and Stodtz, E., *J. biol. Chem.*, 1945, 160, 217.
9. Mckenzie, H. A., *J. sci. ind. Res., Australia*, 1945, 18, 181.
10. *Methods of Analysis*, Association of Official Agricultural Chemists, Washington, D.C., 11th Ed., 1970.
11. Huelin, F. E., *Fd Res.*, 1953, 18, 633.
12. Huelin, F. E., Cogglola, I. M., Sidhu, G. S. and Kesett, B.H., *J. Sci. Fd Agric.*, 1971, 22, 540.

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AN INTEGRATED DISTINGUISHING TEST FOR AFLATOXIN

Based on the photo-sensitivity and slow chromatographic mobility of aflatoxin in ether on silica gel, an integrated method to distinguish aflatoxin B_1 from other nonspecific fluorescent compound is worked out.

Majority of the chemical methods followed for the detection and estimation of aflatoxin in food materials are based on the fluorescent characteristics of the toxin.[1-3] A few others, however, are based on its absorption characteristics[4,5].

Aflatoxin, occurs in many food materials and hence the need for a routine check is obvious. Since they often carry compounds fluorescing like aflatoxin, simple confirmatory tests that fit into routine examinations are required. Chemical confirmatory tests that are worked out,[6,7] require isolation and purification of the suspected compound. For routine examinations involving a large number of samples, there is a need for much simpler tests. This communication describes a procedure to distinguish aflatoxin from non-specific fluorescent compounds. The method is based on two important properties of aflatoxin; namely (i) poor mobility of aflatoxin on thin layer chromatoplates with diethyl ether as the developing solvent[8] and (ii) photo-decomposition of aflatoxins by ultraviolet light into slow moving and fast moving compounds on thin layer chromatograph (TLC)[8,9].

For standardising the technique, infected groundnuts, chips of tapioca tubers, urine of healthy as well as sick children were extracted with chloroform. Groundnuts and tapioca chips (50 g of each) were extracted as per the method described by Pons and Goldblatt[3]. Urine samples (24 hr collections) were first purified by mixing with 20 per cent lead acetate (100:10) and filtered. Filtrate was extracted 3-4 times with 20 ml chloroform. Chloroform extracts were evaporated on a water bath till the volume is reduced to about 1 ml. The concentrates were used in these studies.

The photo-reaction characteristics of non-specific fluorescent compounds and aflatoxin B_1 are studied. The non-specific bluish purple fluorescent compound from urine as well as tapioca chips are purified by spotting their chloroform extracts on TLC coated with silica gel G and activated at 100°C for 30 min. Purified compounds are spotted on TLC plate along with standard aflatoxin B_1. The plates are first developed with diethyl ether (dry) for 30 min in a tank saturated with the same solvent. The plates are air dried and bluish purple fluorescent spots are identified under long wave UV light and recorded. After prolonging the exposure to UV light for another 15 min, the plates are redeveloped with 5 per cent methanol in chloroform as the solvent. The plates are again viewed under UV light and the spots are marked.

Figure 1 shows the photograph of a chromatoplate wherein the extracts of groundnut, child's urine, monkey's urine and suspected tapioca chips (tuber of *Manihot utilissima*) are spotted and developed with 5 per cent methanol in chloroform. The results show that these extracts contain bluish fluorescent compounds (indicated as BP in the photograph) running close to aflatoxin B_1 on the TLC.

Figure 2 is the photograph of the chromatoplate spotted with the non-specific compounds (chloroform extracts of monkey's urine and tapioca) with standard aflatoxin B_1 and developed with dry ethyl ether. It is

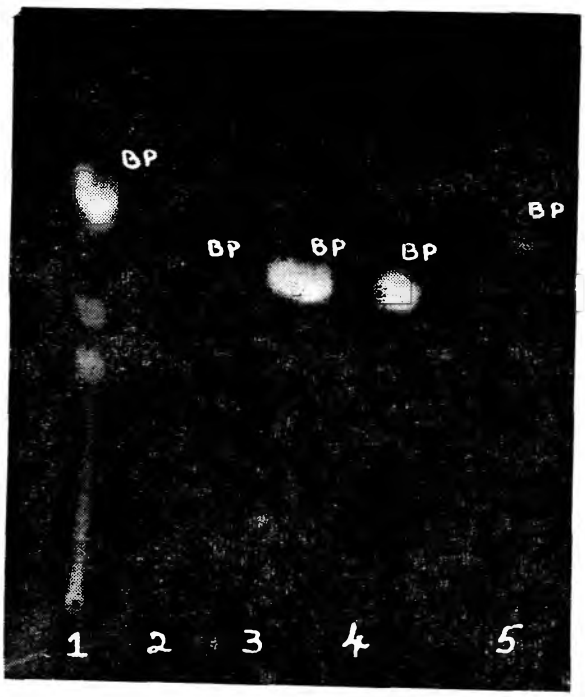

FIG 1. Thin layer chromatograms of chloroform extracts o 1, groundnut, 2, child's urine, 3, monkey's urine 4, tapioca 5, standard aflatoxin B_2 BP, Bluish purple. Quantities spotted are arbitrary (about 30 μl from 1 ml).

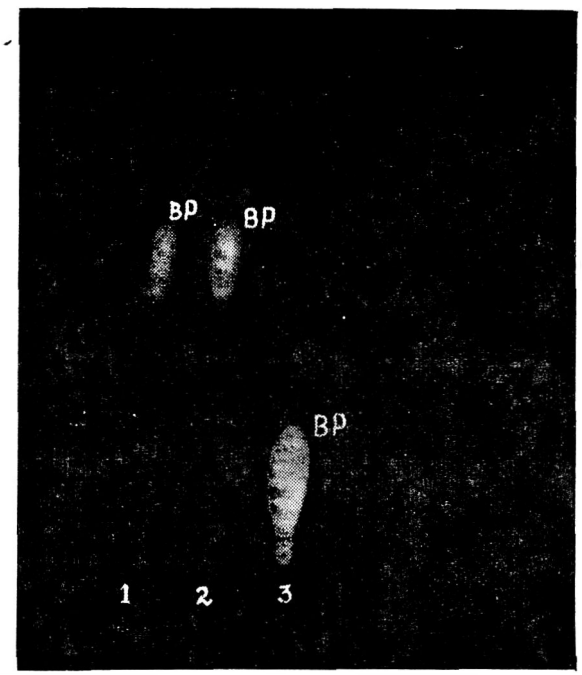

FIG 2. Thin layer chromatograms of chloroform extracts o` 1, monkey's urine, 2, tapioca 3, standard aflatoxin B_1; BP = Bluish purple. Quantities spotted are arb itrary (about 30 μl out of 1 ml will suffice) .

seen that aflatoxin moved only a little, while the non-specific compounds have moved far away from aflatoxin. Thus a preliminary ether run helps to distinguish non-specific fluorescent compounds.

The behaviour of other fluorescent compounds such as those found in the urine is a little different. They move up slightly on the TLC with diethyl ether, run and occupy the same position as B_1, when the plate is developed with 5 per cent methanol in chloroform. This may lead to erroneous conclusion. A second test is therefore necessary to spot out such non-specifics compounds.

The second test is based on the property of aflatoxin B_1 decomposing into two compounds on exposure to UV light having different mobilities on the chromatogram. This can serve as a useful distinguishing test for aflatoxin B_1.

Figure 3 is of the chromatogram exposed to UV light prior to methanol: chloroform solvent run which shows two spots originated from the initial spot of the standard. On the other hand, non-specific compounds give single spots showing thereby that standard toxin has decomposed to two spots while the other fluorescent compounds have not.

The two tests, namely: (i) preliminary development of the chromatograph with ether, and (ii) exposure of the spots to ultra-violet light prior to development with the methanol-chloroform solvent can be adopted

FIG 3. Thin layer chromatogram of chloroform extracts of groundnut, monkey's urine, and tapioca.
1, 5 groundnut extract, 2, 6 monkey's urine extract 3, 7 tapioca extract; 4, 8 standard aflatoxin B_1; BP= Bluish purple; arrow indicates the breakdown product of aflatoxin B_1.
Plate first developed with diethyl ether. Then 5, 6, 7, 8 covered with plastic sheet pasted with black paper without disturbing the spots. 1, 2, 3, 4 exposed to UV for 15 minutes. Plate redeveloped in 5% methanol in chloroform.

as tests complementary to each other for distinguishing aflatoxin B_1 from other non-specific fluorescent compounds. The integrated procedure worked out for adoption in routine analysis is as follows:

The chloroform extracts of the unknown samples are spotted in one half of the plate with standard toxin spot. This sequence is duplicated in the second half of the plate. The full plate is first developed with dry ethyl ether. Covering the second half with the plastic sheet pasted with black paper (taking care that the spots are not disturbed), the spots in the first half are observed under UV light. Evidence of any spot moving out of the origin above 0.1 Rf, suggests that it is a non-specific compound. This sample is marked. Exposure to UV light of this half of the plate is continued for 10-15 min. Then the full plate is developed in 5 per cent methanol in chloroform. The full plate is now observed under UV light and the bluish purple spots in the two halves are marked and compared with each other.

Evidence of photodecomposition of the sample spot similar to that of the standard (extra spot in the UV exposed portion which is not present in the covered half) is a clear indication of aflatoxin B_1 in the sample.

References

1. Broadbent, J. H., Cornelius, J. A. and Shone, E., *Analyst, Lond.*, 1963, **88**, 214.
2. Coomes, T. J., Crowther, P. C., Francis, B. J. and Shone, E., *Analyst, Lond.*, 1964, **89**, 436.
3. Pons, W. A. and Goldblatt, L. A., *J. Am. Oil Chem. Soc.*, 1965, **42**, 472.
4. Nabney, J. and Nesbitt, B. F., *Analyst, Lond.*, 1965, **90**, 155.
5. Mayura, K. and Sreenivasa Murthy, V., *J. Ass. off. agric. Chem.*, 1969, **52**, 77.
6. Andrellos, P. J. and Reid, G. R., *J. Ass. off. agric. Chem.*, 1964, **47**, 801.
7. Pohland, A. E., Yin, L. and Dantzman, J. G., *J. Ass. off. agric. Chem.*, 1970, **53** 101.
8. Jones, B. D., *Methods of Aflatoxin Analysis.*, Tropical Product Institute, 1972, G-70, 17.
9. Adrellos, P. J., Beckwith, A. C. and Eppley, R. M., *J. Ass. off. agric. Chem.*, 1967, **50**, 346.

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PARTITION OF AFLATOXIN DURING SEPARATION OF DIFFERENT CONSTITUENTS OF GROUNDNUT KERNEL

During oil separation from the ground· nels in an expeller or a hydraulic press, about 85-90 per cent of aflatoxin present in groundnut kernels goes with cake and 10-15 per cent with the oil.

Groundnut, as a major oilseed crop in the country, has received the utmost attention for maximum extraction and utilisation of the oil and its meal. With the discovery of aflatoxin as a common fungal toxic metabolite occurring in groundnuts, safety of their consumption is often questioned. There are several reports on the aflatoxin content of expeller pressed cake[1] and oil[2], but there is no information on the pattern of partitioning of the initial toxin among the constituents of the kernel during their separation. This is an important information which will help in predicting the aflatoxin concentrations in oil and cake that are obtained from a batch and which will suggest the necessity of any measure to be adopted to produce low toxin oil and cake. This communication presents results on the partition of aflatonin oil and cake from kernels pressed in an oil mill as well as in the Carver Laboratory Hydraulic Press (Summit,New Jersey, U.S.A.) under controlled conditions.

Representative samples of kernel and cake were collected in 6-7 replicates from batches of 6 ton kernel, following the bulk-sampling method described by Jones[3] and analysed for aflatoxin content. The oil samples were drawn from the tank in triplicates where the oil corresponding to a particular batch was collected, after thorough mixing. The average values of each batch are presented in Table 1. Laboratory trials were conducted by pressing 500 g of the kernel n a Carver Laboratory Hydraulic Press and determining the quantitative yield of the oil and cake. Representative sample of kernels (500 g) was powdered separately for aflatoxin analysis of whole kernels. Triplicate samples were taken in each batch. These were analysed for aflatoxin and average values for each batch are presented in Table 2. Laboratory sampling and estimation of aflatoxin content of kernel, cake and oil were performed by Pons method[4].

In the experiments with hydraulic press, accurate record of yields of cake (63 per cent) and oil (36 per cent) could be obtained. But in trials carried out in the

TABLE 1. PARTITION OF AFLATOXIN BETWEEN OIL AND CAKE OBTAINED FROM KERNELS PRESSED IN AN OIL MILL

Product		Total aflatoxin B_1 (g) Batch A	Batch B	Batch C
Kernel	6 tons ..	1.26	0.72	0.90
Cake	3.36 tons ..	1.08 (86)	0.64 (89)	0.76 (85)
Oil	2.52 tons ..	0.19 (15)	0.12 (16)	0.15 (16)

Upto 2% losses encountered on kernel weight basis.
About 93% of oil is extracted from kernel in the mills and is filtered through hydraulic filter press.
Figures in the parenthesis are percentage values.

TABLE 2. AFLATOXIN PARTITION BETWEEN OIL AND CAKE OBTAINED FROM KERNELS IN A CARVER LABORATORY HYDRAULIC PRESS

Product		Total aflatoxin B_1 (mg) Batch I	Batch II	Batch III
Kernel	500 g ..	0.20	0.035	46.5
Cake	315 g ..	0.18 (90)	0.031 (88.5)	41.8 (90)
Oil	180 g ..	0.025 (12)	0.005 (14.5)	6.5 (14)

Upto 1% loss encountered on kernel weight basis.
About 85% of oil is extracted by the Carver Laboratory Hydraulic Press.
ures in the parenthesis are percentage values.

mill, the yields of cake and oil could not be recorded accurately. The total toxin values tabulated for these fractions were based on the average yields of different fractions over a period of time. This was found to be 56 per cent for the meal and 42 per cent for the oil. Toxin input and total toxin yields in different fractions were obtained as a product of the toxin concentration per unit weight and total quantities of each item.

It is seen from the results that during oil separation in an expeller, bulk of the toxin present in the kernel will be in the cake. The percentage of toxin in cake samples collected in mills as well as in the laboratory press ranges from 85–90 of the initial toxin. The values for protein, whey and residue from cake during isolation of protein have been reported elsewhere[5]. Since the pattern of partition of the toxin in the samples collected in the mills as well as from the laboratory press is almost identical, it may be said that 10–15 per cent of the initial toxin of the kernel goes with the oil, while the rest remains with the cake. The uniformity of its occurrence in oil may be due to the limits of its solubility in oil.

References

1. Gibson, W. W. C., *Vet. Rec.*, 1962, **74**, 99.
2. Dwarakanath, C. T., Sreenivasa Murthy, V. and Parpia, H. A. B., *J. Fd Sci. Technol.*, 1969, **6**, 1.
3. Jones, B. D., *Methods of Aflatoxin Analysis*, Tropical Products Institute, Foreign and Commonwealth Office, London, 1972, 49.
4. Pons, W. A., Jr., Cuculla, A. F., Lee, L. S., Robertson, J. A., Franz, A. O. and Goldblatt, L. A., *J. Ass. off. anal. Chem.*, 1966, **49**, 554.
5. Basappa, S. C., Shankar Murti, A., Sreenivasa Murthy, V. and Parpia, H. A. B., *Indian J. Technol.*, 1972, **10**, 395.

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A NOTE ON NEW CHROMOGENIC TLC SPRAY REAGENTS FOR THE DETECTION OF SOME ORGANO CHLORINE PESTICIDES

A number of chromogenic reagents were screened for the qualitative detection of organochlorine insecticides in defence service rations. Silvernitrate—Bromophenol blue and morin solution (3, 5, 7, 3, 4′—pentahydroxy flavone) were found to be effective and useful. A semi quantitative estimation of insecticides is suggested based on the observed linear relationship between the log wt and square root of the area of the coloured spot on thin layer chromatography.

In the course of preliminary feasibility studies on the analytical survey of pesticide residues[1-6] in service rations, two chromogenic TLC spray reagents were evolved for the qualitative detection of organochlorin pesticides of Defence interest.

The following pesticide standards were used: p, p′—DDT (99.61±0.002 per cent pure; γ-BHC (99.89±0.037 per cent pure; p, p′—TDE (DDD) (99.27 per cent pure; α-Endosulfan (99.8 per cent pure; β-Endosulfan (99.8 per cent pure; call from National Physical Laboratory England); Aldrin (92.5 per cent pure; Shell Research Ltd., England); Endrin (95.0 per cent pure; Shell Research Ltd., England); Methoxychlor (Analytical Standard, El Dupont Nemours & Co., USA); Dieldrin (Technical; NOCIL, Bombay) and Heptachlor (Technical; Krishi Rasayan, Calcutta).

Analytical reagent grade solvents were used for dissolving the pesticide standards (bene ene for p, p' DDT, p'p'-TDE, γ-BHC, α- and β-endosulfan and heptachlor; chloroform for endrin; acetone for aldrin and methoxychlor). Pure analytical grade and redistilled hexane,

TABLE 1. COLOUR DEVELOPED BY NEW CHROMOGENIC SPRAY REAGENTS ON TLC PLATES CONTAINING ORGANOCHLORINE PESTICIDES

Spray reagent	Pesticides	Colour developed on TLC plate: Day light	U.V.light	Rf×100
$AgNO_3$—bromophenol blue[1] ..	Aldrin	Bl gr	No spot	98
	Dieldrin	Yl gr	,,	93
	Endrin	Yl gr	,,	94
	γ-BHC	Colourless	,,	—
	pp′—DDT	Bl gr	,,	95
$AgNO_3$—bromophenol blue[2] ..	Aldrin	Bl gr	,,	98
	Dieldrin	Yl gr	,,	45
	Endrin	Yl gr	,,	60
	γ-BHC	Light Yl gr	,,	65
	pp′—DDT	Bl gr	,,	95
$AgNO_3$—bromophenol blue[3] ..	Aldrin	Bl gr	,,	95
	Dieldrin	Yl gr	,,	10
	Endrin	Yl gr	,,	22
	pp′—DDT	Bl gr	,,	85
	Heptachlor	Gr bl	,,	90
$AgNO_3$—bromophenol blue[3] ..	pp′—DDT	Bl gr	,,	83
	pp′—TDE	Bl gr	,,	55
	γ—BHC	Yl gr	,,	33
	α—Endosulfan	Gr	,,	22
	β—Endosulfan	Gr	,,	5
Morin[3] ..	Aldrin	No spot	Yl	86
	Dieldrin	,,	Bright Yl	13
	Endrin	,,	Bright Yl	20
	γ-BHC	,,	Dull Yl*	38
	pp′—DDT	,,	,,	75
	Methoxychlor	,,	,,	8
	Heptachlor	,,	,,	75
	Aldrin	,,	Yl	90
	Dieldrin	,,	Bright Yl	15
	Endrin	,,	Bright Yl	22
	γ—BHC	,,	Dull Yl*	40
	pp′—DDT	,,	,,	80
	Methoxychlor	,,	,,	10
	α—Endosulfan	,,	,,	22
	Heptachlor	,,	,,	75
	β—Endosulfan	,,	,,	5

Bl: Blue; Gr: Green;

1, Solvent: Hexane saturated methanol; 2, Solvent: Cyclohexane: Chloroform (80:20); 3, Solvent: Petroleum ether: CCl_4 (50:50). TLC glass plates 20×20 cm: Silicagel G, 0.3 mm; length of run 10 cm.

The background under $AgNO_3$—bromophenol for different solvents was violet in day light and pinkish violet in UV.

The background for Morin was light yellow in day light; pinkish green in UV light. *Violet after 2 min.

acetone, petroleum-ether (60-80°C), carbon tetrachloride, cyclohexane and chloroform were used for thin layer chromatography.

Five thin layer chromatographic plates (20×20 cm) were coated with silica gel G slurry in water (8 g in 16 ml) in an indigenous Camag type applicator (Acme Products, Bombay) and the 0.3 mm thick layer was dried for one hour at 110°C. Five to seven organochlorine pesticide standards, listed above (10 μg in 10 μl solvent), were spotted on each TLC plate individually and as mixture in some cases. Ascending type TLC was carried out in a glass chamber containing the solvent system, cyclohexane-chloroform (80:23) or petroleum ether carbon tetrachloride 50:50) after presaturation of the chamber with the respective solvent system for ten minutes. The time taken for the solvent front to advance 10 cm was about 50 min. The TLC plates

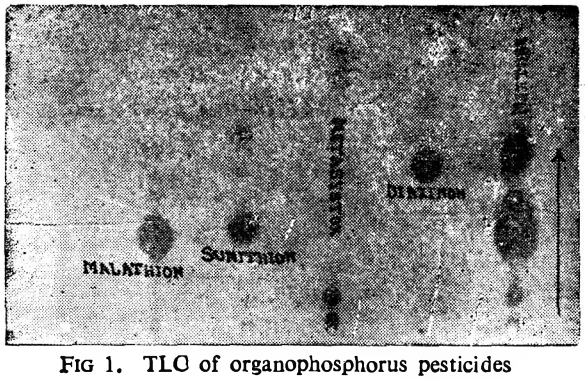

FIG 1. TLC of organophosphorus pesticides

were taken out and individually sprayed with one of the following chromogenic spray reagents and the colour developed by the pesticide spots was recorded (Table 1) both in daylight and after irradiation with ultraviolet light: Morin (3, 5, 7, 3′, 4′-pentahydroxy flavone) in ethanol (0.1 per cent), bromophenol blue (0.2 g) in acetone (50 ml) mixed with aqueous silver nitrate solution (2.0 per cent); other chromogenic spray reagents like bromothymol blue, p-dimethyl aminobenzaldehyde, diphenyl carbazide, dithizon and rhodamine B were also used but these were not as effective as morin or silver nitrate — bromophenol blue in the detection of these pesticides.

It is observed from Table 1 that on spraying with silver nitrate—bromophenol blue reagent, the organochlorine pesticides could be differentiated based on their Rf values and by the green spots of varying intensity. Similarly the organochlorine pesticides studied could be distinguished, with morin reagent and under UV light.

It was also observed that spraying with mild alkali or acid converts the yellow colour, developed by morin, into violet and enhances the intensity of the greenish colour developed by silver nitrate-bromophenol blue in day light. Exposure to bromine vapours (1 min) led to fading away of the colour of the spots.

Besides it was also observed that there is a progressive increase in spot area (40--83 sq. mm) with increase in concentration of the pesticide (aldrin; 5 to 40 μg) with a parabolic relationship between log wt and area and linear relationship between log wt and square root of area; this was observed using either morin or silver nitrate bromophenol blue as the spray reagent. This indicates their potential utility for semi-quantitative estimation of these pesticides.

The authors are thankful to EI Dupont nemours U.S.A. and Shell Research, U.K., for the supply of free pesticide samples. The authors are grateful to Dr P. K. Ramachandran, Director, for his keen interest.

References

1. Mukherjee, G., Mathew, T. V., Mukherjee, A. K. and Mitra, S. N., *J. Fd Sci. Technol.*, 1971, **8**, 152.
2. Das, D. K., Mathew, T. V. and Mitra, S. N., *Res. Ind.*, 1970, **15**, 108.
3. Stahl, E., *Thin Layer Chromatography*, Springer Verlag, Berlin, 1965, 363.
4. Bobbitt, J. M., *Thin Layer Chromatography*, Reinhold, Lond., 1963, 151.
5. Beynon, K. I. and Elgar, K. E., *Analyst*, 1966, **91**, 143.
6. Stolman, A., *Progress in Chemical Toxicology*, Academic Press, New York, 1963, Vol. II, 321.

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BOOK REVIEWS

Food Analysis: Theory and Practice, by Yeshajahu Pomeranz and Clifton E. Mcloan, AVI Publishing Company, Inc. West Port, Connecticut, 1971, pp. viii+669.

Inspite of the rapid additions during the last decade to the already voluminous literature on methods of food analysis, relatively few books cater to the requirement of undergraduates and researchers particularly interested in the theoretical principles involved in the methodology. The present volume meets this long felt need to a great extent. In addition to explaining, the background and principles of analytical methods it gives a comprehensive account of theories required for clear understanding of the physico-chemical aspects of instrumental assay.

The book has been divided into three sections. First section having four chapters deals with methods of reviewing literature, representative sampling, preparation of samples, reliability of procedures and presentation of results. For the benefit of the student and the new researcher included in chapter one is a very exhaustive and impressive list of reference books and periodicals covering the complete ambit of food science research reportage.

Section two, to which bulk of the book is devoted has 27 chapters covering details of methods and instrumentation for measurement of spectra in different regions, colour identification, fluorescence and phosphorescence, flame photometry and atomic absorption, X-rays, potentiometry, coulometry, conductivity and polarography and mass spectroscopy besides column, paper, thin layer and gas liquid chromatography and tracer technique. This section also gives salient features of various extraction techniques, centrifugal separations, densimetry, and rheology. The last three chapters in this section deal with serology, immunochemical and immuno electrophoretic methods and techniques of enzymatic and microbiological assay. The physico-chemical principles of instrumentation have been explained and discussed in a very lucid manner. The concepts of rheology and food colour have also been brought out extremely well.

In a compilation of this nature the authors always face the problem of selection of methods and techniques for inclusion from the vast amount of literature available on food analysis. The different types of methodology as given in the book are a very fair representative of the wide spectrum of tools available to the modern analytical food chemist. To that extent the authors have done a commendable job.

Seven chapters in section three describe the application of the analytical methods and determination of important chemical components of foods such as carbohydrates, lipids, nitrogenous compounds, ash, etc., is also their objective and sensory evaluation.

The authors have covered practically all aspects of analytical procedures, principles involved and their application to food analysis. The book is eminently suited as a text for students specialising in food science and technology and extremely educative for research workers since the theoretical aspects of food analysis along with their usefulness, and limitations, have been stressed and dealt out in more detail, rather than the methodology. For a more inquisitive worker authors have added an exhaustive light of bibliography at the end of each chapter. The book deserves a wide readership and deep study by students and research workers alike.

T. R. SHARMA

The Proceedings of the Symposium on Progress and Problems in Pesticide Residue Analysis: edited by Bindra, O. S. and Klara, R. L., Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab, p. 36.

The Book covers the papers presented at the Symposium held at the Punjab Agricultural University, on 18–19th December 1971. The papers have been divided under Sessions: Present Situation of Pesticide Residues in Food, Feed and Soil; Problems in Pesticide Residue Analysis; Techniques, Equipment and Chemicals; Development of Pestice Residue Tolerance. The Plenary Session made 19 useful recommendations.

The main proceedings of the Symposium is aptly preceded by a review article on the work done in India on pesticide residues by the Editors. In general the theme of the Symposium has been well covered by the papers presented at the Symposium and included in the proceedings. Within 62 contributions, wide areas have been covered. Under the Progress and Problems in Pesticide Residue Analysis attention has been focussed on the future need in the field. Critical reviews have been made on the aspects of the present status of residues in food, feed and soil and the pro-

blems related to its monitoring, detection, isolation-estimation and characterization, toxicological and pharmacological problems associated with the residue and the need of regulatory analysis have been emphasised.

A scaning over the titles will show that due attention is being given by research workers on the selective protectants against general wide spectrum pesticides of high mammalian toxicity; monitoring of residues in soil, feed, food and water; critical discussion on chemical, physical and biological assay methods; persistence of residues in treated soil and crops; metabolism of pesticides in man, fish and poultry and legal tolerances.

In addition to the 336 pages of running matter printed in the book, Authors Index has been supplemented. It would have been worthwhile to include a Subject Index because of the importance and topical nature of the subjects covered under the Proceedings. The Errata is fairly large, but nevertheless the Editors will have to be complemented for bringing out the Errata along with the publication of the book.

This particular book will serve complementary to the previous publication entitled 'Pesticides' of the Academy of Pest Control Sciences, Mysore, which included the Proceedings of the first Symposium in the field. This particular volume comprising of the Proceedings of the Symposium in Ludhiana, will be a valuable addition to the Libraries of the Agricultural Universities, Research Institutions, Technological Institutions, Departmental Libraries of the Ministries and also Academic Institutions.

S. K. MAJUMDER

Freezing and Storage of Fish, Poultry and Meat: International Institute of Refrigeration, 177, Boulevar, Malsherbes, 75017, Paris, pp. 385.

The book includes the papers presented before two Scientific and Technical Commissions of International Institute of Refrigeration. The deliberations of one Commission dealt with application of refrigeration to preservation of agricultural and food products and behaviour of these during storage. The deliberations of the other Commission consisted of technical characteristics of freezing and storing installations.

The presentations of fish covered freezing technique (plate, immersion and liquid nitrogen), glazing, frozen storage, frozen storage changes, stabilizing frozen storage by additives like spices and nitrous oxide, histological changes and comparative aspects of shelf-life of thawed *vs.* fresh fish. Papers on poultry ranged from ageing, freezing, microbiology, alterations in structure, comparison of freezing in calcium chloride, brine and blast freezing, quality of frozen whole eggs. The section on meat covered meat surface emissivity in radiation chilling, characterisation of ice crystals formed during different freezing conditions like immersion in liquid nitrogen, dry ice and a quick freezer at —30°C and still air at —18°C, water retaining capacity as affected by temperature and rate of freezing, change in sarcoplasmic proteins on long term frozen storage bacteriological infection in chilled beef and distinguishing thawed meat from chilled/fresh by oxalic-acetic transaminase of glutamic acid and by electrical conductance measurements. The miscellaneous section developments on freezing in liquid carbon dioxide, glazing of frozen foods, time-temperature tolerances of foods, direct freezing of meat as against two stage freezing, vacuum heat thawing of frozen foods, liquid nitrogen, freezing of pancreas for preparation of insulin, mathematical formulae and graphic calculations for determination of temperature of product during freezing or thawing and economics of chilling/freezing.

The compilation contains a large amount of valuable data of interest to research workers interested in chilling, freezing and storage of meat, fish and poultry.

B. R. BALIGA

The Carriage of Refrigerated Cargoes: International Institute of Refrigeration, 177,Boulevard,Malsherbse, 75017, Paris, 1973, pp. 173.

This Manual, brought out by the International Institute of Refrigeration presents useful information on the refrigerated marine transport of food in cargoes.

The subject matter is covered in 4 chapters devoted to the General Principles of Refrigeration Equipment, Operation and Maintenance, Transport Requirement of Horticultural Products, Transport Requirement of Animal Products and Ship's Store Maintenance.

Chapter I on Basic Principles includes the effect of process parameters such as compressor suction pressure discharge pressure, compressor speed, defrosting method on the performance of refrigeration plants. Chapter II covers General Storage Condition of Horticultural Products, Fruits and Vegetables. Data on carbon-, dioxide produced, heat of respiration, freezing damages

packaging needs are given individually for fruits such as apple, pears, grapes, peach and citrus fruits, tropical fruits such as banana and pineapple, vegetables like tomato melon, potato, onion, etc. and nursery stock like flowers, trees, shrubs, etc. Chapter III discusses various aspects of Animal and Poultry Products like growth of microorganism, cleanliness, temperature control, mass loss and atmosphere control. Problem of frozen packaged meat products are also indicated

Though intended as a guide for the Engineers concerned with the care and maintenance of cargoes for refrigerated transport of food, the Manual has clearly brought out all aspects of refrigerated storage and transport of foods in a small handy volume. It will be of interest and benefit to those engaged in all fields of food storage and transport. Like other publications of I.I.R. the get up and binding of the Manual are good. It will be an useful addition to the Libraries of all food technological laboratories.

P. N. SRINIVASA RAO

Food Composition Table for Use in East Asia: U.S. Department of Health, Education and Welfare, and Food and Agricultural Organisation of the United Nations, Rome, Italy, 1972, pp. 334.

This publication is the most comprehensive and up-to-date of the Asian food composition tables published so for. It consists of two parts: Data on proximate composition, calcium, phosphorus, iron, sodium, potassium, retinol, beta-carotene equivalent of retinol, thiamine, riboflavin, niacin, ascorbic acid and percentage of inedible portion in foods are presented in Part I, the available data on amino acids, fatty acids, other B-vitamins and trace elements are presented in Part II. Areas from which data on food composition were obtained for the preparation of the food table are: Burma, Thailand, Cambodia, Laos, Vietnam, Mainland China, Malaysia, Singapore, Indonesia, Philippines, Hong Kong, Taiwan, Korea and Japan.

This publication is the outcome of a joint research project for compilation of a comprehensive food table for use in East Asia, sponsored by the U.S. Department of Health, Education and Welfare and Food and Agriculture Organisation of the United Nations. Documents of this kind are valuable for evaluation of local food consumption and for planning nutritional improvements and optimum utilization of local food resources by national as well as international agencies.

S. VENKAT RAO

Soft Drink Manufacture, by Gillies, M. T., Noyes Data Corporation, Mill Road at Grand Avenue, Park Ridge, N. J. 07656, 1973, pp. 336.

PThe book under review is a new addition to a series on Food Technological subjects periodically published by the Noyes Data Corporation. It is an assembly of technical information on newer inventions or innovations pertaining to soft drinks, culled from recent (about 1957 and later) U.S. patent literature. The book is organised into eight sections covering all the significant aspects of soft drinks industry.

Section I gives a detailed coverage of the apparatus for carbonation of beverages, involving the use of solid and liquid carbon dioxide as well as the electro-magnetic and supersonic energy. Improved dispensing machines for the carbonated and flavoured beverages, for the factory and for the home are also described.

Section II on 'Carbonated Beverages' covers in considerable detail (*via* U.S. patents) the various facets of the tricky technology of dry carbonated beverages. The problem of preventing the carbon dioxide precursors from interacting till the 'Beverage Powder' is dissolved in water appears to have been successfully overcome by improved coating and packaging technologies. Of particular interest is a novel technique of using-certain organic carbonatinig agents (p. 73) like bis-O-carboxy tartaric or gluotaric anhydride which are hydrolysed by water with concomittant release of CO_2 for carbonation and tartaric or glutaric acid for acidulation of the soft drink. Other aspects deajt with include concentrated beverage base, improvements in foam and carbon dioxide retention and the use of certain special yeasts for carbonation of non-alcoholic carbonated beverages from fruits.

Section III relates to the Acidulants for Citrus Drinks and the various newer techniques which enable some of the unconventional and less soluble acids like fumaric and adipic to be used wih advantage. It is however, not certain if some of the newer acidulants like phenyl phosphonic acid have got clearance from FDA and other agencies.

The next two sections deal with additives for and production of citrus drinks and should intrigue and fascinate both the food scientist and the manufacturer. The use of ammoniated glycyrrhizin as well as of maltol as synergestic sweetening agents should be of some interest in view of the rising cost of sucrose (in this country) and of the restriction and ban on saccharin and cylamates respectively. Selectively and partialyl

oxidised starch hydrolystate described in a patent, to provide both the sweetener and the acidulant (gluconic acid) is interesting. The technologies for utilising carotenoids as well as certain flavylium salts for colouring soft drinks are worth noting at a time when the general trend is to favour natural colours over synthetic coal tar colours. The clouding agents, alternative to the now restricted B.V.O. (brominated vegetable oils) based on natural products (citrus peels and rags, p 166; albedo, p 171; citrus seeds, p 175, glyceryl abietate, p 187) would certainly be welcomed by soft drinks manufacturers. Polyol benzoates (*e.g.*, glyceryl tribenzoate, p. 177) may also be regarded as a clouding agent of essentially natural origin. The elimination of "ringing" by use of Plyronic F-127 (p 211) is another approach to solve the same problem. Various pulping agents such as carrageenans, algin, karaya and locust bean gums, and regenerated collagen are also covered in concerned subsections.

Subsections on flavoring compositions and flavor enhancers are of special importance from the consumer acceptability standpoint. Some interesting aspects dealt with include the use of orange oil "terpenes" in appropriate stabilized form, solid particulate orange flavor (oil dispersed in dextrin matrix), lemon-sweet orange oil combination for synthetic lemon flavor in tablet or emulsion form, use of acetals or mannitol-encapsulated acetaldehyde as acetaldehyde source for an enhancing effect in certain fruit flavor—based beverages, carmel-flavor oil-water dispersions for root beer and cola type beverages, Pluronic-F-127 *plus* gum carriers for terpene containing dehydrated flavors and a synthetic Mandarin flavor based on hymol-N-methyl methyl anthranilate formulation.

Section V of 'Citrus Drink Production' lays emphasis on aspects of mechanisation in citrus drinks manufacture and in addition describes processes for soluble citrus fruit powder, an orange base, etc.

Section VI on 'Slush Drinks'—both carbonated and non-carbonated semi-frozen types containing finely divided 'soft' ice is mostly concerned with mechanical devices for their production including those inpackaged form for the home refrigerator.

Points of interest in Section VII on 'Miscellaneous Beverages' include stabilized grape drink using fumaric or adipic acids to minimise the browning reaction between methyl anthranilate and reducing sugars, use of certain additives like hydrofurans, fatty acids, ester, lactones, aldehydes and also maltol for strawberry flavor formulations, unusual comibinations like fruit-juice *plus* milk and the technology of stabilizing them citrus-licorice combinations, the interesting aqueous extract from soya bean flake having an acid composition similar to natural orange juice and its conversion to an inexpensive orange drink and maple sap drink.

The final section 'Miscellaneous Packaging' contains some fancy items like beverage in a straw and other dispensable articles like paper cups and spoons, suitably coated with the dry beverage mix and ready to use with plain or carbonated water.

The purpose of the publication set out in the foreword, *viz.*, to serve as a source of detailed information of Soft Drinks and as a guide to US patent literature on this subject, has been broadly fulfilled. By exposing the reader to the numerous technical possibilities, the book helps in generating new ideas for R & D. One feels that a section on treatment of water the major ingredient of any soft drink as well as on quality control would have added to the usefulness of the book. Following the customary NDC pattern, the table of contents itself serves as a subject index. The patent, inventor and company name indexes provided would be of help in locating the desired information.

The book is relatively free from errors. A few minor ones which came to the notice of the reviewer are listed below: p 163 1.1 filter for fil*l*er; p 76 last line, glycine for N-Carboxy glyciner; p 188, 1.6 from bottom 0.34 for 0.84; p 200, 1.25 ether for other; p 207, 1.2 vinylidence for vinylidene; p 269, 1.10 from bottom acidul*e*nt; p 274, 1.11 from bottom from for form.

In an area in which books are sparse and information *via* journals is sparingly little, the appearance of this publication is opportune and highly welcome. It will be a very useful addition to the libraries of research and educational institutions dealing with food science and technology as well as to those of manufacturers of soft drinks or ingredients thereof.

N. P. DAMODARAN

Root Crops by Kay, D.E., Tropical Products Institute, London, England, 1973, pp. 240+xxxv; Price £ 1.50.

The digest under review is the second in the "Crops and Product Digest Series." Information on 41 root crops is presented, each crop is dealt under 20 standard headings which include scientific and common names, form and habitate, agricultural aspects including culti-

vation conditions, planting methods, pests and diseases, harvesting and yield, uses of main, subsidiary and by products, chemical and nutritional aspects, processing and lastly production and trade.

Root crops with their high carbohydrate yield per unit area and ability to survive and grow in very marginal lands made them the most popular crops in areas where there is great pressure on agricultural land due to increasing population. It will not be out of place if we trace the origin of agriculture itself to the cultivation of these root or tuber crops.

The tuber crops have got extensive applications ; some are valued much as staple food crops. Most of them find place as vegetables, a few contain valuable nutrients, and majority of them provide only starch with very insignificant quantity of nutrients, and quite a few of them are valued for their medicinal qualities. The byproducts obtained during processing and the waste products have been utilized as feed, if not as food.

The exhaustive coverage of information from diverse sources especially about the minor and unfamiliar root crops which are very much localised in cultivation will serve as a useful guide to those interested in introducing these tubers in newer areas having congeneal climate for their establishment. The publication will also help to stabilise their cultivation in areas where a declining tendency in their production is observed during these years due to increasing cost of cultivation ; information given on the future course of action to be pursued to make a particular crop more paying for the farmer will be of great use in this connection. However, inclusion of diagrams or sketches of these tubers under appropriate heads would have definitely enhanced the usefulness of the publication by closely acquanting the reader with some of the localised, unfamiliar, minor crops.

The headings selcted although cover all aspects about each crop, should have been more flexible ; storage and distribution of crop deserve separate heads instead of giving along with harvesting and cultivation conditions respectively. Confusion sometimes prevails when one reads through the uses of the crop especially in distinguishing the main and subsidiary uses of the crop. As in p 52 extraction of starch, tuber flour and alcohol are included under secondary and waste products (although they are obtained from the main crop—tuber, and not a byproduct) and contrarily similar products (*e.g.*, tapioca starch and cassava flour) are included under subsidiary use in p 30. Quite often one encounters similar situation in other crops too.

The publication needs some corrections in quite a few cases such as in the conversion from inches to centimeters as in p 16, $37\frac{1}{2}$—60 in (*150—200* cm), p 52, 0.4 in (*1—2* cm) ; p 57, 30—36 × 18 in (*60—90* × 45 cm); p 100, 18—24 in (*30—60* cm) ; p 140, 4 ft (*0.6* m) ; p 145, 30—50 in (*75—100* cm) ; p 217, 40 lb (*9* kg) ; p 240, 10—15 in (*25—30* cm) ; p 241, 4 × 4 in (*15* × *15* cm). Wrong use of scientific names such as *Areca cathecu*, *Trigonella foenum—graenum* and *Cyamaposis psoraloides* in p 62 and *I-arbinose* in p 174 and vitamin B 20 Y (?) per cent in p 54 also need correction.

It is strange that some root crops like sugar beet, carrot, chicory and turnip are not included when radish could find a place in the publication.

There is some discrepancy in the values reported for the nutritive flour from chaufa in p 53; the nutritive flour contains lower amount of protein (3.5 per cent) as compared to the tuber flour which has a much higher protein content of 4.4—6.3 per cent. In some countries tapioca form staple food; so much so much work has been done on the enrichment of flour and preparation of enriched macaroni ; a referene to these would not have been out of place in this context. These items definitely show the wide scope for improving the publication in the next print.

Although the major pests and diseases attacking the crops are discussed at length, it would have been more practical and appropriate if the control measure for these also included instead of giving for a few.

The publication will prove useful for both the agriculturists and technologists as it covers both these aspects and also to the persons interested in plant introduction.

K. A. RANGANATH

Minutes of the Meeting of the Bangalore Chapter of the Association held on 16 July 1974

On the request of the Secretary, Dr. Dastur was kind enough to take the Chair. Introducing Dr. T. N. Ramachandra Rao, Shri Chandrasekhara said that it gave him much pleasure to introduce one of his eminent colleagues who has done very good research work in microbiology. He is the Chairman of the Discipline of Microbiology and Sanitation at the Central Food Technological Research Institute, Mysore. He has been responsible for developing many products of industrial importance. Before joining the CFTRI, he was in-charge of the type collection of microorganisms at the National Chemical Laboratory, Poona. As President of the Association, he has been very active in furthering the interest of the Association. It was indeed a great honour to the Bangalore Chapter that Dr. Ramachandra Rao agreed to address the members.

Dr. Ramachandra Rao introducing his talk said that to overcome the protein malnutrition which is wide spread in the country, various suggestions have been put forth. One of them is to use the non-conventional microbial organisms. Bacteria has about 60 to 70 per ceut of protein on dry basis while the other organisms have slightly lower percentage of protein. The earlier work at MIT had resulted in the extraction of a low per cent of protein and since it was not economical, the work had been stopped. But later work has shown much better yields. Considerable work has already been done on the utilization of hydrogen as the substitute for organisms capable of photo synthetic activity. But in this there are problems like the explosive nature of hydrogen when it combines with oxygen. We do not have equipment to carry out research work of this type. Fungi has been suggested as food for live-stock and possibly for humans also. It is possible to grow fungi on a cheap carbohydrate source and improve its protein content. Then it could be used along with other food materials as extruded products. In Japan, soft drink based on algal extract (chlorella) is being sold as "Yakult." This is a lactic fermented drink and is supposed to be very health giving. Much work has been done on *Spirula* which was obtained from Africa. Plants with a capacity of 20 tonnes per day are now available for the manufacture of this. There are countries who are even willing to set up such plants in any developing country if they would like to have it. Yeast has been suggested as a human food for a very long time. Plants with large capacities for production of yeast are now working. In Japan though they were able to set up one such big plant the people were against the idea of using yeast as a food material. Hence the plans were given up. Petroleum yeast made considerable head way and both the German and the French have been leading in this field. The recent escalation of petroleum costs has to some extent dampened the research. The petroleum protein is of high quality but the draw-back in it is that the RNA content is high (8 to 12%). Recent work however has succeeded in removing a large quantity of neuclic acid by fractionation. The neuclic acid fraction is used for making flavouring agents. It is possible that yeast can play quite a big role in production of protein if only part of the molasses could be preserved for the production of yeast. It is also possible to grow yeast, which will have a high fat content. Basic work on this has been completed and is possible now to set up pilot unit. The fat in this yeast has nearly the same composition as vegetable fat. Even if it cannot be immediately used for edible purpose, it can be used for other industrial purposes, thus sparing other fats for edible purpose.

The talk was followed by a very interesting discussion. Dr. Dastur in his concluding remarks thanked Dr. Ramachandra Rao for the very interesting talk.

Seminars

A talk on "*APV Paraflow Plate Type Heat Exchanger*" was arranged by the Eastern Regional Branch of the Association in collaboration with Indian Institute of Chemical Engineers, Calcutta Regional Centre, on 17th August 1974 at Jadavpur University Campus, Calcutta-32. The talk was delivered by Mr. M. R. Bhattacharjee of the Chemical Engineering Project and Marketing Division, the APV Engineering Co. Ltd., Dum Dum.

The AFST, Mysore, in collaboration with CFTRI arranged a Seminar on "*Some Aspects of Sensory Evaluation—Training of New Panels.*" The Seminar was held on August 7th, 1974; the speaker was Mr. Paul Erner Andersen, Food & Nutrition Adviser, Danish International Development Agency (DANIDA), Ministry of Foreign Affairs, Copenhagen, Denmark.

Symposium on "Fish Processing Industry in India"

Considerable progress is being made in planning the above symposium which will take place in the second week of February 1975. An Organising Committee with Dr. B. L. Amla as Chairman has been formed to co-ordinate the various activities while another Committee with Dr. D. P. Sen as Chairman is tirelessly working in chalking out the plan of action. The Symposium is expected to attract a large number of delegates from India as well as abroad. Marine Products Export Development Authority, who is holding the Second Indian Seafood Trade Fair from 7th to 9th of February 1975, at Cochin, has promised full co-operation and help in the conduct of the symposium.

Proceedings of Seminar on Frozen Food Industry

The proceedings of the above Seminar held on 3rd to 4th March 1973 at Calcutta, has been published by the Eastern Regional Branch of the Association. The above publication contains the papers presented by various speakers during the Symposium which was divided into 3 Sessions. The Technical Session I dealing with Raw Materials has 4 papers presented by Messrs. K. Chidambaram, Amba Dan, M. Ghosh and N. B. Das Gupta. The Technology part was covered in Technical Session II. The speakers in this Session included Messrs. P. Chattopadhyaya, Harold Davidge and Drs. N. C. Ganguli, R. K. Rastogi, M. A. Krishnaswamy and M. N. Moorjani. The Marketing Aspects have been covered in Technical Session III. Messrs. R. L. Mitra, A. R. Sutaria, J. J. O'Connor, P. K. Reddy, M. Ghosh and J. K. Maheswari presented appropriate papers in this particular Session.

The above publication can be had from the Hon. General Secretary, Eastern Regional Branch, Food Technology and Biochemical Engineering Department, Jadavpur University, Calcutta-700032 at a price of Rs. 20 per copy for non-members. For members of the AFST, copies are available at a nominal price of Rs. 5.

Symposium on Adulteration in Foods

The Bangalore Chapter of the Association has decided to hold a symposium on Adulteration in Foods on 14th December 1974 at Bangalore. The Symposium will last for a day and there would be one Session which will deal with different aspects of food adulteration like Adulteration in Raw Materials, Adulteration in Processed Foods, Prevention and Standards, Adulteration as It affects Manufacturers, Retailers, Consumers and Recommendations and Conclusion. Persons interested in participating in the symposium may contact Mr. Charles A. Wesley, Convener, Symposium on Adulteration in Foods (Food Craft Institute), S. J. Polytechnic Building Campus, Seshadri Road, Bangalore-2.

General Body Meeting

The General Body Meeting of the Association is expected to be arranged at New Delhi under the auspices of Northern Zone. A Symposium on Fruit and Vegetable Processing is also being contemplated on the occasion of the General Body Meeting which is expected to take place in the second week of March 1975. Further particulars can be obtained from the Hon.General Secretary of Northern Zone of AFST.

New Members

Mr. H. G. Ramanandan,Nandu Chemical Industries, Mytri, Siddapur (N. K.) 581355, Karnataka State.

Mr. M. P. Sanat Kumar, Yeskay Corporation, Kalpetta P.O., S. Wynad, Kerala State.

Dr. P. P. Kurien, C.F.T.R.I., Mysore-570013.

Miss Thangamani, Defence Food Research Laboratory, Jyothinagar, Mysore-10.

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Mr. D. T. Pandya, 558, New High Way-1B, Hauppauge, N.Y. 11787.

Mr. Gauri Shankar, Department of Biochemistry and Food Technology, H.B.T.I., Kanpur-2, U.P.

Mr. Vimal Kumar Jain, Department of Biochemical Engineering and Food Technology, H.B.T.I., Kanpur-2, U.P.

Mr. Girdhar Datt Sharma, Modern Bakeries India Limited, Industrial Estate, Kanpur-12, U.P.

Mr. Rajendra Prasad, 3A/27, Azadnagar, Nawabganj, Kanpur, U.P.

Mr. M. S. Baniwal, Modern Bakeries India Limited, Industrial Area, Kanpur-12, U.P.

Mr. Abhinandan Kumar Jain, S/o Shri Sadhan Prashad Jain, P.O. & Town Nakur, District Saharanpur, U.P.

Mr. Rajeshwar Dayal Tyagi, W.Z. 63, Basai Dara Pur, New Delhi-15.

Mr. Vipin Kumar Gupta, 13/6, Shakti Nagar, Delhi-7.

Mr. Yog Raj Bhatla, 129/2, D.C.M., Railway Colony, Delhi-6.

Mr. K.P. Singh, 186-A, Chhipi Tank, Dr. Choudhary's Lane, Meerut City, U.P.

Mr. K. D. Singh, Vill. & P.O. Ramgarh Kiriyat, *via* Chunar, District Mizrapur, U.P.

Mr. Krishna Kumar, C/o Sri Vijai Kumar, Kirana Merchant, P.O. Ayodhya, Dist. Faizabad, U.P.

Mr. Rakesh Kumar Rastogi, S/o Shri Jagadish Saran Rastogi, Beni Madhev Kuncha, Farrukhabad.

Mr. Anoop Kumar, 124-B/689, Govind Nagar, Kanpur-6, U.P.

Mr. M. S. Ramakrishna, Defence Food Research Laboratory, Jyothinagar, Mysore-570010.

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Mr. Sudhir Bhalachandra Sule, Naarden (India) Limited, Post Box No. 8920, Saki Naka, Bombay-400 072.

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Mr. S. V. Dravid, Kissan Products Ltd., Old Madras Road, Bangalore-16.

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Mr. Bheemeswara Rao Talluru, CFTRI, Mysore-570013.

Jyoti Kumar Sarkar, 39B, Sadananda Road, Calcutta-700026.

Mr. K. S. Pillai, Trade Links Corporation, xxxi-489, T.D. Road, Ernakulam, Cochin-11, Kerala State.

Dr. Krishan Gopal Gupta, Microbiology Department, Punjab Agricultural University, Chandigarh, Punjab.

Mr. S. Javare Gowda RPEC, Indian Institute of Technology, Kharagpur, W.B.

Major M. S. Uban, 83, Road 2-b, Rajendra Nagar, Patna-800016.

Mr. M. Mahadevaswamy, CFTRI, Mysore-570013.

Mr. K. Premakumar, Canning & Food Preservation, Food Craft Institute, Vidyanagar, Hyderabad.

Mr. Kantilal N. Patel, North American Lab. Co. Inc., 1717, West 10th Street, Indianopolis, Indiana-46222, U.S.A.

Mr. M. Chandra Mowli, Export Inspection Agency, Karnataka Bank Building, III Floor, Kodialbail, Mangalore-575003.

Mr. A. George Varkey, CFTRI Experiment Station, Trichur, Kerala.

Mr. A. V. Bhat, CFTRI Experiment Station, Trichur, Kerala.

ERRATA

Hypocholesterolemic Effect of Sardine Oil and Oil-sardine (*Sardinella longiceps*) Fish, by D. P. Sen, C. S. Bhandary, Indira A. S. Murthy, S. Narasimha and M. P. Pai.

Vol. 11, No. 3, 1974, Page 115. Table 3, column 3, Line 3.

2.97 should be read as 2.07.

FISI IA

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Papers are invited l in Central Food Technological Researcl aper should have an abstract not exceeding . Secretary latest by 15*th November* 1974.] ers will be screened by an expert committe

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1. The abstract c uld consist of about 200 words. It paper.
2. The detailed p. ed) or 2,000 words.
3. All the units in
4. Either the tables or the figures only need be given. They should be numbered in arabic with their titles typed in capital letters.
5. The number of tables/figures should be severely restricted and as far as possible only summary tables should be given ; they should be typed in separate sheets of paper and attached at the end of each paper. Figures should be on tracing paper; photographs should be clear glossy prints with legends pasted on the back.
6. As far as possible please cite only review articles as references. References should be indicated in the text by their respective serial numbers given as super script. The pattern of reference citation should strictly be as follows :

Journal : N. B. Shankaracharya, S. Anandaraman & C. P. Natarajan, *J. Fd Sci. Technol.*, 1973, **10** (4), 179.

Book : R. M. Amerine, R. M. Pangborn and R. E. Roessler, *Principles of Sensory Evaluation of Foods.* Academic Press, New York, 1965, 48.

All communications may be addressed to :

Hon. Exec. Secretary,
AFST, CFTRI,
Mysore-570013.

INSTRUCTIONS TO CONTRIBUTORS

1. Manuscripts of papers should be typewritten in double space on one side of the paper only. They should be submitted in **triplicate.** The manuscripts should be complete and in final form, since no alterations or additions are allowed at the proof stage. The paper submitted should not have been published or communicated anywhere.

2. Short communications in the nature of letters to the editor should clearly indicate the scope of the investigation and the salient features of the results.

3. Names of chemical compounds and not their formulae should be used in the text. Superscript and subscripts should be legibly and carefully placed. Foot notes should be avoided as far as possible.

4. **Abstract:** The abstract should indicate the scope of the work and the principal findings of the paper. It should not normally exceed 200 words. It should be in such a form that abstracting periodicals can readily use it.

5. **Tables:** Graphs as well as tables, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. Nil results should be indicated and distinguished clearly from absence of data.

6. **Illustrations:** Line drawings should be made with *Indian ink* on white drawing paper preferably art paper. The lettering should be in pencil. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size. Photographs must be on glossy paper and contrasty; *two copies* should be sent.

7. Abbreviations of the titles of all scientific periodicals should strictly conform to those cited in the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.

8. **References:** Names of all the authors should be cited completely in each reference. Abbreviations, such as *et al.*, should be avoided.

   In the text, the references should be included at the end of the article in serial order.

   Citation of references in the list should be in the following manner:

   (*a*) *Research Paper:* Menon, G. and Das, R. P., J. sci. industr. Res., 1958, 18, 561.

   (*b*) *Book:* Venkataraman, K., The Chemistry of Synthetic Dyes, Academic Press, Inc., New York, 1952, Vol. II, 966.

   (*c*) *References to article in a book:* Joshi, S. V., in the Chemistry of Synthetic Dyes, by Venkataraman, K., Academic Press, Inc., New York, 1952, Vol. II, 966.

   (*d*) *Proceedings, Conferences and Symposia:* As in (*c*).

   (*e*) *Thesis:* Sathyanarayan, Y., Phytosociological Studies on the Calcicolous plants of Bombay, 1953, Ph.D. thesis, Bombay University.

   (*f*) *Unpublished Work:* Rao, G., unpublished, Central Food Technological Research Institute, Mysore, India.

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