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ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS

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A Review

Environmental Pollution with Pesticides

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Manuscript Received: 4 December 1974

INTRODUCTION

It is estimated that on worldwide basis about 20,000,000 tons of organic chemicals enter the environment annually. The solvents such as methanol, ethanol, alcohols, ethers, chlorinated solvents account for about half of the chemicals entering the environment, detergents amount to 1.5 m tons and organic pesticides to about 1 million tons[1]. A report of the U. S. Department of Agriculture has indicated that a complete stop in the use of pesticides might result in a 25 to 30 % drop in crop and livestock production. United Nations Food and Agriculture Organization plans to reduce food losses by pests to 20–30 % as part of a drive to achieve four-fold increase in food supplies[2]. The production of pesticidal chemicals in the U. S. amounted to 1050 million pounds during 1967 and total sales were valued at $ 787 million and more than 60,000 pesticide formulations based on over 500 individual compounds have been on the market for use. The pesticides used for plant protection in Japan amounted to about 150 million U. S. dollars, which was nearly 2.5 times as much as the amount consumed in 1950[3]. Substantial quantities are also used in the rest of the world, especially in Canada, Great Britain, USSR and Western Europe and the consumption of pesticides is spectacularly increasing in Asia, Africa and South America.

The production and use of pesticides in agriculture, public health and forestry results in contamination of soils, water, air and vegetation. Chemical pesticides are posing a great threat to environment. Yet the benefits derived from their use by way of increased food production and prevention of diseases represent an equally strong argument for their continued use.

Information available at present show that the pesticide contamination in water amounts to a few parts per billion [5-13]. On rare occasions higher concentrations of pesticides appear in Potable waters usually as a result of industrial pollution. Pesticides such as BHC, Toxaphene can impart undesirable taste and odour to water at levels 5-20 ppb[8]. Studies by Grazenda and Nicholson[14] have shown the pattern of distribution and magnitude of insecticide residues in a water shed and its adjacent cotton farms. The cotton field soils contained in ppm, Toxaphene (0.16–1.6), DDT (0.02–0.53), and BHC (0.01–0.38). The untreated water from the adjoining streams contained the following insecticides (in ppb): Toxaphene (0-176), BHC (0-256), DDT (0-9) and DDE (0-4). Residues of DDT and DDE in mud collected from the same basin ranged (in ppm): DDT (0.006–4.62), DDE (0.002–1.77). The bottom fauna in the same basin contained the following ranges of pesticides (in ppb): DDT (20–500), DDE (10–610), Toxaphene (0–1100). Fish collected from the same basin contained in their edible portion (in ppm) DDT (0.04–0.93), DDE (0.01–0.90), Toxaphene (0.1–1.6) and BHC (0.01–0.04). Nicholson and co-workers[15] investigated the run-off from a peach orchard into a farm pond. They found 1.9 ppm of parathion in the mud pond and 0.01-1.22 ppb in the water. Tarzwell and Henderson[16] estimated the dieldrin content of run-off water from an area treated with dieldrin at 4.66 lb/acre. The first run-off contained 0.13 ppm and the third 0.01 ppm. Heavy mortality in the Mississippi river in northern Alabama following heavy rains indicated that the toxic materials were washed from the pesticide treated soil to the stream. Metcalf[17] analyzed water samples from 100 locations in U.S. They ranged in the following order (ppb): Endrin, 0.000–0.094; Dieldrin, 0.000-0.118; DDT, 0.000-0.087; and DDE 0.000-0.018. Though the levels of pesticides are minute, it can add a significant quantity to the human body along with other sources such as food and air. A recent report states that the discharge of pesticide residues from the Central Valley into San Francisco Bay has been estimated at about 2 tons per year[18]. As shown by Westlake and Gunther[19], if one drinks two litres of water per day containing 10 ppb of pesticide, one ingests 0.02 mg per day. But the drink-

ing water in the U.S. contains 0-2 ppb and according to national quality network's report, the intake of pesticide from this source is less than 0.004 mg per day.

Soil: Soils are contaminated by either aerial spraying of crops or by applying pesticides directly. A survey of 35 orchards in southern Indiana, revealed that DDT residues being present more than 100 lb. per acre[2]. March[20] has summarized in his comprehensive review the type and magnitude of contamination of insecticides in soil. Most of these compounds persist in soil for several years and are not affected by leaching. He further deals with the insecticide residues in fruits, vegetables, dairy materials, fish and other forms of wildlife. There are many reviews [21–25] which cover the various aspects of pesticide contamination including water, soil, food and animals. The pesticide magnification illustrated by these residues is more than 3000-fold[2] in biological systems. Such level of accumulation in the bodies of animals through food chain may impair coordination and behaviour to point of toxic manifestation.

The accumulation of chlorinated hydrocarbons, especially DDT and DDE in human beings of different countries is furnished [20,26,27]. Cases of illness and epileptoid convulsions to persons constantly exposed to Dieldrin during spraying operations have been reported in India and other countries[28] Civilians and military personnel in India with no occupational exposure to DDT have been shown to contain 12.8 to 31 ppb DDT in their fat[29]. This level of DDT is significantly higher than the corresponding figures for individuals in the USA, where an average level of 12.6 ppb DDT has been reported[30]. This high level of DDT in Indian population is contributed to the dietary pattern of the people of the country where the average dietary intake of DDT of an adult has been calculated to be about 0.2664 mg per day[31]. According to the report, the greatest hazard of pesticide used in India at present arises in the use of DDT and BHC in grain by unscrupulous grain merchants to prevent insect infestation[32].

Research in the Tarai area of Uttar Pradesh, India, shows an average DDT content of 0.5281 ± 0.4093 ppm in the body fat of farm animals; 0.8454 ± 0.4093 ppm in poultry feed; 0.6878 ± 0.5194 ppm in dairy concentrates; 0.5805 ± 0.6478 ppm in hays and other feeds; 11.5000 ± 1.915 ppm in jowar seed; 1.472 ± 1.643 ppm in maize seed; 1.2447 ± 1.316 ppm in wheat seed; 0.395 ± 0.202 ppm in paddy seed; 0.4088 ± 0.227 ppm in edible grains; 0.39 ± 0.025 ppm in poultry eggs; 0.0032 ± 0.0026 ppm in milk; 0.40 ± 0.14 ppm in butter and 0.4600 ± 0.3676 ppm in vegetable samples[33].

There are reports that cattle, and even tigers in India are dying due to pesticide poisoning. Deaths of young Sahiwal and Jersey calves due to accidental poisoning by Gammexane have recently been reported[34].

Moreover, due to lack of knowledge of proper use and poor education, people in developing countries are often applying pesticides quite indiscriminately. Such careless use is creating serious problems, with little comparison in developed countries. Ironically, it is making the less harmful pesticides even more harmful. In 1958 more than 100 fatal cases in India, 74 cases including 67 deaths in Syria, and 20 deaths in Jordan from exposure to parathion have been reported. By contrast, the total number of deaths caused by parathion in the USA since the commercial introduction of this chemical in 1947 was less than 100[35]. According to a report of the Central Bureau of Investigation, India, as many as 4,536 persons died in this country in 1965 alone on account of carelessness in handling poisonous substances.

Investigations conducted for BHC residues on the bark, and stem, of coffee plant, coffee bean, soil and water from the ponds and tanks in coffee estates revealed that the coffee seeds were free from residues while accumulation of insecticides in the soil was more at the surface and almost negligible beyond 6″ depth. The insecticide residue in drinking water was high in comparison to tolerance levels fixed by USDA[36]. The level of BHC found in certain varieties of leafy vegetables available in Mysore market (India) varied from 10.5-20 ppm. It was almost 2 to 4 times more than the *USDA* permissible limit (5 ppm). The authors have suggested a simple, practical and economical technique for the decontamination of this residue in order to bring to the *USDA* limit[37]. The analysis carried out by Majumder *et al.*, on different market samples obtained from Hassan (India) area revealed that BHC in pulses varied from 3.68–15.5 ppm, DDT in wheat varied from 11.1–26.9 ppm and potatoes contained 68.51 ppm[38]. On an average in an individual about a tenth of a gram of DDT residue and measurable amounts of Dieldrin and other chlorinated hydrocarbon pesticides accumulated in the body.[2] During 1962, human milk analysed by the Department of Public Health, California, for pesticide content ranged from 0-0.12 ppm for DDT and 0-0.25 ppm for DDE. The highest level of total DDT plus DDE was 0.37 ppm[39]. The adverse effects of these pesticides in the human body has not been fully established but are considered of no significant consequence. Although specific instances can be cited of persistence of certain organophosphorous and carbamates from four weeksse to veral months, these

pesticides do not appear to cause significant long term residue problems even with their continued use. The greatest hazard is by the use of herbicides which persist in soil and injure crops, months or even years after application.

Contamination of air by pesticides: In view of the ability of pesticides to vapourize into the atmosphere from the treated soil, crop and other materials, the atomosphere is the likely source of dispersal. The major features of this dispersal from the time the pesticides are released to the atmosphere until they accumulate in marine animals are reviewed by Frost Justin[24].

AIR-DRIFT

As a result of agricultural applications and mosquito control programmes pesticides are present in the air for varying times and drift for varying distances. Indirect evidence of the persistence of insecticides is found in the contamination of feed, food stuffs and surface residues. Akkerson *et al.*[25] reported about the drift of chlorinated hydrocarbon sprays applied by aircraft and factors governing the amount of contamination on the adjacent forage crops. They observed that 1 lb of pesticide gave 8 ppm residue in the fields of 100 feet distance. The same insecticide was detected at 0.01 ppm level at 20,000 feet downwind under the same windy conditions. They found from this study that the amount of drift residue is directly proportional to the amount of active chemical applied. A drift from an application of ½ lb DDT per acre resulted in 0.01 ppm at 3000 ft distance whereas a 1½ lb. application deposited DDT residues of 0.04 ppm at the same distance. Air pollution due to BHC in the Matsuyama area was investigated by means of glass tube filled with Florisil (60–100 mesh) coated with 5% glycine and attached to a small pump[29]. The recovery of BHC (alpha, beta and gamma-isomers) dieldrin and p.p'-DDT was 98-100%. In spring, the BHC concentration in rice fields before spraying was 0.127 μg/cu.m, it was 0.054 μg/cu.m. in urban area. After spraying, the concentration in the city it increased to about 1.38μg/cu.m. The increase was not directly due to the spraying, but as a result of the insecticide evaporating from the surface of rice paddies and plants. The pollution was relatively lower on rainy days.

Studies conducted by Risebrough *et. al.*[30] have shown that concentrations of chlorinated hydrocarbons in airborne dust carried by the trade winds from the European and African land areas to Barbados range was less than 1 to 164 ppb. The lower limit of the average content of 1 cu.m. of air was 7.8 times 10 to the minus 14th gram. The amounts of pesticides contributed to the tropical Atlantic by the trade winds appear to be comparable to those carried to the sea by major river systems. The transportation of pesticides by air to the marine environment is approximately calculated and data presented. The operating characteristics of some new spray machines used in plant protection were investigated. The working conditions of operating personnel and the degree of atmospheric pollution by pesticides in the surrounding area were determined. During the sprinkling of the field by various pesticides, the pesticide concentrations in the tractor cabin at a distance of 100 m from the machine and in the atmosphers 30 min later were determined[31]. The amount of pesticide penetrating into the operator's cabin was significantly high. Fenitrothion in the air was monitored daily during a four week period when 700,000 lb of the pesticide were applied by aircraft to 3 million acres of pulpwood forest in New Brunswick, Canada[32]. Air samples were collected in Florisol filter columns linked in series with bubblers containing glass distilled dimethyl formamide. Steady increase in atmospheric phosphorus content was found early and late in the application programme but levels were not sufficient to constitute a health hazard to the local population. The amount of insecticide collected daily represented less than 0.01 mg per kg body weight. Although meteorological data could not be correlated with the insecticide collection and location, contamination appeared to involve a dominant effect. Ware *et al*[33]. found that ground application of methoxychlor with a mist blower resulted in greater drift. Target alfalfa had 127 and 111 ppm of insecticide. Alfalfa ½ mile downwind had 0.27 and 0.14 ppm from the mist blower and plane respectively. Glass plate collections at 165, 330, 660 and 120 ft downwind were in agreement with alfalfa residues. Total airborne insecticide collected at 165 and 220 ft downwind in ethylene glycol air scrubbers from the mist blower was six times that of the aerial application. Abbott, *et al*[34]., analysed air samples of London by GLC and indicated the presence of alpha-BHC, gamma-BHC, dieldrin, p-p'-DDT, TDE. The highest concentration found (in parts per 10 to the 12th power w/w) was 18-21 for dieldrin and 5-11 for gamma-BHC. Examination of rain water for pesticide residues in the atmospheric environment in London showed the presence of dieldrin, p-p'-DDT, and alpha and gamma isomers of BHC. These results suggested that atmosphere carries either as vapour or by occulsion on dust particles, small amounts of organochloride pesticides in common use in the United Kingdom and that they are scrubbed out by rain and snow. Preliminary results showed the presence of

organochlorine pesticides in the range of 10-20 ppm[35]. Hindin *et al.*,[36]., observed that DDT and Ethion, when applied to an irrigated plot, were present in detectable quantities as long as 2 weeks after application. Thirty per cent of DDT and 35% of the diazinon were found 8 feet above the ground level during application in 1963 while 22% of DDT and 49% Ethion were found at this height when applied during 1964. The authors have shown in their investigation that insecticides are lost through drift, photochemical decomposition owing to exposure to sunlight. The investigations by Kraybill[37] have revealed measurable amounts of DDT and chlordane in agricultural communities and DDT and malathion in urban communities. Concentrations of DDT ranged from below detectable level to 23 ng per cu.m. for rural samples and from below detectable level to as much as 8000 ng/cu.m. for urban communities. Cohen *et al*[38]. reported that an experimental agricultural plot at Coshocton, Ohio was sprayed with 4 lb per acre of atrazine and 2 lb per acre of esteron 99. The rain water after 3 weeks showed a total organic chlorine content of 0.3 ppb with atrazine and 2.4-D ester amounting to about 0.1 ppb each. Since the soil one mile away contained 6 ppm of atrazine, it can be calculated that no more than 16-17 mg of soil, per liter of rainfall, would be sufficient to yield the 0.1 ppb of atrazine found in rain water. These data reveal that dust particles containing pesticide could exist in atmosphere and get redeposited by rainfall on land areas remote from the site of original deposition of pesticides.

Yoe[39] reported that spray droplets ranging from 10–50μ in diameter usually produce the greatest ground contamination several miles from the source of application, while the droplets of 100μ usually do not present a drift hazard unless winds are high. About 80% of particles are deposited within short distances when they are larger than 200μ, while droplets of less than 5μ do not produce appreciable deposits and drift for many miles. Such small droplets probably constitute respiratory hazard. Yates and Akesson[40] have demonstrated the importance of particle size upon the drift potential of pesticides. They observed that when a pesticide dust of 10μ size was released about 10 feet above the ground level at 3 mph downwind, it drifted about a mile, whereas those only 2μ in diameter were carried 21 miles. Droplets of 50μ in diam drifted not more than 200 ft under the same conditions. To prevent contamination of the neighbouring hayfields with tedion dust application, Maccollom[41] recommended at least a 200-foot bufer zone when the wind velocity is 3 mph or less. Tabor[42] indicated from his data that the air over both rural and urban communities contain pesticides arising out of mosquito control and agricultural applications. The air samples were collected by standard high volume air samplers kept at one mile away from the area of actual pesticide application. Analysis by gas chromatography revealed measurable amounts of DDT and chlordan in the agriculture communities. Concentration of DDT ranged from below detectable levels to 23 ng per cu.m for the rural samples and from below detectable levels to as much as 8000 ng per cu. m for the urban communities. During 1963, several air samples were analysed from 4 Californian cities. All but two showed measurable amounts of DDT[27].

Bamesberger[43] sampled air in two fields for 100 days in 1964 which showed measurable amounts of aerosol and gaseous 2, 4-D compounds. Akesson and Yates[44] have demonstrated the importance of particle size upon the drift potential of pesticides. They observed that when a pesticide dust of 10μ size was released about 10 feet from the ground level at 3 mph, it drifted about a mile whereas those only 2μ in diam were carried 21 miles. Droplets of 50μ in diam drifted not more than 200 ft under the same conditions. Maccollom[45] utilized air blast equipment using particle size from 20–200μ in diam. Emulsifiable formulations or spray oils will increase the doplet size regardless of pressure or orifice size. Wettable powders yield smaller droplets. The author found that the most satisfactory results to control drift hazard in spray treatment is to reduce the droplet sizes ranging from 40–100μ1 in diam.

Willis *et al*[46]., have shown that a mixture of 42% DDT and 16% DDD was incorporated into commerce silt loam to a ½ ft depth and also the surface was applied to achieve an average concentration of 42.3 ppm of DDT and 16.2 ppm of DDD. Atmospheric concentration gradients and cumulative recovery for each pesticide were then monitored continuously for 6 months at 10 and 30 cm above the water or soil surface of uncropped, flooded and non-flodded plots. Within the first 2 days, the atmospheric concentration of DDT at 10 cm dropped from a maximum value of 1977 to 58 ng/cu.m. above the flooded plot and from 2041 to 100 ng/cu.m. in non-flooded spot respectively. It was found that flooding treatment effectively retarded the volatilization of pesticides. Kiigemagi *et al*[47]., have indicated that the loss of organophosphorus pesticides due to evaporation or drift during application to soil was in the range of 13–40%.

Maccollom[48] have presented data for determining the effect of topography and weather on the drift of dust from aerially sprayed orchards. The results

showed that particles less than 10μ comprised the greatest percentage of total particultes collected at the maximum sampling distance. Smaller particles collected at the maximum sampling distance. Smaller particles remained airborne for prolonged periods. Visualization of dust particles was made by fluorescent pigment. Maccollom studied the movement of pesticide particles from target areas to neighboring forage crop fields. Proper conditions for the air application of pesticides were determined by investigating the effect of air flow patterns on land where forage residue was contaminated by dust drifts. The analysis showed that concentration of insecticide in the air was greater than that being deposited on the filters. To prevent drift hazard, it was suggested that pest control materials be applied during wind velocities of 4–5 miles per hr and in the absence of inversions.

Pesticides in rain water: Ochiai[49] evaluated the concentration of BHC in rain water and atmosphere after the insecticide was applied to the ground during rainfall. The results showed that even in urban areas where agricultural activity was small, BHC existed in rain water leading to the surmise that there was extensive pollution due to BHC. The high BHC content in rain water during summer, rather than in winter, was considered to have some relationship with the spraying period. But there was no relationship between the BHC concentration and meteorological conditions. Experiments showed that BHC was present in rain water in dissolved state. Cohen and Pinkerton[50] analysed rain water which contained two pesticides applied previously in areas one mile away from the sampling site. Organic chlorine and sulphur containing compounds were detected. Additional evidence for the finding of organochlorine pesticides in rain water was provided by Wheatley and Hardman[51]. The samples of rain water were collected for a prolonged period and were analysed by TLC and GLC. The data revealed the presence of organochlorine pesticides in the atmosphere and subsequently in the rain water resulting from the volatilization of pesticides from the upper surfaces of pesticide treated soils. Further it indicated that pesticide laden dust in the atmosphere must have been precipitated to earth by sedimentation and rainfall. The average concentration of format in rain water samples collected over 1 month during the period of November 1964 to February 1965 was for BHC, 100; for dieldrin, 20; and for p-p' DDT, 3 parts per 10^{12} parts of rain water, whereas the samples collected only during the periods of rainfall in January and March 1965 averaged 29 and 3 parts per 10^{12} parts of rainwater respectively. The conclusion made by Weibel[52] with similar findings amply justify that agricultural chemicals are continuously contaminating the entire atmosphere.

Kaye *et al*[53]. have assessed the total DDT burden in the atmosphere of the world. Taking the amount of air around the world as 5×10^{15}m. tons and concentration of DDT $1\times10''$, there might be 5×10^{4} or 50,000 tons of DDT circulating in the atmosphere of the world. If it all gets deposited suddenly on land or sea beneath, it would amount to less than 1 g per hectare (Or one 80th of an ounce per acre) which is a minute fraction of an agricultural dose.

Breidenbach[54] collected air samples during the pesticide spraying season in the peach growing sections of Georgia and South Carolina, the citrus and vegetable growing areas of Florida, the cotton growing regions of Louisiana and Mississippi. They showed measurable amounts (ng per cu.m.) of one or more of the following insecticides in the various 24-hr particulate samples DDT, endrin, Heptachlor, malathion, ethion and 0, 0-diethyl S-p-chlorophonyl methyl phosphondithioate (Trithion insecticide).

Studying the melted ice from Antartica, Peterle[55] found in one analysis 40 ppm of DDT in 10^{12} water, and supposed that this might imply a total over the whole continent of 2.4×10^{3} metric tons. This works out at about 3 g per hectare, not very far from the air burden per hectare over London as indicated by a single sample. The occurrence of insecticides in a place like Antartica must originate only from the atmosphere. Akhmedor[56] studied the air pollution by "metaphos" (0, 0-dimethyl-0-4-nitrophenyl thiophosphate) after the insecticide was used to treat the fields in Uzbek SSP.

Fields of 7.41 and 12.35 acres were sprayed with a 30% metaphos emulsion. Taking meteorological factors into consideration it was concluded that the sanitary protection zone for a 7.41 acre field should be at least 2460 feet and for a 12.35 acre field, the zone should be at least 3272 feet. The recommended maximum single dose permissible in atmosphere was 0.008 mg/cu.m.

Frost and Ware[57] compared various methods of application, weather conditions and locations in four field experiments. A mist blower was employed for the ground application and 1.5 lb. methoxychlor in 3 gallons of spray was applied. A Piper/pawnee monoplane equipped with 56 nozzles applied 1.5 lb of methoxychlor in 7 gallons of spray per acre at 80 mph. Under these conditions an aerial application gave less drift downwind than the comparable mist blower. Under 5 mph wind velocity, wind and inversion temperature had little influence on drift from high clearance sprayer application. High inversion temperature caused greater drift in aerial application.

Deposits on glass plates, with ground application, were considerably less with a larger nozzle. The effects of weather, topography and formulation on the drift movement of agricultural toxicants dusted on orchards from an aircraft are considered in relation to the problem of toxicant residue on dairy animals forage[58]. The authors recommended from their studies that dusting be accomplished during such times when the wind velocity is below 5 mph and when no inversion condition exists, so that the adjacent dairy animal pastureland does not get contaminated. The potential for pesticide drift increases with increased distance between the nozzle and the target, smaller spray droplets, higher wind velocities and lower humidities. Keeping the nozzle low will also reduce the wind effects[59]. Droplet size is increased by operating at lower pressures. The relationship between the surface deposition of insecticide and atmospheric humidity is studied and was found that on low humidity days these sprays evaporate as soon as they leave the nozzle.

It is reported[60] that the average amount of DDT and its analogues in rain water from seven widely spread stations in the rural areas of Britain, all around the year, was 7.9 in 10^{11} ranging from 1.2 to 21 in 10^{11}. Similar amounts of gamma-BHC and smaller amounts of dieldrin were also found. It is evident from the foregoing results that the atmosphere is contaminated with insecticides while the thought that man is continually breathing air with this burden of DDT might be disturbing. Actually, it is possible that ordinary person will lose more DDT than he will gain by respiration. On the basis of current surveys, the concentration of DDT in his fat may well be between 1 in 10^6 and somewhat over 1 in 10^5 compared with the supposed air concentration (based on a single sample) of 1 in 10^{11}. Too little is known of the properties of DDT in these circumstances to permit a definite statement of gain or loss to the air. Risebrough,[61] observed that though PCB have not been detected in samples of airborne particulates but their observed distribution in sea indicates that they are dispersed by wind currents and that their fallout pattern is similar to that of DDT compounds. DDE is accumulating in significant amounts in marine food chains. Its distribution shows that coastal areas are not the primary source of contamination. A quantitative approach to the problem of aerial transport of chlorinated hydrocarbons to the sea has been made by analysing the airborne particulates which contribute to the marine sedimentary deposits. The results obtained indicate that wind transport can account for the observed distribution of DDT compounds in California waters and that the amount of chlorinated hydrocarbons entering the tropical Atlantic as fallout from the northeast trades is comparable to that entering the sea from a major river system.

Methods for the determination of Pesticides in Air: Dranovskaya *et al.*[62] developed a spectrophotometric method of determination of DDT in air. The pesticide was absorbed in the air in n-hexane or on silicagel. The solution containing the pesticide was analysed spectrophotometrically for absorption at 236 nm and DDT content was determined with the air of calibration graph. The method is claimed to be fast, accurate and highly sensitive. Concentrations of DDT as low as 0.15 mg/cu.m. can be detected. Grigorescu *et al.*[63] have standardised a method to determine BHC residues in air. Air was caused to flow through chloride free toluene at 10–15 cu.m./min. It was treated with a 1% solution of ferric ammonium sulphate in 15% HNO_3, a 0.1% solution of mercuric sulphocyanide in 96% ethanol and 0.1 N NaOH dissolved in ethyl alcohol. The inorganic chlorides and chlorine in the atmosphere will not interfere in the determination. Random analysis of samples indicated that the margin of error can range from $\pm$10% with an average error of 3% and time required for the determination was 20 min.

Gonskaya *et al.*[64] standardised optimum conditions for analysing organsphosphorous insecticides in air and aerosol containers. The aerosol container method uses the decomposition of the insecticides with nitric and sulphuric acids in the presence of potassium permanganate and the reduction of molybdic acid to molybdenum oxide which has a blue colour. This in turn reacts with phosphoric acid to yield a complex that has a bright blue colour in an acid medium. The air sampling is made with aluminium oxide rather than the usual silica gel. The method is simple, reduces analysis time and avoids the confusion resulting from the blue colouration from a reaction between silica gel and the reagents used. Takade *et al.*[65] analysed residues of organophosphorous pesticides (Baycid-0, 0-dimethyl 0-(4-methylthio-m-tolyl l) phosphorothioate and disyston-0, 0-dimethyl, by TLC and GLC equipped with flame photometric or alkaliflame thermionic detector. The sensitivity was in the order of 0.005 ppm. Tessari *et al.*[66] have put forward a simple and inexpensive method for analysing pesticides in air samples. The method involves quantitative extraction of pesticide from a nylon chiffon cloth screen exposed to the atmosphere for 5 days followed by clean-up with aluminium oxide and florisil column chromotography. Recovery of organo chlorine pesticides from test screens ranged from 86–100%.

Lahmann, and Henzel[67] standardised a simple

method to quantitatively evaluate pesticide in the air and rain water by GLC. The maximum concentration of DDT in rain water was 15.5 mg/100 sq.m. (undissolved) and 401 mg/100 sq.m. of dissolved matter. In general the survey in Berlin area showed that the concentrations were of the same magnitude as those in Great Britain and U.S.

For the insecticide determination in atmospheric air, a high volume glass fibre filter was used[68]. The sampler draws on about 80 cu.m. air/hr. A maximum of 1.35 ng/cu. m. gamma-hexachlorocyclohexane and of 8.45/ng/cu.m. DDT were found in the 24 hr. samples of atmospheric air in Berlin. Willis *et al*[69]. standardised optimum conditions to monitor the atmospheric concentration of endrin in the field. Air is drawn through a porthole of a stainless steel boom at a rate of 1 litre/min, by a vacuum pump into an ethyleneglycol trap which removes pesticide vapours from the air. By this device more than 90% endrin in air could be accounted for.

Summary

During the past two decades, a huge volume of synthetic pesticides has been dispersed in soil, water and air and many of these compounds are now found far from the area where they were applied. In many instances the translocation of pesticide may be attributed to food or water as the transmission vehicle. Humans in remote areas have also been contaminated with pesticide residues. Pesticide residues in fish, birds and humans in isolated areas of the world can, in some cases, be attributed to distribution through the food chain. However it is apparent from the rapidly accumulating literature that dispersion by air transport must also be considered. The main agents of pesticide contamination in air are air-spraying operations. It has been amply demonstrated that the air over both rural and urban communities contains pesticides. Symptoms resulting from the drift of pesticides have been recorded on grapes 8–12 miles away from the point of application and the drift of tracers used in air pollution studies has been authenticated as far as 22 miles from the source. Many of the organochlorine pesticides are detected and quantitatively assessed in rain water in several countries. These data lead to the hypothesis that pesticides can exist in the atmosphere, absorbed on soil particles, and could be re-deposited by rainfall washout on land areas remote from the site of original deposition of pesticides. The pesticides can also enter the atmosphere by volatillization. The movement of unchanged DDT from the treated crops and trated soil into atmosphere has been suggested to contribute to the widespread contamination of the environment by this substance.

Much of the studies relating to pesticide contamination of air, due to agricultural and mosquito control operations, has been conducted in highly advanced countries, employing the most sophisticated and sensitive analytical methods like gas chromatography, I.R. and mass spectroscopy. But very few references are available in literature to show the magnitude of air pollution due to pesticides application in developing and underdeveloped countries where enormous quantities of pesticides are being used to control insect infestation in foods as well as in the mosquito control programmes. Secondly, pesticide spray machines have to be suitably fabricated as to cause minimum air drift during operation. Such a study is urgently needed to protect the environment from these dangerous microchemicals.

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Studies on the Keeping Quality of Boiled Milk at home and under Sterile Conditions*

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Studies were made on eight samples of boiled milk kept under home and sterile conditions at room and refrigeration temperatures for their keeping quality. Boiled milk samples revealed the presence of heat resistant organisms, which multiplied rapidly and reduced the keeping quality of milk with-in 24 hrs. stored at room temperature (18–22°C). However, these heat resistant organisms could not multiply at refrigeration temperature and hence it was possible to store boiled milk upto 72 hrs. at refrigeration temperature without deterioration of milk quality.

Analysis of boiled milk samples by various tests viz. SPC, psychrophilic count, coliform count, enterococcal count, and staphylococcal count, when kept in sterilized utensils revealed the presence of only heat resistant organisms but when the boiled milk samples were stored under home conditions at room temperature, psychrophiles and enterococci were also detected. This indicated that these were the contaminats from the containers used for storing the milk samples.

Introduction

In-sanitary conditions in the production and handling of milk and other dairy products have been associated with many milk borne diseases among consumers. Iya[1] pointed out the prevelance of highly insanitary conditions of production and the numerous sources of infection to which milk is exposed during its production, processing and distribution to the consumers in India. Such type of milk may be expected to be a potential vehicle for communicating various diseases to the consumers. Since, in India, a general practice to boil the milk before consumption is employed the chances of infection by pathogens are greatly minimized and side by side keeping quality of milk is also enhanced.

In a previous communication, Kumar[2] *et al* studied the keeping quality of raw milk in home and sterile conditions. In the present study attempt has been made to study the keeping quality of boiled milk stored at room and refrigeration temperature to simulate home and sterile conditions.

Materials and Methods

Pooled raw milk samples from the distribution vehicle were collected in one litre quantity in sterilized bottles. These milk samples were given one instant boiling. After boiling, the milk samples were divided in two parts. The half of the sample was kept at room temperature (18–22°C) and the other half in refrigerator maintained at 4°C. Four such boiled milk samples were stored in tap washed, unsterilized one litre glass bottles and studied for keeping quality. The other 4 samples stored in one litre sterilized bottles (chemically cleaned bottles sterilized at 180°C for 30 minutes in a hot air oven), were also studied for their keeping quality. The resulting 16 subsamples were studied at 0, 24, 48, and 72 hours intervals for standard Plate count (SPC), psychrophilic count, coliform count, enterococci count, staphylococci count, methylene blue reduction time (MBRT), titrable acidity (TA. %) and clot-on-boiling (COB) tests. These tests were performed according to the methods recommended by standard methods for the examination of Dairy Products[3] Indian standards Institution[4] and Chalmers[6].

Results and Discussion

Results of analyses of boiled milk samples stored at room and refrigeration temperature under home conditions have been presented in Tables 1 and 2 and those handled, under sterilized conditions have been presented in Tables 3 and 4.

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Resulte of analyses of boiled milk samples stored under home conditions revealed that a few of the organisms could survive boiling. These might be some of the sporeformers, thermoduric or thermophilic organisms which multiplied rapidly during storage at room temperature. But at refrigeration temperature their number was not so high as at room temperature, indicating their low rate of multiplication at refrigeration temperature. Psychrophils were found to occur both at room and refrigeration temperatures. Since all of the psychrophilic organisms are killed by pasteurization (Witter,[6]), their destruction at boiling point is obvious, therefore, their presence in boiled milk indicated nothing but post-boiling contamination.

TABLE 1. AVERAGE BACTERIAL FLORA, MBRT, COB & TITRABLE ACIDITY (TA) OF BOILED MILK KEPT AT ROOM TEMPERATURE UNDER HOME CONDITIONS AT DIFFERENT TIME INTERVALS

Tests	0 (hr)	24 (hr)	48 (hr)	72 (hr)
SPC/ml.	52	11×10^5	48×10^5	205×10^5
Psychrophiles/ml	Nil	2×10^3	8×10^3	12×10^3
Enterococci/ml	Nil	25	30	12
MBRT (in hrs)	5	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$
COB	—	+	+	+
TA (%)	0.16	0.29	0.57	0.71

TABLE 2. AVERAGE BACTERIAL FLORA, MBRT, COB & TITRABLE ACIDITY (TA) OF BOILED MILK, KEPT AT REFRIGERATION TEMPERATURE UNDER HOME CONDITIONS AT DIFFERENT TIME INTERVALS

Tests	0 (hr)	24 (hrs)	48 (hrs)	72 (hrs)
SPC/ml.	52	2×10^2	26×10^2	287×10^2
Psychrophiles/ml	Nil	22	152	570
MBRT (in hrs.)	5	5	5	5
COB	—	—	—	—
TA (%)	0.16	0.16	0.16	0.16

TABLE 3. AVERAGE BACTERIAL FLORA MBRT, COB & TITRABLE ACIDITY (TA) OF BOILED MILK KEPT AT ROOM TEMP UNDER STERILIZED CONDITIONS AT DIFFERENT TIME INTERVALS

Tests	0 (hr)	24 (hrs)	48 (hrs)	72 (hrs)
SPC/ml.	18	21×10^3	134×10^3	166×10^3
MBRT (in hours)	5	3	1.6	1.4
COB	—	+(2)	+(3)	+(3)
		—(2)	—(1)	—(1)
TA (%)	0.15	0.17	0.22	0.33

(Figures in the parenthesis indicate the number of samples tested).

TABLE 4. AVERAGE BACTERIAL FLORA, MBRT, COB & TITRABLE ACIDITY (T A) OF BOILED MILK KEPT AT REFRIGERATION TEMPERATURE UNDER STERILIZED CONDITIONS AT DIFFERENT TIME INTERVALS

Tests	0 (hr)	24 (hrs)	48 (hrs)	72 (hrs)
SPC/ml.	18	11	37	37
MBRT(in hours)	5	5	5	5
COB	—	—	—	—
TA (%)	0.15	0.15	0.15	0.15

The presence of psychrophiles in boiled milk, stored under home conditions, was suspected from the storing vessel which was not sterilized and also from the environment as the container was not screw capped. In the present work the findings that boiled milk, when stored in sterilized container did not reveal the presence of psychrophiles supported the view that the container was the main source of psychrophiles in the boiled milk and these organisms can multiply at room temperature also. The presence of enterococci in boiled milk samples kept under home conditions at room temperature after 24 hrs. of storage suggested that they also came from the container and multiplied at room temperature. Absence of enterococci in boiled milk stored at refrigeration temperature under home conditions might be due to inability of contaminants to multiply at refrigeration temperature. Since psychrophiles, coliforms, enterococci and staphylococci could not be detected in boiled milk samples up to 72 hrs of storage under sterilized conditions at room temperature as well as at refrigeration temperature, it may be assumed that they were destroyed by boiling.

The MBRT and titrable acidity of boiled milk samples stored at refrigeration temperature under home and sterilized conditions remained constant and all milk samples remained negative for COB tests up to 72 hrs. Therefore, on the basis of observations recorded in the present work it may be recommended that boiled milk can be stored upto 72 hrs. at refrigeration temperature without any appreciable change in its quality.

Results of analyses of boiled milk kept at room temperature under sterilized conditions showed the presence of some heat-resistent organisms only. Psychrophiles, coliforms, enterococci and staphylococci could not be detected throughout storage. The Standard Plate Count was probably due to some of the sporeformers that survived boiling and multiplied during storage. With the increase of these organisms during storage, titrable acidity showed an increasing trend while MBRT decreased correspondingly. Thus it became apparent that the survival of thermoduric or thermophilic organisms has a great influence on MBRT, and titrable acidity of milk and ultimately affected the keeping quality of milk at

room temperature. Of the 4 such milk samples analysed, 2 became positive for COB test within 24 hrs. The remaining two were negative for COB having MBRT of 5 hrs. Only one sample remained COB negative upto 72 hours as it was free from thermoduric organisms. Henson[7] observed the growth of thermophiles in milk causing off flavours and high acidity. It was interesting to note that heat resistant organisms were also present in boiled milk samples stored at room temperature under home conditions and they were probably responsible to make the milk samples COB positive within 24 hrs. of storage. The MBRT was also brought down suddenly to ½ hour after 24 hrs of storage and there was a rapid increase of titrable acidity. Therefore, it was evident that these organisms were largely responsible for lowering the keeping quality of boiled milk stored at room temperature.

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Effect of Polyphosphates on Cooking Quality of AFD* Mutton

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The present process of cooking for the production of pre-cooked freeze dried mutton results in the release of large quantity of gravy or cook out and the consequent loss of colour and juiciness of the end product, resulting in a low level of acceptability. A modified method of cooking is described in which the above drawbacks are overcome to a considerable extent by treatment with inorganic polyphosphates.

For the production of precooked, Accelerated Freeze Dried (AFD) meat on a large scale, the present method is to cook the mutton after deboning and removing the superficial fat, in spring loaded cooking forms, popularly known as ham moulds. Each cooking form has a holding capacity of 4 kg of dressed mutton. The cooking forms are heated in an autoclave at 85°C for five hours at the rate of one hour for the heating of form and one hour for every kilogram of mutton. Care is taken to ensure that during this operation, there is no building up of overhead steam pressure. At the end of the cooking process, it is found that 35–40% (by weight of dressed mutton) of "Cook out" is released. This loss of gravy is reported to produce the characteristic "Woody Texture"[1] of the freeze dried meat and a consequent lower level of acceptability, as compared to the meat cooked from fresh mutton.

In order to utilise gainfully this cook out, attempts were made to concentrate the released gravy and add back to the cooked mutton before freeze drying. But this did not improve the product to any significant level. Studies have been therefore initiated to evolve a standard process to improve the acceptability of pre-cooked AFD meat by increasing its Water Holding Capacity (WHC). This has been attempted by the use of inorganic phosphates/polyphosphates and chelating agents like EDTA so that cooking release volume could be restricted. The use of phosphates for increasing the WHC is well known and has been investigated by several workers[2, 3]. Existing data is, however, mostly confined to pork, ham, beef and poultry meat like turkey and chicken. The present communication reports the effect of polyphosphates on the WHC of mutton as also whether or not the enhanced WHC as a result of this treatment is retained on subsequent cooking and freeze drying.

Materials and Methods

Raw material: Fresh mutton as available in the local market was used in all the experiments. Mutton after deboning and removing the superficial fat was cut into chunks of approximate dimensions 3.75× 3.75× 1.25 cm.

Pretreatment: This was carried out by dipping the chunks in solutions containing various concentrations of EDTA, sodium hexametaphosphate, sodium tripolyphosphate and mixtures of sodium tripolyphosphate and sodium chloride.

After treatement for a specified length of time the solution was drained off and the mutton was stored at 5°C overnight.

Temperature and period of cooking: Cooking form, of dimension 26.8× 14.0× 16 cm, loaded with treated and control mutton samples were heated in a cabinet provided with steam inlets and outlets. Temperature was regulated by adjusting the rate of steam. Temperature of cooking was accurately determined with the help of copper-constantan thermocouple which was kept in the geometric centre of a representative sample[3] the meat piece was again kept at the core of the form. The period of cooking was calculated from the time the centre of the representative piece reached the required temperature. After cooking, the meat was allowed to cool to room temperature, and the volume of gravy, thrown out, was measured by decantation. The volume of gravy itself was taken as a measure of WHC[4]. The gravy was added back to the cooked meet and the samples were stored at 5°C

*AFD—Accelerated freeze dried mutton.

overnight for possible reabsorption of the liquid by the meat.

pH: pH of the meat was determined with the help of a pH meter, before and after cooking.

Texture measurement: Cohesiveness of cooked samples was estimated using a texturometer, developed by General Foods Corporation, USA[5]. Samples were cut to a thickness of 1.25 cms and the cohesiveness was evaluated by calculating the ratio between the areas of first two chews of the plunger.

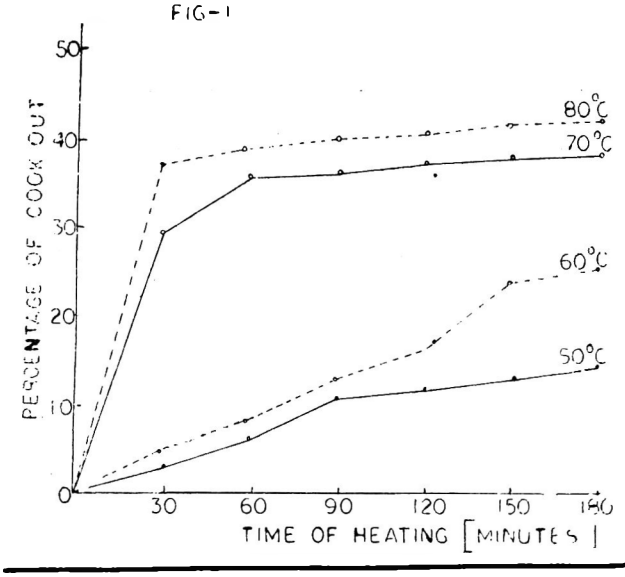
Freeze-drying: The meat, both treated and control, was frozen in a blast freezer and finally radiant freeze dried at a maximum surface temperature of 55°C. The freeze dried end product was evaluated for rate of rehydration[6]. The rehydrated mutton was converted into a curry and was submitted to organoleptic evaluation by a panel of trained judges.

All the chemicals used were of analytical standard except sodium tripolyphosphate which was of commercial grade. All determinations were run in duplicate.

Results and Discussions

All the results have been presented as a percentage of control since it is well known that there is a large variation in muscle quality from sample to sample. This handicap of wide fluctuations in the condition of raw material has been reported by earlier workers[7] also.

Results shown in Figure 1, confirm the earlier observations[8] that the two important factors influencing the WHC are the temperature and time of heating. In order to minimise the gravy loss, heating of mutton, till the geometric centre of the representative piece, reached 50°C, was tried. This resulted in minimum cook out. However, cooking was incomplete. Also heating at 50°C for prolonged period of 3 to 5 hrs, did not yield a well cooked product. Higher cooking temperature of 70°C resulted in the release of 35, 40 and 45% gravy (by weight of mutton) in 3, 4 and 5 hr. heating respectively.



In order to restrict the amount of cook out and to achieve the desired degree of cooking preferably at a lower temperature the effect of EDTA and sodium hexametaphosphate was tried in the first instance. The results are recorded in Table 1. It will be observed that neither of the additives increases the WHC.

The effect of sodium tripolyphosphate (TPP), as presented in Table 2; indicates that the treatment of raw mutton, effectively decreases the cook out after cooking at 60°C for 180 min and the degree of cooking achieved is also more than in the control. Solution containing 7.5 to 10% TPP gives the maximum WHC and any concentration above 12% shows a tendency towards more loss of gravy though it is less than the control.

TABLE 1. COOK OUT FOR EDTA AND SOD HEXAMETA PHOSPHATE TREATED MUTTON

(After cooking at 60°C for 180 min)

Concentration of Solution used	Cook out (% by Wt. of mutton)	Cook out as % of Control
EDTA		
(1) 1.0 %	25	100.0
(2) 2.0 %	30	130.0
(3) 5.0 %	30	130.0
(4) 10.0 %	36	92.3
Sodium Hexameta Phosphate		
(1) 1.0 %	40.0	123.0
(2) 10.0 %	48.5	124.0
(3) 15.0 %	25.0	129.0
(4) 20.0 %	26.0	134.0

TABLE 2. COOK OUT FOR DIFFERENT CONCENTRATIONS OF SODIUM TRIPOLYPHOSPHATE

(After cooking at 60°C for 180 min)

Concentration of solution	Cook out (% by wt. of mutton)	Percentage of Control
1.0 %	24.5	100.0
5.0 %	20.0	70.4
7.5 %	18.5	67.4
10.0 %	16.5	63.4
12.5 %	24.8	80.0
15.0 %	27.0	87.0
17.5 %	27.0	87.0
20.0 %	27.0	87.0

The effect of TPP on cook out for four different temperatures of heating, namely 60, 70, 80 and 90°C for periods from 30 to 180 min is given in Table 3. The cook out progressively increased with time and temperature. It can be observed that the polyphosphate treated samples gave much less gravy as compared to the corresponding control (untreated samples). This confirms the efficacy of polyphosphate at all temperatures and periods of heating.

The failure of EDTA and sodium hexametaphosphate to increase the WHC may probably be due to the fact that the initial pH was not adjusted[9] and also due to the absence of specific configuration and distribution of charges such as occur in long chain polyphosphate[10].

Effect on pH: Polyphosphate treated samples showed distinctly higher pH initially and after cooking. On treatment with polyphosphate, the pH is increased by 0.5 unit more than the control initially and heating subsequently increases the pH by nearly 0.7 unit in the case of phosphate treated samples and 0.5 unit in untreated samples. The maximum pH is reached within 30 min of heating and remains constant on further heating. The change of pH which is on the alkaline side may be one of the reasons for increased WHC[11].

Texture profiles: The changes in cohesiveness for the treated and untreated samples are reflected in Table 4. The cohesiveness for the treated samples i.e. the work done to break the internal bonds, is markedly lower than the untreated samples irrespective of the initial variation of samples. This indeed was expected because phosphate treated muscle systems soften much easily than untreated ones, and polyphosphates permit more rapid processing[12]. Since at temperatures higher than 65°C, myofibrillar and globular proteins get coagulated[8], a cooking temperature of 60°C for 180 min was selected for treated mutton samples.

Period of treatment with tripolyphosphate: Cook out for treatment for different periods with 10% solution of sodium tripolyphosphate and subsequent cooking at 60°C for 180 min is presented in Table 5. The volume of gravy decreases with increasing time of contact from 30 to 120 min and shows a tendency towards increase for 150 min contact and beyond. Indefinite soaking, therefore, does not produce any beneficial effect.

Effect of addition of sodium chloride: Addition of sodium chloride to sodium triployphosphate is known to improve the swelling and WHC[13]. Table 6 shows the beneficial effect of sodium chloride particularly at .5%. Concentration more than 1% sodium chloride was found to give adverse effect on the palatability of the end product.

Evaluation of treated and untreated samples, after freeze drying: Polyphosphate treated freeze dried samples have better reconstitution as compared to untreated and hence possess more juiciness and tenderness (Table 7). This is further confirmed by the higher rating by a trained panel of judges when curry prepared from the freeze dried product was submitted for organoleptic evaluation. (Table 8).

From the above discussion it can be concluded that the treatment of mutton in 10% sodium tripolyphosphate with ((or without 0.5% to 1%) sodium chloride

TABLE 3. COOK OUT, FOR COOKING AT VARIOUS TEMPERATURES AND VARIOUS PERIODS FOR CONTROL AND SAMPLES TREATED WITH 10% TPP SOLUTION

Temp. °C / Period of cooking (Min)	60			70			80			90		
	Control (% by wt. of mutton	TPP (% by wt. of mutton	% of Control	Control	TPP	% of Control	Control	TPP	% of Control	Control	TPP	% of Control
30	6.0	1.5	25	23.5	16.0	69	35	24	68.5	36	30	83.5
60	9.5	4.0	42	28.0	18.0	66	38	25	69.5	42	28	67.5
90	15.5	8.5	55	31.0	21.5	69	39	23	70.5	42	30	71.5
120	15.5	8.5	55	31.5	23.5	74	39	23	70.5	43	31	71.5
150	21.5	11.0	66	36.5	27.6	75	39	29	74.0	43	31	71.5
180	26.5	13.0	49	36.5	27.0	75	43	29	67.5	43	31	71.5

Note: TPP—Treated with 10% solution of TPP.

(by weight of dressed mutton) solution for 2 hours and subsequently cooking at 60°C for 3 hrs produces a product superior to the existing one for the production of pre-cooked accelerated freeze dried meat, from lamb mutton.

Acknowledgement

Authors are thankful to Shri V. Subramanian for his help in freeze drying the samples.

TABLE 4. COHESIVENESS, AS MEASURED WITH A TEXTUROMETER AFTER VARIOUS TEMPERATURE FOR VARIOUS PERIODS

Temp. °C	60		70		80	
Time (min)	Control	TPP	Control	TPP	Control	TPP
30	0.65	0.64	0.77	0.70	0.89	0.74
60	0.63	0.56	0.78	0.70	0.88	0.74
90	0.60	0.54	0.75	0.66	0.88	0.66
120	0.60	0.54	0.75	0.60	0.80	0.66
150	0.60	0.54	0.66	0.55	0.77	0.62
180	0.60	0.54	0.58	0.55	0.68	0.46

Note:—1. TPP—Treated with 10% solution of TPP for 120 min.

Note:—2. *Corresponding values after freeze drying*

Control — 0.53
TPP — 0.39
TPP+Nacl— 0.36

TABLE 5. COOK OUT FOR TREATMENT FOR DIFFERENT PERIODS WITH 10% SOLUTION OF TPP

Treatment period min.	Cook out as % of control	pH
30	88	7.25
60	75	7.60
120	65	7.60
150	80	7.60
Overnight	80	7.80

(After cooking at 60°C for 180 min.)

Note: Cook out for control is 18.5%.

TABLE 6. COOK OUT FOR COMBINATION OF DIFFERENT CONCENTRATION OF SODIUM CHLORIDE WITH TPP (10% SOLN.) AFTER TREATMENT FOR 120 MIN. (After cooking at 60°C for 180 min.)

Nacl Conc. %	Cook out % of wt. of mutton	Cook out as % of control.
0.0	14.3	60.8
0.5	11.8	50.0
1.0	14.3	60.8
1.5	13.6	57.7

Note: The corresponding value for the control is 23.5

TABLE 7. COMPARATIVE RECONSTITUTION FOR CONTROL, SAMPLES TREATED WITH 10% SOLUTION OF TPP AND 10% SOLUTION OF TPP+NACL FOR 120 MIN.

Time min.	Control % Water absorbed	Control % re-const.	TPP % Water absorbed	TPP % re-const.	TPP+Nacl % Water absorbed	TPP+Nacl % re-const.
5	64	85.5	66	88.5	64	85.5
10	65	87.0	68	91.0	68	91.0
15	71	95.0	69.5	93.0	69.5	93.0
30	71	95.0	70	94	71	95.0
60	71.5	95.5	71	95	71	95.0
120	71.5	95.5	75	100	74	99.5

TABLE 8. ORGANOLEPTIC EVALUATION

Quality Attribute	Control sample	TPP +NaCl	TPP
Colour	0	1	1
Flavour	−2	+2	+2
Juiciness	−1	+2	+2
Overall grading	−2	+2	+2

Note: Score

Like definitely	Like mildly	Neither like nor dislike	Dislike mildly	Dislike definitely
+2	+1	0	−1	−2

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Microbiological Quality of Market Milk in Hissar City

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A total of 94 milk samples were collected from four sources of milk supply to the Hissar City, during Winter (January to March) and Summer (May to June). These samples were analysed as raw and lab-pasteurized milk. Total bacterial population was found maximum in milk of Govt. Livestock Farm (GLF) followed by confectioners, milk-vendors and Animal Farm (HAU) milk. Maximum microflora, after pasteurization, were present in confectioners and GLF milk, showing the poor type of sanitary conditions of milking and utensils cleaning. Organisms tested were found to be maximum in summer months with the exception of spores, in case of milk-vendor's milk.

Microbiological quality of market milk in various cities in India has been reported by different workers[1-3]. No such study has been reported for Haryana where the temperature is maximum in summer[4]. Therefore this study was undertaken to find out the microbiological quality of market milk in Hissar City, from various sources of milk supply, in different seasons.

Materials and Methods

Ninty-four samples of raw milk collected were examined for various micro-organisms both in raw and laboratory pasteurised conditions. Out of 94 samples, 15 were collected from Animal Farm, Haryana Agricultural University (HAU), 17 from Government Livestock Farm (GLF), 34 from milk-vendors and 28 from confectioners.

Samples of milk were collected from vendors at 6 different major road barriers. Samples of milk from the confectioners were collected from 5 different zones of the city. The samples of milk from Animal Farm (HAU) and GLF were collected at the points of final disposal of milk (raw milk) to the consumers. Samples were collected during Winter (January to March) and Summer (May to June).

Duplicates samples were collected carefully in sterilized milk sampling bottles of quarter-litre size. Ice-pail was used to bring down the temperature immediately. Laboratory pasteurisation was done in a water-bath, at $63.0° \pm 0.2°C$ for 30 min. in sterilised pluged 100 ml. conical flasks and immediately cooled to 10°C in ice-bath[5].

Standard procedures were used for enumeration of SPC and coliforms[5] (violet-red-bile-agar) and spores, staphylococci and yeasts & molds[6].

Results and Discussion

(*a*) Microflora in Raw-Milk: The results of microflora in raw-milk are presented in Table 1. SPC was found to be highest in GLF milk samples, followed by milk samples from confectioners, milk vendors and Animal Farm. Studies of Pal and Sinha[1] and Vijai & Saraswat[2] showed lowest bacterical counts in the organised dairy milk. Results obtained with milk samples of Animal Farm (HAU) are in agreement with the findings of these workers[1, 2] but highest count in GLF milk is quite contrary in this study. This may possibly be due to contractor system of milk disposal under less sanitary conditions. Also no chilling facility was available at GLF, the single major source of milk supply in the city. The highest range was in confectioner milk, i.e. 6×10^4 to 2490×10^4 per ml. This illustrates the variations in sanitary production of milk. According to ISI[5] the milk produced at Animal Farm (HAU) can be graded as 'Good' and milk produced by GLF as 'Poor' and the other two sources as 'Fair'.

Maximum numbers of coliform were found in GLF milk followed by confectioners, Animal Farm and milk-vendors. Only presumptive test was carried out for coliform organisms. According to ISI[5] standards, milk from all sources can be graded as unsatisfactory. The high coliform densities especially in GLF milk, revealed insanitary conditions of production and

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TABLE 1. QUALITY OF RAW MILK SAMPLES FROM VARIOUS SOURCES

Test	Animal Farm	Govt. Livestock Farm	Milk Vendors	Con-fectioners
SPC (1×10^4/ml)	76 (3–248)	623 (76–1,895)	121 (9–1,505)	303 (6–2,490)
Coliforms (1×10^2/ml)	64 (0.4–259)	462 (2–2,150)	30 (0.45–112)	138 (0.15–2,490)
Staphylococci (1×10^2/ml)	78 (2–292)	721 (16–2,000)	286 (3–4,840)	532 (8–5,200)
Spores (1×10^2/ml)	0.46 (0–1.2)	0.71 (0–1.45)	2.05 (0–25)	2.17 (0–30)
Yeasts & molds (1×10^2/ml	1.2 (0–4)	14 (0–37)	1.0 (0–11)	3.0 (0–19)

*Figures in brackets indicate range.

TABLE 2. QUALITY OF LABORATORY PASTEURISED MILK FROM VARIOUS SOURCES

Test	Animal Farm	Govt. Livestock Farm	Milk Vendors	Con-fectioners
SPC (LPC/ml) (at 63°C) (1×10^2/ml)	40 (1.5–120)	132 (28–330)	88 (11–446)	157 (5–1330)
Coliforms (1×10^1/ml)	—	—	—	—
Staphylococci (1×10^2/ml)	0.21 (0–0.9)	0.34 (0–1.4)	0.31 (0–1.5)	0.29 (0–1.0)
Yeasts & molds (1×10^1/ml)	—	—	—	—

*Figures in brackets indicate range.
LPC: Laboratory Pasteurisation Count

TABLE 3. SEASONAL DISTRIBUTION OF VARIOUS MICROFLORA IN RAW MILK FROM VARIOUS SOURCES

Test	Animal Farm		Govt. Livestock Farm		Milk Vendors		Confectioners	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
SPC (1×10^4/ml)	13 (3–41)	118 (35–248)	492 (76–1080)	771 (92–1895)	40 (9–100)	213 (14–1505)	95 (6–322)	511 (20–2490)
Coliforms (1×10^2/ml)	16 (0.4–48)	99 (8–295)	235 (2.5–950)	664 (20–2150)	22 (0.45–67)	42 (1.2–112)	28 (0.15–70)	248 (1.7–2490)
Staphylococci (1×10^2/ml)	21 (2–75)	115 (17–292)	524 (16–2000)	897 (23–1980)	44 (3–117)	558 (33–4840)	120 (12–751)	944 (8–5200)
Spores (1×10^2/ml)	0.35 (0–1.2)	0.53 (0–1.15)	0.64 (0–1.45)	0.79 (0–1.25)	2.3 (0–25)	1.75 (0–1.14)	0.83 (0–4.5)	3.43 (0–30)
Yeasts molds (1×10^2/ml)	0.76 (0–1.7)	1.5 (0–4)	12 (0–25)	16 (8–37)	0.57 (0–1.7)	1.5 (0–11)	2.23 (0–19)	4 (0–14)

*Figures in brackets indicate range.

TABLE 4. SEASONAL DISTRIBUTION OF VARIOUS MICROFLORA IN LABORATORY PASTEURISED MILK FROM VARIOUS SOURCES

Test	Animal Farm		Govt. Livestock Farm		Milk Vendors		Confectioners	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
SPC (LPC/ml)63°C (1×10^2/ml)	11 (1.5–33)	59 (28–117)	112 (23–257)	142 (30–325)	40 (11–128)	141 (21–446)	37 (3–86)	277 (55–1330)
Coliforms (1×10^1/ml)	—	—	—	—	—	—	—	—
Staphylococci (1×10^2/ml)	0.05 (0–0.2)	0.32 (0–0.9)	0.32 (0–0.9)	0.36 (0–1.4)	0.14 (0–0.65)	0.34 (0–1.5)	0.22 (0–0.79)	0.37 (0–1.0)
Yeasts & molds (1×10^1/ml)	—	—	—	—	—	—	—	—

*Figures in brackets indicate range.
LPC—Laboratory Pasteurisation Count.

handling of milk. Mc Callum[7] findings also indicated that coliform test was the most reliable for detecting poor quality of raw milk.

Staphylococci population (using Staphylococcus Medium 110) has also been maximum in GLF milk & minimum in Animal Farm milk. However, spores showed a different trend than other microorganisms. Maximum number was present in confectioners milk and minimum in Animal Farm milk. Confectioners generally get their milk from vendors and trend observed may be due to contamination of milk from the cans of the vendors, which are not seamless. But yeast and molds were maximum in GLF milk and lowest in vendors milk. Higher number of yeasts and molds seems to be directly related to the higher densities of other organisms.

(*b*) Microflora in Laboratory Pasteurised Milk: The results are presented in Table 2. Coliforms and yeast & molds were absent in all the samples (1:10 dilution). Absence of coliform showed satisfactory quality of Laboratory Pasteurised milk as per ISI-Standard[5].

The highest SPC was in confectioners milk and minimum in Animal Farm milk. According to ISI standard[5], appreciable numbers of bacteria in laboratory pasteurised milk indicates contamination from poorly sanitized utensils

Staphylococci number was also mixmum in GLF milk and minimum in Animal Farm milk. The large number of staphylococci may be regarded with suspicion, but definite diagonosis have not been carried out in the present study. Heinemann[8] also reported salt-tolerant micrococci in grade 'A' pasteurised milk (140°F for 30 min) on Medium 110 and observed less than 20 colonies/ml.

The destruction after laboratory pasteurisation was more than 99 percent in all the case.

(*c*) Seasonal Distribution of Microflora in Raw Milk: The results have been presented in table 3. SPC was always found to be more in summer. The same trend was reported by Pal and Sinha[1]. Coliforms, staphylococci, yeast & molds also followed the same trend in the present study.

Spore formers showed the same trend with the exception of vendors milk. The finding are in aggrement with Verma *et al*[9] and El Sadek & Attia[10]. The reverse trend in vendors milk has also been supported by the findings of Pal & Harper[11] and Abo-Elnaja[12] probably due to carelessness in the maintenance of sanitary conditions during winter.

(*d*) Seasonal Distribution of the Microflora after Laboratory Pasteurisation: Table 4. Shows the distribution after pasteurisation. All the organisms were more in Summer than in Winter. Confectioners milk showed the highest number of all types of microflora during Summer showing the poor type of sanitary conditions.

Summer season is well-known for poor quality milk and development of low cost chilling facilities on crash programme is a need of the day.

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Changes in Free Amino Acids of Arhar Dhal (*Cajanus cajan*) on Processing

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Arhar dhal (*Cajanus cajan*) is investigated for its free amino acid content and also for other water soluble non-protein constituents. It has been observed that nearly 40% of the ninhydrin positive constituents comprise of peptides, which are mostly glutamyl peptides of phenylalanine, tyrosine, alanine, isoleucine and leucine, as shown by liberation of corresponding acids after hydrolysis with 2N HCl. All the natural amino acids were found to be present, glutamic acid being highest followed by asparagine and glutamine. During processing of the dhal, a slight increase in peptides was found and part of alanine appears to be bound to proteins in a way that is released by the mild hydrolytic conditions of processing. Amide ammonia content after processing with enzyme treatment showed a slight increase. Arginine was one of the amino acids found in higher amounts than others (7.5 μmol/g).

Leguminous seeds[1,2] particularly *Phaseolus* sp and groundnut[3,4] have been investigated for their free amino acids, with respect to changes in them during germination. Only one dhal in common use, green gram, (*phaseolus vulgaris*) has been examined for its free amino acid content[5]. Arhar dhal (*Cajanus cajan*) has been reported for its changes in amino acids and proteins and the changes in them after a processing treatment involving precooking and dehydration.[6,7] But this important dhal of the Indian dietary has not been reported for its free amino acid contents and the changes in them during processing treatments. In this paper the dhal is examined for its free amino acids and other non-protein nitrogenous components that are also water souble and for the changes they undergo during a processing treatment.

Materials and Methods

Dehusked Arhar dhal obtained from local market, was processed according to the procedures previously described in connection with development of precooked dehydrated dhals with reduced cooking time[8]. The raw dhal (RD), dhal prepared by precooking and dehydration (PD) and dhal prepared by treating with papain during the processing (PTD) were powdered and sieved through 100 mesh to minimise sampling errors and used for extracting the water solubles as described below.

Aqueous extracts: A modified procedure of Dent[9] was followed for preparation of water extracts. Two grams of the powdered dhal were taken in a 250 ml flask with narrow long neck such as volumetric flask and extracted with 10 ml of water for 30 min with occasional shaking. To the aqueous suspension was added 40 ml of redistilled ethyl alcohol to make it 80% alcohol. About 200–220 ml of chloroform was added to the alcoholic extract in the flask and contents gently swirled to prevent excessive emulsification. The aqueous and alcohol-chloroform phases were allowed to separate overnight and the aqueous extract used for further investigation.

Amino acid determination: One ml of the extract was diluted with 0.2N citrate buffer of pH 2.2 and 0.5 ml of diluted sample applied directly on the two resin columns of Beckman 120°AC Amino acid analyzer and analyzed for the amino acids using conditions recommended for physiological fluid analysis*, excepting that Resins PA 35 and AA 15 were used. The concentration of the various amino acids in the extract was calculated using appropriate instrument constants and when the identity of amino acid or peptide is not known, an average constant was used. The values are expressed as μmol/g of dhal, after applying an appropriate factor that was calculated using internal standards as described below.

Internal standards: The two amino acids, norleucine and homoarginine, which do not occur naturally have been used as internal standards[10]. Their recovery during the extraction has been determined and the concentration of the various amino acids and peptides calculated on the basis of 100% recovery of added standards, one each for the two

*Beckman Technical Bulletin ATB-034 December 1966 Spinco Division, Beckman Instruments Inc, Palo Alto, Cal.

resin columns. The values expressed are average of two determinations.

Amide ammonia determination: Amide ammonia was determined by estimating ammonia in the extract before and after hydrolysis with 1N HCl at 100°C for 3 hr as recommended by Kasai[11]. The ammonia in solution was determined by Conway microdiffusion technique and using Berthelot's reaction as recommended by Fawcett and Scott[12] using Beckman microanalytical system.

Results and Discussion

The method adopted here for the extraction of free amino acids viz., extraction with 80% alcohol and adding chloroform has a great advantage over other methods in that both extraction and deproteinization necessary before analyzing on the ion-exchange resins is accomplished in one step. The method has been compared with other methods and found most suitable[13] for estimation of all amino acids excepting the basic amino acids which are presumably bound to the acidic groups of proteins and not released[14, 15]. Hence internal standards, nor-leucine and homoarginine (one each for each set of determinations, acidics and neutrals and basics) have been added on the principle that the losses in each during extraction by binding to proteins etc. will be same for each of the amino acids in the two sets of determinations. The internal standards also help in the identification of the amino acids on the charts as well as for accounting of errors due to day to day variations and for these reasons also, the practice is routinely adopted[10, 15]. Recovery for the two amino acids during the experiments was 100% for nor-leucine and 93–98% for homoarginine. Table 1 gives the values for all the amino acids determined for the three dhals analyzed viz., raw dhal; processed dhal and dhal processed using enzyme treatment. Values for the amino acid composition of the proteins of dhal are also given in the table for comparison.

Data available in literature regarding the free amino acid content of leguminous seeds[1, 5, 16] show that the values obtained in this investigation are nearly the same (0.2 to 20 μmols/g). Further it has also been observed that when this is compared with the amino acid composition of the proteins of the dhal itself, no regular pattern can be observed and this has also been the finding of other investigators as summarized by Steward and Bidwell[17]. The values observed by us for arginine in particular and basic amino acids in general are comparatively high and might be due to the improved extraction in the method adopted by us. The values remain more or less unchanged during processing treatment being same 7.5 μmols for arginine, 0.9, 1.0 and 1.1 for lysine and 1.3, 1.2 and 1.1 for histidine. It will be seen that the contents of a few more amino acids such as proline, glycine and tyrosine also remain unchanged whereas for valine, leucine and isoleucine there is a slight but significant decrease after processing treatment, perhaps due to leaching losses. Exceptionally high losses in phenylalanine alone among all the amino acids may be noted (3.4 to 1.2 μmol/g). On the other hand aspartic acid and alanine contents increase more than four times and two times respectively after both processing treatments. The increase in the value for aspartic acid must be from aspargine hydrolyzed during the wet heat processing conditions. Glutamic acid also shows a similar increase, although slight, from 18.5 to 21.8 presumably after a release from glutamine. An increase seen in the value for alanine may be compared with no corresponding increase in that of β-alanine.

A preliminary analysis of the peptide constituents has been done and data given in Table 2. All ninhydrin positive constituents that show an absorption peak which cannot however be related to known elution volumes of amino acids listed in Table 2 are given in this table and their composition given on the basis of data available from literature[5, 18, 19]. Their composition cannot be certain unless the fractions are isolated and analyzed. For the purpose of this paper, only their peptide nature has been confirmed by hydrolysis with 2N HCl and data given in Table. 2. It will be seen from the table that fractions 1 and 2 disappeared after hydrolysis by 2N HCl and fraction 4 showed a decrease. The decrease is only about half but considering the large amount (16.5 μ moles released together with Fraction 5) and perhaps a slow rate of hydrolysis, it is still significant and confirms that the fractions are of peptide nature. The values for the amino acids after hydrolysis are given in Col 4 of Table 1. Aspargine has been found to be absent, and nearly 10 times increase in aspartic acid and ammonia found simultaneously. There was an increase in amino acids that constitute these peptides[3] glutamic acid, phenylalanine showed high increase, β-alanine and β-amino isobutyric acid moderate; valine, glycine, isoleucine low. The slight decrease in the values for histidine and arginine perhaps lies in the known susceptibility of basic amino acids to acid treatment. One compound tentatively identified as galactosamine is found to appear after hydrolysis and there is no report of this compound occurring as part of a peptide. Excepting for this, all other information such as elution volumes, the increase in the values for amino acids points to the conclusion that the peptides found belong to the category of glutamyl

TABLE 1. AMINO ACID COMPOSITION (μMOLS/G) OF THE WATER EXTRACTS OF RAW DHAL (RD) AND DHAL PROCESSED BY DIFFERENT METHODS

Amino acid	RD	PD	PTD	Raw hydrolyzate	Total protein** g/16g N.
Phosphorserine	0.9	1.1	1.0	–	–
Urea	0.4	0.6	.5	.4	–
Aspartic acid	0.8	3.8	3.3	9.6	9.8
Threonine	–*	–*	–*	0.8	3.7
Serine	–*	–*	–*	(1.4)	6.0
Aspargine	19.	23.6	22.2	–	–
Glutamic acid	18.5	21.8	21.3	v. high	23.7
Proline	1.6	1.6	1.6	1.6	5.3
Glycine	0.7	0.7	0.6	1.2	4.2
Alanine	1.4	2.8	2.5	1.3	4.4
Cystine	Tr	Tr	Tr	Tr	0.8
Valine	1.8	1.4	1.4	3.3	3.8
Methionine	Tr	Tr	Tr	0.5	0.9
Isoleucine	0.4	0.2	0.3	0.7	2.8
Leucine	0.8	0.4	0.4	0.9	7.0
Tyrosine	0.4	0.4	0.3		2.0
Phenylalanine	3.4	1.6	1.2	15.	8.6
β-alanine	0.25	0.25	0.3	1.9	–
β-amino isobutyric acid	0.3	0.3	0.3	2.5	–
Tryptophan	–*	–*	–*		
Lysine	0.9	1.0	1.1	1.2	7.0
Histidine	1.3	1.2	1.1	1.0	3.7
Arginine	7.5	7.5	7.5	7.0	6.8
Ammonia amide	12	11	16	(12)	

*Could not be determined; Tr., Traces
**Ref. No. 7.

peptides generally found in plant materials[20]. These peptides constitute more than 40% of the ninhydrin positive components in the water extract and thus glutamic acid and glutamyl peptides account together for nearly 60% of the non-protein nitrogen. The changes these constituents undergo and the physiological role they play in plant economy has been the subject of many papers but investigations on them with respect to storage, ageing, colour changes, etc. would appear to be of practical significance.

The peptide 1 appears to be N-carboxymethyl-β alanine reported by Kasai *et al*[11] in green gram. Presence of peptide bound β-alanine is confirmed because its concentration goes up after acid hydrolysis of the water solubles. Only its concentration does not go up under the conditions of processing whereas alanine shows a reverse behaviour. Further experiments to isolate the peptide and study its behaviour under conditions of processing are necessary before any explanation can be offered regarding this anomaly.

Because of the ubiquitous nature of ammonia contamination, ammonia in extracts has been determined separately to obtain values for free ammonia and total ammonia before and after hydrolysis with 2N acid and by difference bound (amide) ammonia estimated. It was found to increase slightly for papain treated dehydrated dhal, over raw dhal or dehydrated dhal without papain treatment. The same trend, seen in the case of aspargine and glutamine, would confirm that under processing conditions the release of these two into water solubles contributes to the increased amide ammonia found, but how far the increase with processing and enzyme treatment is significantremains to be investigated. The data

TABLE 2. CONCENTRATION OF NINHYDRIN POSITIVE CONSTITUENTS OTHER THAN AMINO ACIDS WITH THEIR ELUTION VOLUMES AND POSSIBLE PEPTIDE NATURE IN AQUEOUS EXTRACTS OF DHALS PROCESSED BY DIFFERENT METHODS (μmots/g)

Fraction	Elution volume ml	Composition	RD	PD	PTD	Raw hydrolyzate
Peptide 1	60	N-Carboxy methyl β Alanine	5.7	0.9	1.5	—
,, 2	83	γ-Glutamyl leucines	0.8	1.4	1.1	—
,, 3	110	Citrulline	—	—	—	0.4
,, 4	160	γ-Glutamyl phe., tyr., met., val.	40–50	40–50	40–50	6.5
,, 5	175		—	—	—	10.
Fraction 6	—	Galactosamine	—	—	—	1.6

presented here would indicate that changes presented in alanine, amide ammonia during processing and also the higher levels of arginine found employing the method used by us may be of significance for a further study.

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Sugars, Organic acids, Amino acids and Invertase Activity of Juices from 22 Grape Varieties

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Twenty two grape varieties were examined for their sugars, non-volatile organic acids and soluble amino-acids pattern. Juice characteristics such as colour, consistency, total soluble solids, titrable acidity, pH and invertase activity were recorded in view of their importance in identifying varieties with suitable processing characters. Wide variations in these constituents were observed. The use of such informations as a possible index of quality of grape used for processing is discussed.

The composition of grapes largely determines the physical and chemical characters of grape products. Based on the biochemical constituents which is dependent on region, climate etc, varieties of grapes can be recommended for different processing. Based on such informations maturity standards[1-5] have been fixed. Classification of grape varieties on the basis of their composition has also been suggested by a number of workers[3, 6, 7]. In the absence of definite informations on the chemical composition of grapes from our region, a study was undertaken with 22 grape varieties being evaluated, under the grape improvement programme of this Institute. The importance of such informations in establishing varieties in relation to their use is also discussed.

Materials and Methods

Grape bunches (1 to 2 kg) harvested from vines grown under the same climatic, cultural and soil conditions at the experimental station of this Institute, Hessaraghatta were used for analysis. Juice was extracted by crushing berries and squeezing through two layers of muslin cloth. The colour, yield and consistency of juice were recorded. Total soluble solids (TSS) and pH of the juice were measured by a hand refractometer and systronic pH meter respectively. A known amount of juice was titrated against standard alkali and acidity was expressed as g/tartaric acid/100 ml juice. The extraction and assay of invertase in juice samples was carried out according to the procedure described earlier[8]. For the separation of soluble amino acids, organic acids and sugars, 5 ml of juice (heated on a boiling water bath for 10 minutes) was passed through Dowex 50×8 (H^+ form) and Amberlite IR 4B (OH^- form) resin columns successively to absorb amino acids and organic acids respectively. The filtrate after passing through the two columns, was taken for sugar analysis.

Reducing sugars and total sugars after inversion were estimated by standard AOAC methods[9]. Fructose was estimated by resorcinol-thiourea[10] method. The glucose values were calculated by substracting fructose content from reducing sugar values. Sucrose values were calculated by multiplying the difference between total and reducing sugar values by 0.95.

The Dowex column was washed first with distilled water and then the amino acids were eluted from the column using 2N ammonium hydroxide. The eluant was dried under vacuum and the residue was taken up in 5 ml water. Fractionation and identification of amino acids were carried out with two dimensional paper chromatography. Total soluble amino acid was estimated by the method of Hyman, Rosen[11], proline by the method of Ough[12] and arginine by Sakaguchi reaction modified by Gilboe *et al*[13].

The Amberlite resin column was washed with water and the organic acids eluted using 6N formic acid. The eluant was dried over water bath to remove formic acid and the dried residue was taken up in known volume of water. An aliquot was titrated against standard alkali to estimate total non-volatile organic acid content. To separate acids a unidimensional paper chromatographic technique of Lugg and Overall[14] was used. Bromocresol green (0.04%) was used as a spray reagent to locate the acid spots. The area corresponding to malic acid spots were measured graphically and the concentration calculated from the

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area of a known concentration of a standard malic acid spot. Estimations of tartaric acid and citric acid were done by colorimetry procedure of Hill *et al*[15] and Murry *et al*[16] respectively.

Results and Discussion

The juice characteristics of different varieties are presented in Table 1. The juice yield from a given variety depends primarily on the pulpiness, size and seediness of berries. In the present study juice yields varied from 57 to 65 per cent. Black Champa, Convent Large Black, Queen of the vineyards and Thompson Seedless recorded higher percent juice among the varieties studied. This type of information is useful in calculating the yield of wine and juice available from varieties, if processed. The juice colour provides a relative indication of the colour of the wine a particular variety will yield. The anthocyanins in red, purple, blue or black varieties impart a red or purple colour to their juice. Based on the colour intensities, dark coloured beverages are expected from varieties like Alicante Bouchat, Athens, Black Champa, Convent Large Black and Isabella. The juice consistency, again a varietal character, depends on the non-sugar constituents like gums, mucilaginous material, colouring matters, tannins, soluble protein, mineral salts, pectic substances etc. The consistency varied from clear to turbid and varieties like Alicante Bouschat, Bayan Sherei, Beauty seedless, Black Champa, Chenin, Blanc, Convent Large Black, Gamsa, Queen of Vineyards and Thompson Seedless were clear.

The range of values among different varieties studied are: total soluble solids 15.4 to 28.2°B titrable acidity 0.43 to 0.93%, pH 3.3 to 3.8, TSS: acid ratio 18.5 to 45.6, total sugars 12.26 to 24.32% and sugar: acid 13.9 to 44.1. This information is useful in fixing the maturity index and also for classifying them for different uses. Use of TSS: acidity ratio as a maturity index for harvesting was suggested by Amerine[1]. Winkler[17] emphasised the importance of sugar: acid ratio in relation to quality of grapes. Amerine *et al*[18]., has recommended ranges of TSS:, titrable acidity and pH for using them in different types of wines. Thus reliable measurement of sugars, acid present permit an evaluation of maturity, approximate calculation of alcohol obtainable in fermentation, utilization as to wine type, and blending for balance of sugar and acid in the wine and for amelioration. pH is a very important factor in the biological process of fermentation and is responsible for retention of flavouring substances in wines.

TABLE 1. JUICE CHARACTERISTICS OF GRAPE VARIETIES

Sl. No.	Name of the variety	Juice yield ml/100g	Juice colour	Juice consistency	TSS °Brix	Acidity gm tartaric acid/100 ml juice	°Brix / acid	Total sugars g/100 ml.	Total sugar / Acid	pH of juice
1.	Alicante Bouschet	61.0	Dark blood red	Clear	19.0	0.64	29.7	14.94	23.34	3.4
2.	Amber queen	59.4	Light brown	Turbid	21.2	0.60	35.9	18.05	30.00	3.5
3.	Anab-E-Shahi	60.0	Yellow green	Medium clear	15.4	0.43	35.8	12.30	28.61	3.5
4.	Athens	60.0	Dark Rose Red	Thick clear	22.4	0.61	36.7	17.78	29.16	3.5
5.	Bangalore Blue	58.0	Reddish brown	Medium clear	17.0	0.92	18.5	12.26	13.30	3.2
6.	Bangalore Purple	59.0	Purplish brown	Light turbid	20.0	0.93	22.1	19.45	20.91	3.4
7.	Bayan Sherei	61.0	Greenish yellow	Clear	19.8	0.67	29.2	17.02	25.40	3.6
8.	Beauty Seedless	60.0	Light dark red	Clear	18.8	0.45	41.8	17.34	38.53	3.3
9.	Black Champa	64.0	Reddish brown	Clear	23.8	0.84	26.7	23.21	27.64	3.6
10.	Black Corinth	57.0	Greyish brown	Thick turbidity	28.2	0.63	44.8	24.32	38.61	3.7
11.	Chenin Blanc	61.0	Yellow green	Very clear	23.0	0.57	40.3	20.41	35.80	3.6
12.	Coarna Rosie	60.0	Rose browinsh	Medium clear	20.8	0.60	34.3	18.64	31.10	3.5
13.	Convent Large Black	63.0	Reddish brown	Clear	24.0	0.82	29.3	23.21	28.31	3.6
14.	Delaware	62.0	Reddish grey	Medium clear	22.6	0.65	34.1	21.15	32.54	3.5
15.	Delight	57.0	Light to dark yellow	Medium clear	23.8	0.54	42.2	22.74	44.10	3.5
16.	Gamsa	59.0	Dull brown	Clear	19.6	0.43	45.6	17.86	41.54	3.6
17.	Isabella	57.0	Dark Brown	Clear	22.4	0.89	24.7	21.40	24.02	3.3
18.	Mrs. Halls	59.0	Light brown	Medium clear	19.2	0.61	31.6	18.24	31.39	3.5
19.	Perlette	58.0	Light grey	Turbid	23.0	0.57	41.7	20.60	36.20	3.5
20.	Queen of the Vineyards	65.0	Greenish yellow	Clear	21.0	0.52	40.1	20.46	30.09	3.6
21.	Rose Giotet	58.0	Light rose red	Medium clear	21.8	0.72	30.8	19.34	26.86	3.2
22.	Thompson Seedless	65.0	Yellowish green	Clear	23.2	0.71	33.5	22.05	31.06	3.6

Sugar pattern in different varieties showed a wide variation (Table 2). Glucose and fructose were predominating sugars and only a little (0.04 to 0.83 %) sucrose was observed. Glucose values ranged from 6.71 to 15.5 %, fructose 4.0 to 9.97 % and Glucose. Fructose ratio 1.07 to 2.02. Similar type of variations has been reported by earlier workers. Carrol *et al*[19]., found 3.52 to 7.7 % Glucose, 3.35 to 9.28 % fructose and 0 to 5.2 % sucrose in 12 varieties of muscadine grapes. Kliewer[20] studied the glucose, fructose ratio of *Vitis vinifera* grapes and reported 0.74 to 0.97 in table varieties, 0.74 to 1.05 in wine varieties and a little sucrose content in all the varieties. Since the consumer acceptance of a particular variety is markedly influenced by sweetness to acid balance and because fructose is sweeter than glucose the study is of interest in selecting suitable varieties for different purposes.

The invertase activity in berries ranged from 0.145 to 1.771. Hawker[21] reported the occurrence of invertase activity in grape berries and showed, using labelled sugars, that sucrose is both hydrolysed and synthesized within berries. The absence of significant negative correlation between sucrose content and invertase activity could be the result of changing activities of both sucrose synthesizing and hydrolying enzymes in berries.

The variations in the concentrations and kinds of organic acid cause both physical and chemical changes in their products and play an important role in flavour, colour and keeping quality of the beverage. Therefore, concentrations of predominating acids were determined and presented in Table 2. Tartaric and malic acids were found to be the major acids. Total non-volatile organic acids ranged from 0.264 to 0.620 %; malic acid 62.2 to 237.0 mg %; tartaric acid 115.1 to 340.0 mg % and tartaric acid: malic acid ratio 0.61 to 3.47. Minor proportions of citric acid was observed. Winkler[17] reported that the major organic acids in grapes were tartaric and malic acids which constitute well over 90 % and only 0.02 to 0.03 % of citric acid. Differences in acidity of grapes result in different types of fermentation and qualities of wine. These differences are also attributed to the ratio of tartaric: malic acids.

The chromatographic separation of amino acids fraction from juices of different varieties indicated very little qualitative differences. In all the chromatograms 9 to 11 amino acids were obtained and tentatively identified as cystine, arginine, serine, glutamic acid, alanine, γ-aminobutyric acid, asparatic acid, leucine, threonine, proline and valine. The total amino acids ranged from 91 to 301 mg %; arginine

TABLE 2. SUGARS, INVERTASE ACTIVITY AND NON-VOLATILE ORGANIC ACIDS OF GRAPE VARIETIES

Sl. No.	Name of the Variety	Sucrose %	Glucose %	Fructose %	Glucose / Fructose ratio	Invertase activity*	Total Non-volatile acids %	Tartaric acid mg %	Malic acid mg %	Citric acid mg %	Tartaric / malic acid ratio
1.	Alicante Bouschet	0.21	9.05	5.67	1.58	1.771	0.428	161.4	193.2	73.0	0.83
2.	Amber Queen	0.20	10.48	7.36	1.42	0.819	0.480	320.0	109.0	t	2.93
3.	Anab-E-Shahi	0.48	6.71	5.10	1.31	0.224	0.350	130.0	141.0	t	0.92
4.	Athens	0.76	8.78	8.20	1.07	0.311	0.325	162.0	151.0	—	1.07
5.	Bangalore Blue	0.70	7.55	4.00	1.88	0.660	0.610	214.0	237.0	t	0.90
6.	Bangalore Purple	0.56	10.90	7.94	1.39	0.591	0.591	154.8	191.9	t	0.81
7.	Bayan Sherei	0.21	9.25	7.36	1.38	0.978	0.264	133.0	114.8	84.0	1.15
8.	Beauty Seedless	0.86	9.39	7.04	1.33	1.117	0.360	115.1	187.8	t	0.61
9.	Black Champa	0.17	15.50	7.52	2.02	0.531	0.357	194.0	144.6	t	1.34
10.	Black Corinth	0.69	13.86	9.76	1.42	0.542	0.510	270.0	143.9	t	1.87
11.	Chenin	0.53	11.37	8.48	1.34	0.255	0.407	180.0	102.7	t	1.75
12.	Coarna Rosie	0.17	12.00	6.40	1.89	0.652	0.278	150.4	63.51	t	2.37
13.	Convent Large Black	0.89	14.69	7.97	1.84	0.393	0.300	126.4	97.9	t	1.29
14.	Delware	0.46	11.23	9.92	1.13	0.485	0.620	340.0	143.2	t	2.37
15.	Delight	0.06	13.73	8.95	1.62	0.557	0.399	152.0	97.3	t	1.56
16.	Gamsa	0.64	10.73	6.49	1.65	0.401	0.370	148.6	108.1	t	1.37
17.	Isabella	0.68	11.79	8.96	1.32	0.320	0.378	172.0	122.9	t	1.39
18.	Mrs. Halls	0.80	9.79	7.62	1.28	0.780	0.386	208.4	109.4	t	1.90
19.	Perlette	0.70	13.73	9.73	1.35	0.858	0.257	135.3	62.2	t	2.17
20.	Queen of the Vineyards	0.40	12.79	7.62	1.68	0.145	0.330	127.0	116.2	9.0	1.09
21.	Rose Ciotet	0.52	10.47	8.32	1.25	1.401	0.570	256.0	73.6	t	3.47
22.	Thompson Seedless	0.89	12.47	8.64	1.44	1.405	0.535	243.3	144.5	t	1.68

* $activity = \frac{\text{mg dextrose}}{\text{mg protein}}/\text{min.}$

TABLE 3. TOTAL SOLUBLE AMINO ACIDS, ARGININE, PROLINE AND ARGININE/PROLINE RATIO OF GRAPE VARIETIES

Sl. No.	Name of the Variety	Total Souble amino acids mg%	Arginine mg%	Proline mg%	Arginine / Proline
1.	Alicante Bouschet	100	15.5	29.4	0.86
2.	Amber Queen	186	45.3	38.0	1.19
3.	Anab-E-Shahi	142	45.0	11.6	3.88
4.	Athens	251	31.6	39.0	0.81
5.	Bangalore Blue	174	30.7	22.8	1.35
6.	Bangalore Purple	174	68.7	49.6	1.38
7.	Bayan Sherei	191	28.0	32.6	0.86
8.	Beauty Seedless	176	81.2	32.6	2.49
9.	Black Champa	169	112.5	38.0	2.96
10.	Black Corinth	241	91.0	81.9	1.10
11.	Chenin Blanc	155	50.0	34.6	1.44
12.	Coarna Rosie	162	54.7	54.4	1.00
13.	Convent Large Black	301	35.5	45.7	0.78
14.	Delaware	242	39.6	40.0	0.99
15.	Delight	146	42.4	73.6	0.58
16.	Gamsa	147	88.1	37.4	2.35
17.	Isabella	142	62.0	34.6	1.79
18.	Mrs. Halls	146	43.9	46.7	0.94
19.	Perlette	91	23.3	69.8	0.33
20.	Queen of the Vineyards	109	25.5	34.5	0.73
21.	Rose Ciotet	222	76.9	31.0	2.48
22.	Thompson Seedless	140	54.7	48.6	1.10

23.3 to 112.5 mg%; proline 11.6 to 81.9 mg% and arginine: proline ratio 0.33 to 3.88. (Table 3). Such quantitative differences in the amino acid content of grapes has been reported by a number of workers[7, 22]. Drawert[23] reported the use of determining amino acids as a measure of maturity of grapes which relates more probably to flavour and aroma of the grape. Nastic *et al*[24]., reported that varieties which had more nitrogen compounds were known to give a high quality wines thereby suggesting the scope of such study in predicting the product quality.

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Evaluation of Some Grape Varieties for Wine quality

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Thirty grape varieties were tested for their enological properties during the year 1973–74. Three types of wines. namely, dry white table, dry red table and red dessert were prepared. The chemical compositions of musts and wines were analysed. White grape variety *Chenin blanc* and a red grape variety *Black Cornichon* produced good quality dry table wines. Dry table wines from 4 white grape varieties, namely, *Excelsior*, *Ova Pagola*, *S. V.* 5276 and *Sauvignon* and from 5 red grape varieties, namely, *Black Champa*, *Gamsa*, *Red Prince*, *Rubired* and *Zinfandel* were found to be of satisfactory quality. Red dessert wine from *Convent Large Black* variety was adjudged satisfactory. The remaining 19 varieties produced substandard wines.

Nearly 1,000 grape varieties introduced from important grape growing regions of the world are in collection at the Indian Institute of Horticultural Research. Hessaraghatta. In addition, 3,000 grape hybrids have been produced by crossing promising varieties. These grape varieties and hybrids are being evaluated thoroughly for their suitability for making different types of quality wines. In an earlier paper[3], a number of grape varieties were reported to be promising for making dry table wines and a variety for making red dessert wine. The present paper deals with the composition of musts and wines of 30 grape varieties and the quality of wines prepared from these varieties.

Materials and Methods

Nine white grape varieties (Table 1) and 21 red grape varieties (Table 2), which ripened during March-April, 1973 at the Vineyard of the Indian Institute of Horticultural Research, Bangalore were used for the present study. The sampling and analytical procedures employed for must analyses were as described by Negi and Suresh[2]. Dry table wines and red dessert wine were prepared according to the procedures described earlier[3]. Analyses of wine samples for their chemical compositions such as per cent alcohol, total titratable acidity, pH, residual sugar, tannins, and extract content were done according to the methods described by Amerine[1] and A.O.A.C.[4]. Organoleptic evaluation of wine samples was conducted by a panel of 5 judges on a score card with a total score of 10.0. Wine samples with an average score range of 7.5 to 8.5 were considered satisfactory, 8.6 to 9.2 good and above 9.3 excellent.

TABLE 1. COMPOSITION OF MUSTS AND DRY TABLE WINES OF SOME WHITE GRAPE VARIETIES

	MUST				WINE						
Variety	TSS °(Balling)	Total titratable acidity*	Balling-acid ratio	pH	Alcohol (%Vol)	Total acidity**	pH	Residual sugar (%)	Tannin (g./100 ml wine)	Extract (g./100 ml wine)	Average acore
Chenin blanc	22.2	0.57	39.0	3.6	10.3	0.50	3.7	0.27	0.067	1.92	**8.7**
Excelsior	22.2	0.75	29.6	3.3	10.5	0.63	3.5	0.28	0.054	1.18	**7.6**
Lake Emerald	24.0	0.50	48.0	3.6	10.8	0.46	3.8	0.33	0.067	1.57	**4.3**
Malvasia Bianca di Candia	19.2	0.66	29.0	3.3	9.3	0.63	3.4	0.21	0.066	2.08	**6.0**
Ova Pagola	20.2	0.43	47.0	3.5	9.6	0.42	3.4	0.47	0.058	1.21	**8.0**
S. V. 5276	24.0	0.54	44.4	3.4	10.4	0.50	3.7	0.22	0.065	1.41	**8.2**
Sauvignon	21.6	0.61	35.4	3.5	9.6	0.57	3.6	0.25	0.057	0.82	**7.8**
Selection-7	18.2	0.54	34.5	3.8	8.9	0.52	3.7	0.16	0.054	1.36	**4.2**
Vratschanski Misket	21.4	0.61	35.1	3.6	9.9	0.52	3.6	0.20	0.067	1.23	6.8

*Grams tartaric acid/100 ml juice.
**Grams tartaric acid/100 ml wine.
TSS Total soluble solids.

Contribution No. 335

TABLE 2. COMPOSITION OF MUSTS AND WINES OF SOME RED GRAPE VARIETIES

Variety	MUST				WINE						
	TSS (Balling)	Total titratable acidity*	Balling-acid ratio	pH	Alcohol (%Vol)	Total acidity**	pH	Residual sugar (%)	Tannin (g./100 ml wine)	Extract (g./100 ml wine)	Average score
Dry Table wine											
Alicante Bouschet	20.0	0.64	31.3	3.4	9.7	0.64	3.4	0.23	0.179	1.10	5.8
Athens	22.4	0.61	38.7	3.4	10.3	0.57	3.5	0.29	0.111	1.21	5.4
Bangalore Purple	19.8	0.46	43.1	3.6	9.4	0.47	3.5	0.24	0.139	1.02	7.1
Black Champa	22.6	0.64	35.3	3.5	10.8	0.61	3.7	0.22	0.117	2.53	**7.7**
Black Cornichon	19.0	0.71	26.8	3.3	9.1	0.70	3.4	0.29	0.152	1.03	**8.6**
Black Round	18.6	0.50	37.2	3.5	8.8	0.40	3.6	0.25	0.098	1.18	5.8
Cardinal	23.2	0.46	50.4	3.5	10.4	0.42	3.5	0.24	0.128	1.72	7.0
Convent Large Black	22.4	0.66	33.9	3.5	10.6	0.61	3.6	0.31	0.146	2.43	5.4
Champanel	19.6	1.25	15.7	3.3	9.4	0.93	3.3	0.22	0.155	3.07	6.8
Dirriat	21.4	0.54	39.7	3.7	9.8	0.50	3.6	0.19	0.140	1.54	7.2
Gamsa	20.2	0.43	47.0	3.6	9.7	0.43	3.6	0.23	0.098	2.55	**8.2**
Kali Sahebi	19.8	0.61	32.5	3.5	9.6	0.57	3.6	0.25	0.116	0.86	6.0
Muscat di Adda	22.0	0.50	44.0	3.6	10.5	0.50	3.7	0.25	0.108	1.49	6.6
Muscat Hamburg	21.4	0.61	35.2	3.5	10.4	0.53	3.7	0.31	0.130	1.62	3.6
No. 104	21.2	0.86	24.7	3.4	10.2	0.75	3.5	0.22	0.153	2.76	6.3
Red Prince	23.2	0.64	39.0	3.6	10.7	0.61	3.6	0.27	0.142	2.20	**7.5**
Ribier	18.6	0.82	22.7	3.3	9.1	0.75	3.3	0.31	0.098	2.10	4.8
Rubired	21.8	1.11	19.6	3.3	9.8	0.84	3.4	0.30	0.153	2.38	**7.9**
S. 8357	22.4	0.98	22.8	3.1	10.4	0.88	3.3	0.22	0.139	2.27	6.4
Tamiioasa Romineasca	20.4	1.24	16.5	3.3	9.7	0.99	3.4	0.19	0.126	2.41	4.8
Zinfandel	20.4	0.75	27.2	3.6	9.8	0.68	3.6	0.25	0.157	2.63	**7.5**
Dessert wine											
1. Convent Large Black	23.4	0.68	30.3	3.5	18.6	0.65	3.7	4.32	0.149	3.2	**7.5**

*Grams tartaric acid per 100 ml juice
**Grams tartaric acid per 100 ml wine.
TSS. Total soluble solids

Results and Discussion

Analytical data on composition of musts and dry table wines from 9 white grape varieties are presented in Table 1. The variety *Chenin blanc* produced good quality wine with an average score of 8.7. Wines from *Excelsior*, *Ova Pagola*, *S. V.* 5276 and *Sauvignon* were rated as satisfactory. Four varieties, namely, *Lake Emerald*, *Malvasia Bianca di Candia*, *Selection-7* and *Vratschanski Misket* produced substandard wines.

The chemical composition of musts and dry table wines from 21 red grape varieties and that of a red dessert wine from *Convent Large Black* variety are presented in Table 2. The wine prepared from the variety *Black Cornichon* was adjudged good. Wines from the varieties *Black Champa*, *Gamsa*, *Red Prince*, *Rubired* and *Zinfandel* were rated as satisfactory. The remaining 15 varities, namely, *Alicante Bouschet*, *Athens*, *Bangalore Purple*, *Black Round*, *Cardinal*, *Champanel*, *Convent Large Black*, *Dirriat*, *Kali Sahebi*, *Muscat di Adda*, *Muscat Hamburg*, *No.* 104, *Ribier*, *S.* 8357 and *Tamiioasa Romineasca* produced substandard wines.

Dessert wine prepared from *Convent Large Black* variety was found to be of satisfactory quality.

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Effect of Starter Cultures and incubation (period and temperature) on the acidity of *dahi* (curd)—Part II

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The present investigation was carried out with an objective to study the effect of starter cultures used in combinations (1:1) of (1) *S. thermophilus*+*S. diacetilactis* (2) *S. lactis*+*diacetilactis* and (3) *L. bulgaricus*+*S. thermophilus* on the acidity of curd using both buffalo and cow milks. Curd from buffalo milk produced slightly more acidity than the curd from cow milk when noted at a particular time of incubation and with a particular quantity of starter culture. Combination (2) produced the least acidity whereas the combination (3) produced maximum acidity among all the three combinations. Acidity increased rapidly upto 16 hrs. of incubation after which the rise in acidity was not very significant.

Curd, popularly known as 'dahi' in India, is a product of milk fermented by bacteria and has been an article of diet and refrshing beverage since time immemorial. Milk converted into curd by inoculating it with suitable starter culture prolongs the life of the milk for some time without any substantial loss of nutrients in hot countries[1]. As much as about 7.8 percent of the milk produced in the country is converted into curd for direct consumption[2].

A good curd should be firm and of uniform consistency with a pleasant acid flavour and aroma. As reported earlier[6], the pleasant flavour in the curd is produced by the selective starter culture which consists of lactic acid producing bacteria, and flavour producing bacteria fermenting lactose and citric acid of milk respectively.

Whereas Joshi and Ram Ayyar[3] isolated flavour producing bacteria from a few samples of milk and curd, which were capable of developing considerable amounts of acetylmethyl-carbinol and diacetyl, Karnad[4] reported that similar organisms named as *S. diacetilactis aromaticus* had the ability of producing 1% lactic acid together with the high amount of acetoin and diacetyl. Beutler *et al*[5] reported that the most acceptable flavour in cultured butter milk was found to occur at a titratable acidity of 0.83 to 0.88% lactic acid and at a minimum content of acetyl methyl carbinol plus diacetyl of 11 mg/100 gm. The present authors[6] have reported earlier the effects of starter cultures and incubation on the acidity of dahi, using *S. lactis*, *S. thermophilbus* and *S. diacetilactis* organisms individually. In the present investigation, the same organisms with *Lactobacillus bulgaricus* in different combinations in the starter cultures have been selected to study their effects on the development of acidity in the curd.

Materials and Methods

The pure cultures of *Sterptococcus lactis*, *S. thermophilus*, *S. diecetilactis* and *lactobacillus bulgaricus* used in the study were obtained in chalk milk medium from the National Dairy Research Institute, Karnal (Haryana). The cultures were used in three combinations *S. lactis*+*S. diacetilactis*, *S. thermophilus*+*S. diacetilactis* and *L. bulgaricus*+*S. thermophilus*, in 1:1 ratio. The mother and bulk cultures were prepared as reported earlier[6]. The incubation temperatures adopted were 30°C for cultures—*S. lactis*+*S. diacetilactis* and 37°C for combinations of *S. thermophilus*+*S. diacetilactis* and *L. bulgaricus*+*S. thermophilus*. Mixtures of two cultures were prepared by growing them separately and adding equal quantities of each culture as it was difficult to maintain mixed seed cultures as one or the other organism overgrew due to the difference in optimum temperature for growth. The materials (cow and buffalo milks) used, the methodology followed and the tests carried out for the evaluation of the curd quality during this study were the same as reported earlier in part I of this paper[6, - 8].

Results and Discussion

Titratable acidities of the curd as determined at each successive time interval and with each con-

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centration of starter cultures, are shown in Tables 1, 2 and 3. In case of buffalo milk, the combination of *S. thermophilus* and *S. diacetilactis* produced maximum acidity of 0.94% in 24 hr with 2.5% as rate of adding starter culture and 0.57% in 8 hr with the least dose of starter culture. With the increase in time of incubation the acidity also increased. Neglecting the quantity of starter culture used, the increase in acidity during intervals of 8 to 24 hr, 8 to 16 hr and 16 to 24 hr of incubation was found to be from 0.58 to 0.94, 0.58 to 0.89 and 0.89 to 0.94 per cent respectively as is clear from Table 1. The increase in acidity during interval of 8 to 16 hr was much more than that during interval of 16 to 24 hr. In case of cow milk curd, the

TABLE 1. EFFECT OF *S. Thermophilus*+*S. Diacetilactis*, QUANTITY OF STARTER CULTURE AND TIME OF INCUBATION ON THE ACIDITY OF CURDS USING BUFFALO AND COW MILKS

Acidity (% lactic acid) at different concentrations of starter culture.

Time of incubation	1.0%		1.5%		2.0%		2.5%	
(hr)	B	C	B	C	B	C	B	C
8	0.58	0.58	0.61	0.60	0.66	0.65	0.60	0.67
10	0.65	0.66	0.68	0.67	0.72	0.72	0.74	0.74
12	0.72	0.72	0.75	0.76	0.77	0.77	0.79	0.78
14	0.77	0.75	0.79	0.79	0.81	0.82	0.84	0.84
16	0.82	0.83	0.83	0.84	0.87	0.88	0.89	0.89
24	0.90	0.89	0.91	0.90	0.92	0.92	0.94	0.93

B = Buffalo milk curd
C = Cow milk curd.

TABLE 2. EFFECT OF *S. Lactis*+*S. Diacetilactis*, QUANTITY OF STARTER CULTURE AND TIME OF INCUBATION ON THE ACIDITY OF CURDS USING BUFFALO AND COW MILKS

Acidity (% lactic acid) at different concentrations of starter culture.

Time of incubation	1.0%		1.5%		2.0%		2.5%	
(hr)	B	C	B	C	B	C	B	C
8	0.60	0.60	0.62	0.63	0.65	0.65	0.68	0.67
10	0.68	0.68	0.71	0.70	0.75	0.74	0.78	0.77
12	0.78	0.77	0.79	0.79	0.82	0.81	0.83	0.82
14	0.82	0.80	0.84	0.84	0.86	0.85	0.87	0.87
16	0.86	0.86	0.89	0.89	0.92	0.92	0.94	0.93
24	0.95	0.91	0.95	0.93	0.97	0.97	0.99	0.97

B = Buffalo milk curd
C = Cow milk curd

TABLE 3. EFFECT OF *S. Thermophilus*+*L. Bulgaricus*, QUANTITY OF STARTER CULTURE AND TIME OF INCUBATION ON THE ACIDITY OF CURDS USING BUFFALO AND COW MILKS

Acidity (% lactic acid) at different concentrations of starter culture.

Time of incubation	1.0%		1.5%		2.0%		2.5%	
(hr)	B	C	B	C	B	C	B	C
8	0.68	0.68	0.71	0.69	0.73	0.72	0.74	0.73
10	0,76	0.75	0.78	0.77	0.79	0.79	0.83	0.82
12	0.83	0.82	0.86	0.85	0.89	0.89	0.94	0.93
14	0.94	0.93	p.95	0.95	0.97	0.96	0.99	0.98
16	0.99	0.97	1.02	0.99	1.04	1.02	1.06	1.05
24	1.12	1.10	1.14	1.13	1.20	1.17	1.22	1.19

B = Buffalo milk curd
C = Cow milk curd

same combination of cultures produced minimum acidity of 0.58% in 8 hr and maximum acidity of 0.93% in 24 hr of incubation. The increase in acidity (0.58 to 0.89%) was rapid upto 16 hr and afterwards the increase in acidity (0.89 to 0.93%) was very slow upto 24 hr, following the same pattern as that of buffalo milk.

In case of buffalo milk used, the mixed culture (*S. lactis*+*S. diacetilactis* produced maximum acidity of 0.99% in 24 hr and minimum acidity 0.60% in 8 hr of incubation. The acidity increased from 0.60 to 0.99, 0.60 to 0.94 and 0.94 to 0.99 per cent during intervals of 8 to 24 hr, 8 to 16 hr and 16 to 24 hr of incubation respectively as is clear from Table 2. The acidity developed rapidly from 8 to 16 hr, afterwards the rise in acidity was not significant upto 24 hr. In case of cow milk curd a minimum acidity of 0.6% in 8 hr and maximum acidity of 0.97% in 24 hr of incubation was noticed as is clear from Table 2. From 8 to 24, 8 to 16 and 16 to 24 hrs of incubation the acidity increased from 0.60 to 0.97, 0.60 to 0.93 and 0.93 to 0.97% respectively, following the same trend of rise in acidity as in the case of buffalo milk. Also this combination of cultures produced less acidity as compared with the previous combination in this study.

The combination of cultures *S. thermophilus*+*L. bulgaricus* produced maximum acidity of 1.22% in 24 hr and minimum acidity of 0.68% in 8 hr as is evident from Table 3. Results showed that this mixed culture differed from other two mixed cultures used in this investigation. The acidity kept on rising as the time of incubation increased and also this culture produced the maximum acidity in comparison with other two mixed cultures, These results have been found in agreement with the findings of Laxminarayana[9] who reported that *L. bulgaricus* alone produces 1.4% acidity in 24 hr. As this culture (*L. bu garicus*) was used with *S. thermophilus* (which produced maximum acidity of 0.84% in 24 hr (Sharma and Jain[6]), combination of these two cultures produced the maximum acidity of 1.22% in 24 hr in the present investigation. Also Pette[17] reported that *S. thermophilus* is less tolerant to higher acidity than *L. bulgaricus*, using 1 to 2.5% incoulum; which also supported the present findings. In case of cow milk curd, this mixed culture under study produced a minimum acidity of 0.67% in 8 hr and maximum acidity of 1.19%, in 24 hr of incubation. The acidity increased from 0.67 to 1.19, 0.67 to 1.05 and 1.05 to 1.19 per cent in intervals of 8 to 24, 8 to 16 and 16 to 24 hrs of incubation respectively, following the same trend as in the case of buffalo milk curd. Also acidity in the curd using this combination developed at the fastest rate as compared with the acid development rate in curds using other two combinations of cultures, in cases of both, the buffalo and cow milk curds.

The results in Tables 1 to 3 showed that the curd from buffalo milk produced higher acidity at a particular time of incubation and at a particular concentration in a combination of starter cultures than that prepared from cow milk under identical conditions.

The results revealed that the curds of both buffao and cow milks under study were, in general, free from coliforms and yeasts and molds. Out of 72 samples of buffalo milk curd, three samples were found positive for coliform tests and five showed the presence of yeasts and molds. Out of 72 samples of cow milk curd, five gave positive presumptive coliform test and four showed the presence of yeasts and molds.

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A Spreader-Roller Device for Objective Evaluation of Rolling Characteristics of *Papad* Doughs

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Use of a spreader-roller device consisting of a metallic roller running down an inclined plane has been described for evaluating the rolling property of *papad* doughs in objective terms of increase in the area of dough strip. The recipes based on blends of different pulses and cereals showed considerably higher increases in area (108–154 per cent) indicating thereby the desirable rolling property of these doughs. In contrast, the doughs based on blackgram with only carbonate or with starches were difficult to roll as indicated by lower increases in area (72–92 per cent).

Till recently, no instrument has been used to express objectively, the hand-feel, dough consistency and rolling property of *papad* doughs based on different recipes. Use of Brabender Farinograph as a tool in objective evaluation of different *papad* doughs has been discussed[1, 2]. However, the stretching or the rolling property of the *papad* dough, which has so far been described only in subjective terms like easy-to-roll or smooth-to-roll could not be expressed in objective terms using Brabender Farinograph. In this communication, a spreader-roller device has been tried as a tool in objective assessment of rolling characteristics of different *papad* doughs containing blackgram.

Materials and Methods

Various *papad* doughs based on blackgram, with or without additives as well as its blends with greengram, horsegram, maize, wheat, rice and different starches were prepared in a Hobart mixer according to the methods described earlier[1]. Of the several blends based on different ingredients tried, only those selected ones and referred to in earlier studies[1] have been included in the present investigations.

Rolling property using a spreader-roller (*Fig.* 1): An inclined wooden plane of 900 mm in length and 100 mm in width was adjusted to an angle of 1°4′, so that a solid steel cylinder weighing 3.8 kg and having a diameter of 90 mm and length of 80 mm could just start rolling down the inclination on its own. The top surface of the incline was covered with a glass sheet, beneath which a centimeter graph paper was placed.

The doughs based on different recipes (obtained by kneading the ingredients in a Hobart mixer) were passed through a hand-operated macaroni extruder to obtain a continuous strip of 1 mm thickness and 40 mm width. From this strip, 10 mm pieces were cut and placed centrally along the length of the incline 400 mm away from the top end. The roller was then allowed to roll down twice over the strip from the top end of the incline to the bottom, so as to obtain a significant increase in the area of the strip.

The final area of the strip stretched under the weight of the roller was measured by tracing the outline of the strip on a centimeter graph sheet. The increase in the area of *papad* strips was taken as the index of the stretching or the rolling property of the *papad* dough. For each of the doughs based on different recipes including the one for blackgram *papads*, with or without sodium carbonate and/or common salt, this trial was repeated ten times. The time taken for the roller to traverse its path along the incline was also recorded.

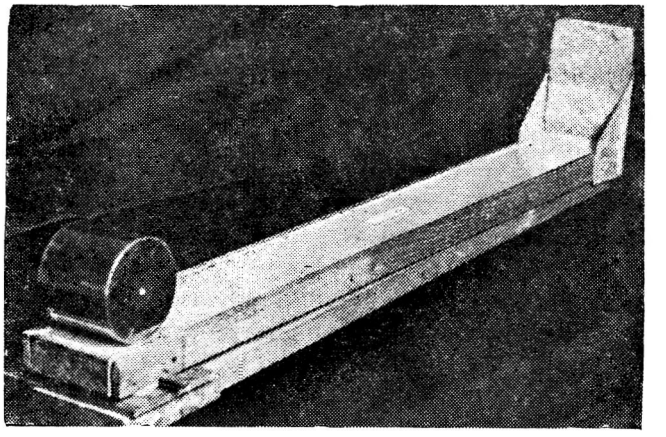

Fig. 1. The steel roller and inclined plane used for recording the increase in area of the strip (seen in the centre of the inclined plane) of *papad* dough.

Results and Discussion

The rolling characteristics of *papad* doughs prepared from different blends of cereals, pulses, starches and additives as indicated by the increase in area of the dough strip are given in Table 1.

Effect of additives on blackgram dough: It is interesting to note that blackgram dough with or without 1 per cent sodium carbonate had practically the same rolling property with an average of 76 per cent increase over the initial area. However, the dough containing sodium carbonate was tough, while the dough without carbonate was fairly soft. Both these doughs were neither cohesive nor easy to roll. As against this, each of the blackgram doughs containing 7 per cent salt alone or 7 per cent salt and 1 per cent carbonate was cohesive and had a comparable increase in the area of 100–110 per cent on an average. This clearly brings out the role played by commonsalt in obtaining a soft and easy to roll dough of desired consistency, as reported earlier[2].

Rolling property of doughs based on different recipes: Depending on the ingredients used for replacing blackgram, different doughs containing other pulses, cereals and starches covered a wide range (72–154 per cent) of increases in area. Most of the doughs were soft and easy to roll.

The doughs containing starches as such or in gelatinised form required higher quantity of water to obtain a dough of desired consistency and rolling property. On the contrary, the increase in the area of these doughs were somewhat lower, viz. 72–92 per cent. In spite of the significantly higher water addition of 60 per cent, the increase in area was minimum in the case of dough based on blackgram and gelatinised starch and was comparable to the tough blackgram dough containing carbonate only. Also, this dough was somewhat tough and difficult to roll.

The maximum increase in area of over 150 per cent was observed in the case of a dough based on 50:50 blend of blackgram dough and wheat may be attributed to the unique combination of mucilaginous and gluten components of the two ingredients respectively.

In spite of the high increases in area of 132 to 137 per cent observed, in case of doughs based on blends of blackgram with rice or horsegram, these doughs were somewhat less cohesive and the edges of the strips had a slightly cracked appearance. Though the final area of blackgram doughs based on blackgram blend with greengram or maize were practically the same (873–883 sq. mm.), the dough containing greengram required 5 per cent less of water than that containing maize.

TABLE 1. ROLLING CHARACTERISTICS OF *Papad* DOUGH PREPARED FROM BLENDS OF CEREALS, PULSES AND STARCH

Sl. No.	Ingredients*		Water (ml.)	Time** (sec.)	Final Area Range (sq. mm.)	Final Area Average*** (sq. mm.)
1	Blackgram flour		45	4.6	673–738	706
2	,,	+Salt (7%)	45	4.5	781–835	804
3	,,	+Sodium carbonate (1%)	45	4.6	691–725	705
4	,,	+Salt (7%)+sodium carbonate (1%)	45	4.5	809–854	833
5	Blackgram flour Greengram flour	(20) + (80)	40	4.5	830–919	873
6	Blackgram flour Wheat flour	(50) + (50)	45	4.4	971–1066	1015
7	Blackgram flour Maize flour	(80) + (20)	45	4.5	864–896	883
8	Blackgram flour Corn starch Gelatinised starch	(70) + (25) + (5)	50	4.5	752–792	766
9	Blackgram flour Corn starch	(80) + (20)	45	4.6	703–750	724
10	Blackgram flour Rice flour	(50) + (50)	45	4.5	934–964	949
11	Blackgram flour Gelatinised starch	(80) + (20)	60	4.5	674–708	689
12	Blackgram flour Horsegram flour	(20) + (80)	45	4.5	913–948	927

*For Sl. Nos. 5–12, figures in the parentheses indicate the proportions of the main ingredients used in the *papad* dough. In addition, Sl. Nos. 5–11 contained 7 parts of salt and 1 part of sodium carbonate, while Sl. No. 12 contained only 7 parts of salt.

**Taken by the roller to traverse a distance of 900 mm along the inclined plane.

***Average of 10 trials. Initial area of the dough strinp: 400 sq. mm..

To summarise, the rolling or stretching characteristics of doughs based on different *papad* recipes can be evaluated objectively using a metallic roller on an inclined plane. The increase in area of the strip under the pressure of roller of known weight can be used as an index of rolling characteristics of the dough. The nature of the ingredients and the additives used in the recipe influenced the increase in area of the dough strip excepting the doughs containing starches. The high increases in area (873–1015 sq. mm.) observed for most of the doughs based on cereals and pulses indicated a soft and easy to roll nature of the dough indicated by subjective evaluation.

The observations also brought out the necessity of salt in the dough for obtaining a dough of desired softness as well as better rolling characteristics No relation was found to exist between the rolling property of the dough and expansion of *papad* on frying

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Keeping Quality of Fresh Milk with Hydrogen Peroxide as a Preservative

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The possibility of enhancing the shelf-life of fresh fluid milk has been investigated. It has been possible to preserve pasteurized milk for a period of about 7–10 days with the addition of hydrogen peroxide in the concentration range of 0.10 to 0.40 g per litre.

The use of preservatives to extend the keeping quality (KQ) and to comouflage the poor standard of unhygenically collected and produced milk is to be discouraged for various reasons[1-3]. However, preservatives like hydrogen peroxide have been suggested in exceptional circumstances in warm countries where, collection, refrigeration, transportation and distribution facilities are far from satisfactory and adequate[1-3]. The proper collection and distribution of fresh milk poses an enormous problem for use by the Army Formations deployed in forward areas because it curdles by the time it reaches them from the supply points located in the hinterland. It was in this context that the work of Nambudripad *et al.*[2] was viewed with interest and further work planned to assess the possibility of its application in case of supplies of fresh milk to the Armed Forces where a 6–7 day period is required for milk to reach certain foremost Army Formations. With this in view, the present studies on the enhancement of shelf-life of milk with the use of varying concentrations of hydrogen peroxide and its effect on different initial bacterial loads were undertaken.

Materials and Methods

The experiments were initiated with the KQ of reconstituted milk as a standard reference. Reconstituted milk was prepared from Amul whole milk powder (spray dried) using the Gaco mini dairy minor (milk recombiner). Raw market milk and dairy pasteurized milk were obtained from the local dairy. The raw milk was a representative mixture of cow's and buffaloe's milk collected at village levels and pooled at the dairy for subsequent pasteurization and distribution.

Laboratory pasteurization was done (holder method) in sterilized one litre conical flasks in batches of 500 ml of milk per flask.

250 ml of milk from each of the above samples was aseptically transferred into 500 ml sterilized/unsterilized conical flasks and hydrogen peroxide of 100 vols. (analytical grade) added after estimating its strength, and dilution to give the final desired concentration in each flask. The hydrogen peroxide milk mixture was then uniformly shaken to give a homogenized product and stored under ambient conditions (30°C). Each treatment had 5 replicates and a control wherein no peroxide was added. The values presented (Tables 1–4) are an average of two such experiments.

The samples were analysed initially and on treatement and storage periodically at intervals of 24 hr for standard plate count (SPC/ml), coliform count/ml, pH, acidity, Organoleptic evaluation and clot on boiling test (COB). The SPC and Coliform count were determined by following the colony count method[5], using plate count agar for SPC and a medium Violet Red-Bile Agar (VRBA) for the Coliform Count. The acidity was determined as lactic acid[6,7]. Organoleptic evaluation was carried out after subjecting the samples to COB test.

Results and Discussion

Reconstituted milk: The reconstitued milk was treated with 0.0188 and 0.025% hydrogen peroxide levels and stored under ambient conditions and at 37°C for its KQ. The initial bacteriological status indicated that the SPC/ml for reconstituted, unpasteurized and pasteurized milk was between 13000 and 24000/ml and 300 and 1200/ml respectively. Coliforms were absent in both the cases. The KQ results indicated that without hydrogen peroxide treatment the samples curdled within 24 hr irrespective of the container (sterilized/unsterilized) used and the storage condition employed. Pasteurization alone without hydrogen peroxide treatment also served no purpose. But when the reconstituted milk was treated with

0.0188% hydrogen peroxide followed by pasteurization the KQ was 24 to 48 and 48 to 72 hr in unsterilized and sterilized flasks, respectively. With 0.025% hydrogen peroxide the KQ was 168 hr in unsterilized flasks (further studies were discontinued since the samples curdled). However, in the case of samples in sterilized flasks, the KQ was 192 hr. (Table 1).

Some experiments carried out to combine heat treatment with peroxide treatment[2], giving a reaction time of 1 to 3 hr after addition of hydrogen peroxide and then pasteurization, indicated a slight improvement in the KQ depending on the concentration of hydrogen peroxide, type of container (sterilized/unsterilized) and the reaction time studied.

Raw market milk: The bacteriological quality of the many samples examined indicated the SPC/ml to vary from 132 million to 260 million and coliforms/ml from 0.27 million to 1.9 million. The pH of the samples was between 6.5 and 6.8 and titratable acidity from 0.177 to 0.188 per cent lactic acid. (The initial SPC/ml and coliforms/ml of the experimental samples varied from 161 to 196 million and 0.78 to 1.5 million respectively.

Raw milk treated with 0.0125, 0.0188 and 0.025% hydrogen peroxide without pasteurization, curdled within 24 hr under ambient conditions, irrespective of the storage container used. But when pasteurization followed peroxide treatment the KQ was 24, 48 and 96 hr in sterilized containers. However, the KQ was further enhanced when peroxide treatment followed pasteurization. The KQ in unsterilized containers was generally very poor. Experiments combining heat treatment with peroxide treatment indicated poor KQ since the samples curdled in 24 hr of treatment. This is attributed to the initial quality of Raw milk of high bacterial load.

The experiments carried out indicated that the heat treatment should invariably be carried out prior to the peroxide treatment to raw milk and not *vice versa*. This is also necessary from the point of view of applicability of the treatment of peroxide to milk as a preservative. Addition of peroxide after pasteurization would be more appropriate since its presence in milk at this stage would keep the qualify better as a preservative for reasons enumerated below:

(*i*) The addition of peroxide to raw milk cannot be employed by individual milk producers to mask the insanitary conditions of production and therefore should not be encouraged.

(*ii*) Retention of the peroxide is more when added after pasteurization as compared to the addition and heat treatment that follows since during pasteurization most of the peroxide is lost due to heat. Rapid decomposition of hydrogen peroxide takes place by the catalase activity associated with high bacterial population and leucocytes of the milk.[1,2]

(*iii*) Pasteurized milk in which most of the catalase is destroyed by heat can be preserved with less hydrogen peroxide than is normally used for raw milk[1]. Hydrogen peroxide required to ensure satisfactory preservation increased with increase in catalase index of milk.[8]

Dairy pasteurized milk: The initial SPC/ml and Coliforms/ml were 1.47×10^5 and from nil to less than 30, respectively. The presence of coliforms in pasteurized milk is attributed to post pasteurization contamination.[9,10] A further reduction in the total count may be possible if double and even triple pasteurization is given.[11]

Treatment with 0.0125, 0.0188, 0.02, 0.025, 0.03 and 0.04% levels of hydrogen peroxide and storage under ambient conditions for KQ revealed coliforms to be absent after treatment. The KQ of the samples increased with increasing concentration of hydrogen peroxide employed (Tables 2, 3 and 4). Samples without hydrogen peroxide curdled within 24 hr. The KQ of milk with 0.0125, 0.0188, 0.02, 0.025, 0.03 and 0.04% hydrogen peroxide was 24, 72, 72, 120, 144 and 168 hr respectively in sterilized contianers. In unsterilized containers with a level of 0.02, 0.03 and 0.04% hydrogen peroxide it was 24, 144 and 168 hr respectively. However, with the latter two concentrations, bitterness and curdling were noticed in a few cases after 144 and 168 hr and further KQ observations were discontinued. In a few other cases, the samples in unsterilized flasks revealed the presence of mould growth in the cream layer after 5–6 days of storage. This has also been reported in the case of KQ under cold storage conditions of normally and aseptically packaged pasteurized milk.[12,13]

The problem of bitterness in milk encountered by us may be attributed inter alia to the protein digesting activity of the aerobic spore formers and *Streptococcus liquefaciens*, which usually results in a decidedly bitter flavour[14]. In a few other cases, the curdling that occured while little or no acid production may be associated with bacterial action on proteins of the milk, noticed in raw milk produced under insanitary conditions and sometimes in pasteurized milk as well and is called sweet curdling.

Laboratory pasteurized milk: The initial SPC/ml and coliforms/ml were 1.3 to 1.55×10^5 and nil respectively. Laboratory pasteurization did not indicate any significant reduction in the Total Plate Count as compared with dairy pasteurized milk but it did show a total absence of coliforms. The KQ of samples treated with 0.0125, 0.0188 and 0.025%

TABLE 1. KEEPING QUALITY OF RECONSTITUTED PASTEURIRIZED MILK WITH 0.025% H_2O_2 UNDER AMBIENT CONDITIONS

Treatment	Before treatment O hr.	After treatment 24 hr	72 hr	120 hr	168 hr	KQ in hr
Milk treated in sterilized flask						
(*a*) SPC/ml	2.6×10^2	6×10	$<3\times10$	$<3\times10$	4.5×10^1	
(*b*) pH & acidity	7.1	7.0	7.0	7.0	6.9	
(% lactic acid)	0.129	0.134	0.141	0.153	0.154	192
Milk treated in unsterilized flask						
(*a*) SPC/ml	2.7×10^2	6.6×10^1	7.5×10^1	4.4×10^2	6.9×10^1	
(*b*) pH & acidity	7.2	7.2	7.2	7.2	7.2	
(% lactic acid)	0.126	0.136	0.142	0.147	0.152	168

TABLE 2. KEEPING QUALITY OF RAW MILK, DAIRY PASTEURIZED MILK AND LABORATORY PASTEURIZED MILK WITH 0.0125% H_2O_2

Treatment	Storage period hr.	SPC/ml	pH	Acidity (% lactic acid)	C.O.B.
Raw market milk+	0	1.61×10^8	6.65	0.182	–ve
H_2O_2+pasteurization	24	3.8×10^5	6.80	0.168	–ve
	48	—	—	—	+ve
Dairy pasteurized	0	1.47×10^5	7.05	0.148	–ve
milk+H_2O_2	24	4.9×10^4	7.00	0.149	–ve
	48	—	—	—	+ve
Laboratory	0	1.55×10^5	6.95	0.151	–ve
pasteurized milk+	24	3.5×10^4	6.75	0.168	–ve
H_2O_2	48	—	—	—	+ve

TABLE 3. KEEPING QUALITY OF RAW MILK, DAIRY PASTEURIZED MILK AND LABORATORY PASTEURIZED MILK WITH 0.0188% H_2O_2

Treatment	Storage period hr.	SPC/ml	pH	Acidity (% lactic acid	C. O. B.
Raw market milk+	0	1.96×10^8	6.75	0.180	–ve
H_2O_2+Pasteurization	24	4.80×10^3	6.90	0.163	–ve
	48	4.60×10^5	6.75	0.168	–ve
	72	—	—	—	+ve
Dairy pasteurized	0	1.47×10^5	7.05	0.148	–ve
milk+H_2O_2	24	3.65×10^2	7.00	0.149	–ve
	48	7.00×10^2	6.95	0.151	–ve
	72	2.41×10^5	6.90	0.163	–ve
	96	—	—	—	+ve
Laboratory pasteurized	0	1.30×10^5	7.00	0.149	–ve
milk+H_2O_2	24	8.00×10	7.00	0.149	–ve
	48	1.20×10^2	7.00	0.149	–ve
	72	2.73×10^3	6.95	0.151	–ve
	96	3.57×10^3	6.95	0.151	–ve
	120	6.10×10^4	6.90	0.151	–ve
	144	2.15×10^5	6.80	0.168	–ve
	168	—	—	—	+ve

TABLE 4· KEEPING QUALITY OF RAW MILK, DAIRY PASTEURIZED MILK AND LABORATORY PASTEURIZED MILK WITH 0.125% H_2O_2

Treatment	Storage period hr.	SPC/ml	pH	Acidity (% lactic acid)	C. O. B.
Raw market milk+ H_2O_2+pasteurization	0	1.61×10^8	6.85	0.165	-ve
	24	3.40×10^2	7.00	0.151	-ve
	48	6.35×10^2	7.00	0.163	-ve
	72	4.50×10^3	6.95	0.165	-ve
	96	2.25×10^5	6.75	0.180	-ve
	120	—	—	—	+ve
Dairy pasteurized milk+ H_2O_2	0	1.47×10^5	7.05	0.148	-ve
	24	9.00×10	7.00	0.149	-ve
	48	1.50×10^2	7.00	0.149	-ve
	72	1.65×10^2	6.95	0.151	-ve
	96	6.95×10^3	6.90	0.154	-ve
	120	4.18×10^4	6.75	0.170	-ve
	144	—	—	—	+ve
Laboratory pasteurized milk+ H_2O_2	0	1.30×10^5	7.00	0.149	-ve
	24	3.00×10	6.90	0.151	-ve
	48	3.00×10	6.95	0.151	-ve
	72	3.00×10	6.95	0.151	-ve
	96	4.50×10^1	6.95	0.151	-ve
	120	6.20×10^2	6.95	0.151	-ve
	144	5.50×10^2	6.95	0.151	-ve
	168	1.06×10^3	6.95	0.151	-ve
	192	1.26×10^3	6.90	0.154	-ve
	216	1.40×10^3	6.90	0.154	-ve
	240	1.75×10^4	6.90	0.154	-ve

hydrogen peroxide levels in sterilized conical flasks under ambient storage conditions was 24, 144 and 240 hr, respectively (Tables 2, 3 and 4).

These experiments clearly show that only pasteurized milk could be preserved for periods of the order of 7 to 10 days (168–240 hr) using hydrogen peroxide as a preservative in the concentration range of 0.025 to 0.04%. Furthermore good quality milk of low bacterial load is desirable for the purpose of preservation which would permit the use of low concentrations of hydrogen peroxide. For consumption in fluid state the maximum permissible limit of hydrogen peroxide is in the region of 0.10 to 0.40 g/litre[1].

It should also be ensured that treatment of milk with hydrogen peroxide is carried out only after the pasteurization process.

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RESEARCH NOTES

EFFECT OF HOMOGENIZATION AND PASTEURIZATION ON CREAMING ABILITY OF BUFFALO MILK

Average percentage cream layer volume in raw standardised buffalo milk was observed to be 6.96. It was reduced to 5.58 and 6.0% by holder and high temperature method of pasteurization respectively. No cream layer was observed in either single or double stage homogenized milk, homogenized either before or after pasteurization. The USPHS index of homo genized standardised buffalo milk was found to be within the acceptable limits as recommended for efficient homogenization.

Cream layer formation in milk is a function of Stocke's law and is explained by the agglutinin and electro kinetic theories[1]. Heat treatment and homogenization commonly decrease the cream layer formation. Marquardt and Dahlberg[2] have presented a graphic illustration of time/temperature relationship in correlation to creaming ability. Heat treatment of milk between 61.05–62.2°C for 30 min resulted in slight but not marked decrease in creaming properties[3] at 62.2°C or higher temperature the effect was more pronounced. Halloran,[3] Halloran and Trout[4] reported prevention of cream layer in milk subjected to homoginization at minimum pressure of 102.7 kg/cm^2. Brueckner[5] observed no advantages in using double stage homogenization over signle stage in preventing the cream layer formation. Market milk is commonly homogenised at pressures of 180 kg/cm^2 and 40 kg/cm^2 at 1st and 2nd stage respectively. United States Public Health Services (USPHS)[6] has adopted the extent of creaming ability in cow milk as an index of homogenization. According to Whitaker and Hilker[7] properly homogenized market milk shows USPHS index between 6 to 9. The work reported in this paper deliniates the effect of pasteurization and Homogenization on standardised buffalo milk since the data in this respect is lacking.

Bulk Buffalo milk from the Experimental Dairy of NDRI, Karnal standardised to 6% fat was used in this experiment.

Pasteurization methods adopted were as follows.

1. *Holder pasteurization*: Standardised milk was held in a water bath maintained at 80°C so as to raise the temperature of milk to 63°C in about 5 min. Later the milk was held in another water bath at 63°C for a period of 30 min.

2. *High Temperature Heating* (*HTH*): Standardised milk was held in a water bath maintained at 80°C such that milk was allowed to come to a temperature of 73°C within 15 min and maintained at this temperature for 15 sec.

Homogenization: The sequence of homogenization was (*i*) Homogenization after Pasteurization (HAP) (*ii*) Homogenization prior to pasteurization (HPP).

Milk was homogenized in Rannie piston type homogenizer[3] the pressures for 1st and 2nd stage being 180 kg/cm^2 and 40 kg/cm^2 respectively. Standardised raw milk samples were preheated to 60–63°C before homogenization and then pasteurized (Holder or high temperature heating). The second lot was pasteurized (Holder and high temperature heating) and later homogenized at 60–63°C. All samples were cooled first with tap water and then on a surface cooler and maintained at 4–6°C for 4 hrs prior to the analytical procedures.

Determinations of percentage cream layer volume: The percentage cream layer volume was measured by taking 99 ml of milk sample containing Formalin (1 ml formalin in one litre milk) in 100 ml graduated cylinder. One ml saturated solution of Sudan III dye in 70 per cent alcohol was added to each of the cylinder and mixed well. The samples were kept at 4–6°C. After 48 hr of quiescent storage the formation of cream layer was noted.

Determination of USPHS Index: The method recommended by USPHS[6] was modified in the absence of the availability of a quart size milk bottle as follows:

Five hundred ml milk samples were transferred to 500 ml milk bottles and sealed with the aluminium cap. Milk samples were then stored in quiescent state for 48 hr at 4–6°C. After 48 hr of storage bottle caps were removed, a stiff wire was passed around the inner surface of the bottle neck to loosen any adhering butter fat. Forty five ml of the top layer were sucked directly from each of the bottle into a graduated pipette and thoroughly mixed and tested for fat content by Gerber method. Fat percentages were also determined by keeping milk in these bottles after proper sampling by Gerber method. USPHS index was determined as fat percentage of the top portion minus fat percentage of the bulk of milk after removal of the top aliquot divided by fat percentage of top portion $\times$ 100.

From Table 1 it is observed that the average cream layer volume was reduced by 19.8 and 14.8 per cent by holder and high temperature pasteurization respectively. The greater reduction in creaming ability of holder pasteurized standardised buffalo milk may be attributed to the denaturation of agglu-

TABLE 1. CREAM LAYER VOLUME (%) UNDER DIFFERENT METHODS OF PASTEURIZATION AND SEQUENCE OF HOMOGENIZATION

Sequence of homogenization	Raw milk	*Unhomogenized* Pasteurization Holder	HTH
H A P	7.38	5.92	6.46
H P P	6.54	5.23	5.54
Average	6.96	5.58	6.00
%Reduction	—	19.8	14.4

In homogenized milk under both methods of Pasteurization no cream layer formation was observed.

TABLE 2. AVERAGES OF USPHS INDEX OF HOMOGENIZED MILK (AVERAGE OF 6 TRIALS)

Particulars	Holder Pasteurization Single stage	Holder Pasteurization Double stage	HTH Pasteurization Single stage	HTH Pasteurization Double Stage
H P P	5.29	3.49	7.19	4.96
H A P	8.30	4.93	8.99	4.59
Av for method of pasturization of homogenizes milk.	Holder 5.50		HTH 6.43	
Av for stages of homogenization	Single stage 7.44		Double stage 4.49	
Av for method of sequence	H.A.P. 5.50		H.P.P. 6.43	

tinin to a greater extent in holder process than in high temperature heating method.

Single stage and double stage homogenization either after pasteurization or before pasturization resulted in loss of creaming ability. This is in agreement with the work reported by Halloran[3] and Halloran and Trout[4] for cow milk and can be attributed to denaturation of agglutinin, increased viscosity of milk and increased casein adsorption on the fat globules after homogenization.

The two methods of pasturization were highly significant in their effect. The holder pasturization reduced the USPHS index on an average by 0.93. Highly significant differences in the index were revealed between stages of homogenization. Double stage homogenization gave lower USPHS values than single stage homogenization. The interaction between different stages of homogenization and different methods of homogenization were insignificant indicating that the effect of single stage and double stage was almost the same in both the methods of pasteurization. Homogenization prior to pasteurization gave lower USPHS index compared to homogenization after pasteurization.

Interaction between different methods of pasteurization and homogenization was found to be insignificant indicating that the effect of homogenization prior or after pasteurization was almost the same on both holder and HTH pasteurization. The interaction effect of the methods of psateurization and stages of homogenization were found to be significant at 5% level. The interaction effect between methods of pasteurization, stage of homogenization and sequence of homogenization was found to be insignificant.

Acknowledgement

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DIMETHOATE RESIDUES ON PEACHES

Residues of dimethoate on peach fruits were estimated after spraying at concentrations of 0.025, 0.050 and 0.075%, for the control of peach fruit fly. The deposits in all cases except 0.075% were below the tolerance limits of 2.0 ppm fixed by EPA. Even in the case of 0.075% the residue was within the tolerance limit 7 days after spraying. The residue further diminished rapidly, the reduction was more during the month of

June than during May. The estimation was done by g as-liquid chromatography using modified flame ionization and electron capture detectors.

Peach (*Prunus persica* L.) is severely attacked by fruit fly (*Dacus zonatus.*) At times the attack is so severe that not a single fruit is left unattacked on a plant. Damiano[1] observed that one or two aplli-cations of dimethoate at 0.05 % were effective against *Ceratitis capitata* on summer ripening peaches. Keiser Irving[2] observed dimethoate effective against *D. dorsalis*, *D. cucurbitae* and *Certatis Capitata* on peaches. Sohi and Bindra[3] used dimethoate spray in 3 concentrations for the control of this pest. It was thought necessary to investigate the implications of the residues of dimethoate on marketable fruits from the same experiment, before clearing it for recom-mendation.

The experiment was conducted during 1972 at Regional Fruit Research Station, Bahadurgarh (Patiala). Two different orchards of peaches were sprayed individually with dimethoate at 0.025, 0.050 and 0.075 %, using 3 litres of spray material per plant. The first orchard was treated on May 26 1972 at the stage of maturity and the second one on June 13, 1972 at the full ripe stage. In each treatment 28 plants were treated out of which 6 plants were taken for observation and sampling. From each plant 10 fruits were taken randomly. Total fruits of a treatment were pooled and representative sample of 500 g was taken to the laboratory. The fruits were collected 0, 7, 14 and 20 days after the treatment in both cases.

The treated fruits were chopped, mixed throughly and 25 g sample was drawn from each lot. For extrac-tion two replications from each sample were taken.

Method of Mills *et al.*[4] for the extraction of chlori-nated hydrocarbon was followed with some modifi-cations. The sample containing 25 g of fruits was taken in a waring blender and was extracted with 75 ml of acetonitrile at high speed. The extract was taken in a Buchner funnel and filtered by suction after giving 3 washings with acetonitrile. The parti-tioning was done first with hexane (to remove pig-ments) in the presence of distilled water. The aceton-itrile water layer was then extracted twice with 50 ml of chloroform. The chloroform was then evaporated and 10 ml of acetone was added to the flask. The estimation was done with the help of gas-liquid-chromatography using both modified flame ionization and electron capture deterctors, (Fig 1). The con-ditions of GLC were essentially the same as described by Bhalla *et al.*[5] and Jaswant Singh *et al.*[6] The retention times were 387 minutes for FID & EC

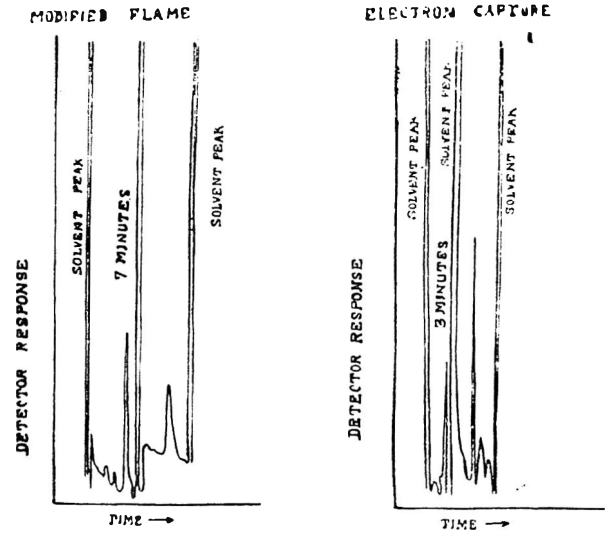

FIG 1 GRAPHS SHOWING THE PEAKS OF DIMETHOATE

respectively. No interference was observed onGL C when a cleaned blank extract was injected. Recovery experiments were also performed to see the efficiency of the procedure, which ranged from 80.60 to 83.30 per cent. The results were confirmed qualitatively by TLC.

The data given in Table 1 show that only in the case of 0 day samples of 1st experiment (0.075 %), the initial deposit was more than tolerance limit of 2.0 ppm fixed by EPA for dimethoate on pear (no data available on peach). In all the other cases either the residues were below the tolerance limits or not detectable. It is further seen that the reduction in initial deposit which occurred within a week after the treatment varied from 36 to 50 %. Only in 0.075 % treatment residue could be detected upto 20 days and about 88 % less than the initial deposit.

In the second experiment the initial deposit due to 0.075 % spraying was 2.0 ppm while in others the quantities were less than 2ppm. Residues could be detected only upto 14 days even in highest does of 0.075 %.

de pietri Tonnelli *et al.*[7] also studied the residues of dimethoate 0.03 % sprayed on peaches to run off. They found the residues of 1.1 ppm after 12 days and 0.5 ppm after 23 days. The higher residues in that case may be due to more amount of spray material used for plant. Chilwell and Beecham[8] detected only 1.1 ppm residue of dimethoate on peaches after applying 0.353 % after 8 days.

de Pietri Tonnelli *et al.*[7] have reported that the half-life values of dimethoate on peaches in June to September period varied from 4 to 12 days. In the

TABLE 1. RESIDUES OF DIMETHOATE ON PEACHES AT DIFFERENT INTERVALS

Days after treatment	0.025% concn.			0.050% concn.			0.075% concn.		
	Residue ppm (FID)	% Reduction	Residue (ppm) (EC)	Residue ppm (FID)	% Reduction	Residue (ppm) (EC)	Residue ppm (FID)	% Reduction	Residue (ppm) (EC)
0	1.0*	—	1.1	1.8	—	1.6	2.5	—	2.7
	0.5	—	0.5	1.3	—	1.4	2.0	—	2.1
7	0.5	50	0.5	1.0	44	1.1	1.6	36	1.14
	ND	—	—	0.4	69	0.3	0.9	55	1.1
14	ND	—	—	0.4	77	—	1.0	60	0.9
	ND	—	—	ND	—	—	0.2	90	0.4
20	ND	—	—	ND	—	—	0.3	88	0.4
	ND	—	—	ND	—	—	—	—	—

*First and second figures obtained for the same day indicate the data collected in the 1st and 2nd experiment respectivily.
Based on mean of two replications.
No peak was obtained when 4 μl out of 5 ml cleaned extract was injected.

present investigation it was found to be 6.87 days in July. This shows that the results are within the range of earlier report.

The estimations were also done with electron capture detector and there was not much difference between both the estimations. (Table 1).

Dimethoate is converted to many metabolites in plants e.g. oxygen analog, desmethyl dimethoate, oxycarboxy derivatives, thiocarboxy derivatives etc. Among them oxygen analog is the important one from toxicity point of view as reported by Dauterman *et al.*[9] Alongwith parent compound, 3 other compounds were also detected by GLC but only one compound was confirmed with TLC. Steller and Pasarela[10] have reported that none of the several colorimetric procedures could distinguish dimethoate from its oxygen analog.

Due to the non-availability of pure-metabilities, different packing materials of GLC columns and flame photometric detector, confirmation and quantitative evaluation of the metabalities could not be done.

It has been observed from the available literature that the amount of oxygen analog of dimethoate present in/on plant parts varies from o ppm (immediately after spraying) to 8–10 PPM (10 to 15 days after spraying). Santi and de Pietri Tonnelli[11] have also stated that the quantity of oxygen analog is usually negligible. However, before this insecticide is recommended studies on the terminal residues are essential.

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SELF COAGULATION PHENOMENON OF PECTIN IN SUNFLOWER

Dilute acid extract of Sunflower heads showed coagulation effect which is probably due to low methoxyl content and high polyvalent cation content of the native pectin.

Sunflower head is a rich source of pectin, content being about 23% on dry weight basis[1]. After removal of seeds from sunflower heads for extraction of oil, the residual portion, considered as waste, could, however, be utilized for extraction of pectin. The object of this study was to find out nature and characteristics of pectin available in sunflower heads.

The sunflower (variety: Romsun record) grown at Oilseeds Research Station (U. P. Institute of Agricultural Sciences) Kalyanpur, Kanpur, was utilized in the present work. The sunflower heads (receptacles) were threshed and then powdered in a laboratory mill. A 5 g. sample was first soaked in sufficient quantity of ethanol (95%) for 15 min. to remove the colour pigments. The mixture was then filtered and the residue was washed first with ethanol-diethylether (1:1) mixture and subsequently with ether alone. The residue was then dried and suspended in 200 ml of 0.05 N HCl and refluxed for 30 min. to extract the pectin. The extraction was repeated two or three times using 100 ml of 0.05 N HCl each time.

The total extract was set aside for some time with a view to remove dust particles, silica etc. by sedimentation. During this holding period, partial gelation of the extract was observed. On extending the holding time the coagulam further coalesced to form bigger lumps which settled down leaving behind a clear solution. The quantity of pectin in the gelled fraction and the total extract, along with their methoxyl contents are presented in Table 1.

On analysing the ash of sunflower heads, it was found that calcium, aluminium, and magnesium make up about 50% of the mineral contents[2] (Table 2).

The coagulation of pectin in the extract could be due to polyvalent cations as well as low methoxyl content of pectin in the extract. Pectins having less than 7% methoxyl content are known to gel in the presence of polyvalent cations even when soluble solid contents of a jelly mixture are 1% or less[3]. The results presented in the Tables 1 and 2 duly support this observation. In order to further confirm the above, a fresh acid extract, which was passed through a suitable ion exchange column, did not subsequently exhibit self coagulation under conditions of holding mentioned above.

The result indicate that sunflower heads are not only a relatively rich source of pectin but in the native state it is different from the pectins derived from other raw materials viz. apple pomace and orange peel.

TABLE 1. QUANTITY AND METHOXYL CONTENT OF PECTIN FROM SUNFLOWER

Type of extract	% Pectin dwb	Methoxyl content %
a) Cold water	6.31	4.34
b) Boiling water	9.45	5.30
c) Dilute acid (0.05 N HCl)	19.37	8.74
d) Self coagulated portion	9.56	6.26
e) Acid extract after removal of (*d*)	9.81	8.19

TABLE 2. ASH ANALYSIS dwb

Ash constitutents on dwb	Percent
Ash	9.70
Calcium	2.70
Aluminium	1.11
Magnesium	0.77
Iron	0.11

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DETECTION OF DATURA IN FOOD AND FEED BY THIN LAYER CHROMATOGRAPHY

A Simple method for identification of Datura seeds (*Datura metal*) L in foods by extraction of alkaloids followed by Thin Layer Chromatography is described. Several cereal and millet samples admixed with Datura were tested and the presence of 0.05 gm of Datura in 10 gm of the material could be detected.

Datura plants have been generally consumed in the form of a brew or tea made from the leaves and crushed seed of the plant[1]. The accidental ingestion of the plant has led to numerous instances of poisoning, particularly among children[2]. Raymond (loc. cit.) described instances of Datura poisoning among the African; this was traced to the consumption of bread made from grains which had become contaminated with Datura seed. Losses of all classes of livestock including horses, cattle, sheep, hogs and chicken have been reported in the literature which has been reviewed by Kingsbury[3]. Mechanically harvested food grains are found to have been contaminated with Datura seeds. In this communication a simple method for identification of Datura in foods by TLC is described.

About 10–50 gm of powdered sample containing about 0.05 gm Datura seed *Datura metal* L is

refluxed with rectified spirit in a boiling water bath with an air condenser for 30 min. The extract is filtered and evaporated. About 5 ml of 1:1 HCl is added to the residue which is warmed and filtered in a separatory funnel. The solution is neutralised with liquid ammonia and extracted twice with 10 and 5 ml chloroform and concentrated to reduce the volume. The extract is tested for alkaloid with Dragendorff's reagent. If the test is positive then the chloroform extract is spotted on a thin layer, thickness 0.5 μ on slides spread with silica gel. It is developed using chloroform+benzene+methanol (4:1:0·5) in ascending way upto 5 cm. After evaporating the solvent the chromatogram is sprayed with Dragendorff's reagent. Two spots will be clearly visible at Rf. 0.18 and 0.5. Atrophine as free base (prepared from atrophine sulphate on treatment with liq. ammonia and extracted with chloroform) is used as reference side by side. The lower spot will be atrophine but it may also include hyoscyamine. Hyoscyamine ($C_{17}H_{23}NO_3$) is isomeric with atrophine $(C_{17}H_{23}NO_3)_4$. The two alkaloids run to the same Rf in theTLC. Atrophine is differentiated by precipitation of the salts and subsequent pharmacological tests on cat's eye.

Several cereal and millet samples admixed with Datura were tested along with control experiments. It has been observed that even as low as 0.05 gm of Datura in 10 gm food, could be identified by this method.

The test can be utilised as a limit test of sensitivity of 0.5% or even less. By the estimationof the alkaloids it is possible to quantify the Datura content since it contains 0.2% alkaloids[5] consisting of atrophine, hyoscymine and hyoscine (also known as scopalomine).

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ESTIMATION OF PROPOXUR IN FORMULATION

A colorimetric and thin layer chromatographic method for estimation of propoxur (2-isopropoxy phenyl N-methyl carbamate) based on alkaline hydrolysis and coupling with diazotised sulfanilic acid is described.

Several carbamates have shown a high degree of activity against a wide range of insects. Propoxur (2-isopropoxy phenyl N-methyl carbamate) is widely used nowadays for household spray. It is mixed with formulation in different amounts.

Residue of Propoxur was determined by colorimetric estimation of O-isopropoxy phenol[1]. Bracha[2] determined it sepectrophoto-metrically by diazotising with 3-nitro aniline 4-sulfonic acid.

In the present study, a colorimetric method based on the alkaline hydrolysis and coupling with diazotised sulfanilic acid is reported. Compared to Bracha's method, the coupling agent is different and the possibility of error is reduced by avoiding the addition of one drop of alkali finally after the volume has been made up.

Colorimetric estimation requires comparatively larger amount of the residue and does not differentiate between the propoxur and its hydrolysis product. Besides, it is only applicable to those samples which are known to contain this particular pesticide. Though colorimetric procedure is simple and a sensitive one, an equally efficient thin layer chromatography method is described along with a colorimetric method.

A) Colorimetric method: Reagents required are *a*) 10 per cent alcoholic KOH solution (freshly prepared); *b*) Diazotised sulfanilic acid (freshly prepared). 4 ml of 0.25 percent sulfanilic acid in conc. HCl is mixed with 5 ml of 0.1 per cent sodium nitrite in water; and c) 0.1 per cent propoxur in acetone.

Different amounts of propoxur (0.1, 0.5, 1, 2 and 3 ml) are taken in separate glass beakers. After the addition of 1 ml of 10 per cent KOH to each, they are kept on boiling waterbath for 30 min. After cooling, the treated samples are taken with 10 ml of water in test tubes followed by the addition of 1 ml of the diazotised solution. It is stirred with a glass rod gently for 1 min. Colour formation takes place immediately. The intensity of the color is measured after 5 min. in a photoelectric colorimeter using a green filter. A line is drawn by plotting the colorimetric reading against concentration. The colour formation follows Beer's law within a range of 0.1 to 10 mg of propoxur with the dilution volume as done here. Incidentally the

lower value of the range is the sensitivity of the estimation. The colour is indefinitely stable at the pH and other conditions of the experiment.

TLC procedure: The thin layer plate is made up of aluminium oxide (0.25 mm thick) activated at 100°C for 1 hr and kept in desiccator. The mobile solvent is ethyl ether-toluene (1:3). Twenty micro litres of the formulation is spotted along with 5, 10, 20, 30, and 50 μl of the propoxur solution on the plate. Then it is dipped in a chamber saturated with the mobile solvent. When the solvent has run upto 15 cm, it is taken out and the residual solvent is evaporated off at room temperature. The plate is then sprayed with 20 per cent alcoholic KOH. After 8–10 min. it is sprayed with diazotised sulfanilic acid. Fine orange coloured spots appear immediately. The outline of the spots is marked with a pin carefully and these are scrapped off into test tubes containing 10 ml of 10% alcoholic KOH. The tubes are shaken occasionally. When all the colour is eluted, the tubes are centrifuged and the intensity of the colour is measured in a photoelectric colorimeter using blue filter. Curiously the blue filter works better here than the green as the shade of the generated colour is slightly different. This is possibly because of adsorbent and the smaller concentrations. Alternatively photo-electric densitometer can be used where unknown amount is found out by comparing with, or referring to standard graph from spots of known concentrations. The method is satisfactory and possibility of interference from other carbamates can be avoided.

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BOOK REVIEWS

Triticale: First Man made Cereal: Edited by Cho C. Tsen, American Association of Cereal Chemists, St. Paul, Minnesota, U.S.A., 1974, pp. 292.

Although genetic crosses between wheat and Rye have been of interest for more than a century, the foundations for its practical applicability were laid by research work mostly in Europe in the period 1930–40. Research was revived in this area in Europe after the Second World war in 1950's but the major breakthrough in evolving agronomically useful varieties of Triticale took place in 1954 in Manitoba, Canada. Later collaborative work between the University of Manitoba group and the Centre Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) at Mexico has given greater impetus in the Triticale breeding field and now programmes for use of Triticale as a field crop are in progress in most parts of the world including Asia, Africa and South America.

The present publication on Triticale (which is a compilation of several articles presented in a symposium jointly organised in November 1973 by the American Association of Cereal Chemists and International Union of Food Science and Technology) is timely, selective yet broad based covering several main and border areas and has useful information to the Food and Agricultural Scientists. In contrast to other publications on Triticale where greater emphasis has been laid on genetics and information of specific value to the plant breeder, the current publication on Triticale has information which is useful not only for the novice in the Triticale field but also to the expert in this area.

The publication is divided into seven chapters covering all aspects of Triticale research such as Triticale as a agronomically useful crop, Breeding and genetics, Morphology of the Triticale kernel, Biochemical & Nutritional aspects of Triticale, Triticale milling and finally use of Triticale as a feed material.

Chapters I and II which deal with triticale in various countries, and Breeding and Genetics have been covered elsewhere in depth (International Development Research Centre, Monograph on "Triticale" No. IDRC—024e Published in 1974) and no new information is presented here. The prospect of Triticale as a field crop in a country like India will depend on many factors particularly a conviction to the farmers that the crop can be grown in areas where wheat does not yield well, resistance to rusts, ergot and other infection and finally good monetary returns to the farmer.

Chapter III has two articles on the morphology of the Triticale kernel as revealed by classical and modern cytological methods such as scanning electron microscopy. An article on "Triticale standards" is of value not only for the internal marketing trade but also provides useful guidelines to a possible import and export trade in this grain.

Chapter IV has seven articles covering most of the basic biochemical aspects of Triticale such as proteins, Tryptic and Chymotryptic inhibitors, Starch and Lipids. It has been written by specialists in these areas and has up-to-date information.

Chapter V dealing with the nutritional value of Triticale is of rather limited coverage and points to the need for more work in this important area particularly in technologically under developed countries where this grain may ultimately be grown for consumption by human population. As cereals constitute the major portion of the diet in these countries it is imperative that one should not only look at the protein value and lysine content of the Triticale grain but consider other nutrients such as vitamins, minerals, etc. Total nutrition should be the goal rather than protein or amino acid nutrition.

Chapter VI. The milling of Triticale into flour, suitability of some of the equipment currently in use for wheat milling, by products of Triticale milling are covered in one of the articles. The other articles deal with the preparation of Bakery products such as Breads, Cakes, Cookies, Muffins, etc. from Triticale flour. An article on the malting of Triticale is also inluded in this chapter.

The last chapter VII contains information where Triticale is evaluated as feed material for Steers and Pigs. It is interesting to note that nutritional studies have demonstrated that lysine is the most limiting amino acid in Triticale and with improved protein content and quality it may become an important feed grain.

The coverage of literature in most of the articles is up-to-date. The reviewer strongly recommends this publication to all Food & Agricultural Scientists.

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Breakfast Cereal Technology: by Roger Daniels, Noyes Data Corporation, Park Ridge, N. J. 07656, USA, 1974, pp. 290, Price $ 36.

Adding to its series of Reviews in the field of Food Technology, the Noyes Data Corporation has brought out this new book on "Breakfast Cereal Technology". The presentation of technical information in the book

generally follows the pattern in their earlier book entitled "Quick Cooking Foods" and as mentioned in the foreword to the book it is essentially a classified guide to the US Patent Literature in the field of Breakfast Cereals.

Breakfast cereals have attained a position of prominence in the daily menu of all advanced countries. Hardly a few would now prepare the cereals everyday from the original raw materials in these developed countries as prepared and precooked cereals of various kinds are manufactured and marketed for consumption as ready-to-eat products. The cereals are presented to consumers in various forms, shapes and sizes and are prepared either from a single cereal source or from a mixture of composite flours chosen and blended for adding to nutrition and broad based appeal. The present book summarises the technical US Patent Literature with regard to the processes for preparation, shaping, drying, and quick cooking of the different types of cereals. Methods for sugar coating, fruit incorporation, nutritional fortification, taste improvement, etc. are also described.

Extrusion Cooking which combines cooking followed by instant drying which is a recent innovation in breakfast technology and for preparation of snack foods is first described. The various patents relating to gelatinization and cooking of the dough followed by extrusion drying and the methods for improving the product texture, compounds that can be used for preventing stickiness of dough, etc are classified and described. The two succeeding chapters relate to puffing of cereal grains for best consumer acceptability. The preparatory treatments used just prior to puffing both with respect to process variables, compositional variations, use of adhesives for improving dough properties are first considered. The actual patented processes used for obtaining different types of puffed materials are next described. The recent improvements in flaking of cereals and composite blends of cereal flours are considered in a separate chapter. The different sophisticated gadgets used for obtaining distinctive and appealing shapes to the breakfast cereals described in the next chapter would especially appeal to those interested in increasing marketability of their products.

Processes relating to manufacture of quick cooking Oats are exclusively treated in a separate section. Recent innovations for reducing the cooking time and improving product texture by processing, and quicker hydration are described in this chapter. The patented literature for improving the hydration and reducing the softening time of other breakfast cereals is also next described and enumerated.

Taste improvement, and enhancement of consumer acceptability by uniform application of sugar and incorporation of natural fruits and other flavours which is a further sophistication in the marketing of breakfast cereals are next described. The last Chapter on nutritional enrichment would appeal to those who would like to give balanced breakfast cereals nutritionally balanced with regard to all the nutrients. It is interesting that such blended cereal combinations are normally compounded from vegetable sources rich in proteins. This would be of special interest to those in developing countries who might like to develop such products for weaning foods for children although not as breakfast foods. The last subject covered relates to making of a French Toast with long stability incorporating the principle of including permisible additives for reducing the concentration free water.

The book has also incorporated an index of companies, inventors and a number index relating to the patents enumerated.

The book is a good reference manual of patents relating to this field and will be of great use to manufacturers, technologists and entrepreneurs in the field of breakfast cereals which has attained the position would be good in later editions if each patent is prefaced by a self contained summary of the process and also the status of the patent with regard to actual commercial application.

H. S. R. DESIKACHAR

Plastics in contact with food: by John H. Briston and Leonard L. Katan. Food Trade press Ltd., 7 Garrick street, London 1974, pp. 466. Price £ 20.

With rapid growth in food industries the demand for various processed foods in consumer size unit packages are ever increasing. Heavy pressure on the existing expensive metal and glass containers and rapid development of plastic technology in 20th century has opened a potential market for the use of these plastics in food packaging. In recent years the protection of food against environmental hazards has been achieved by application of plastics for storage and transportation. The plastic use is varied. At the one extreme there are plastic silos, pallets and sacks; at the other end tiny containers for sophisticated applications such as cosmetic tubes etc., In between the extremes lies the bulk of plastics which come in direct contact with food and provide a safe and economical barrier for the food. However, plastics in contact with foods pose some health hazard problems and have some disadvantages

eventhough in the ultimate the advantages outweigh disadvantages. The present book attempts to dispel some of the misconceptions in relation to hazards and discusses in detail the relative merits and demerits of the various plastics and their interactions when they come in contact with food.

The book is in three main parts; part 1 covers the plastic raw materials and how they are made. Part 2 describes the relation of plastics and food packaging. Part 3 indicates the applications based on the basic knowledge of part 1 & 2 and covers major manufacturing methods and systems relating to plastic articles likely to come in contact with food. The book consists in total of 20 chapters covering aspects such as science of plastics (thermoplastic and thermosets), natural polymers, food hazards, toxicity, migration of constitutents of plastics, tainting of foods involving plastics, laws and regulations covering food application of plastics, manufacturing of plastic films, semi rigid containers by various processes, composites consisting laminates of plastics, closures and their food contact hazards, and finally ornamentation such as labelling, printing on plastics by different techniques. Each chapter is backed by systematic presentation of bibliography for future reading. The Appendix includes glossary of scientific and technical terms concerning plastics, Diffusion and permeability of plastics and plastics waste disposal. Tables in the end give very useful data on the physico-chemical properties of various plastic materials used in food packaging.

The first chapter on "Science of plastics" deals briefly on the nature, structure and strength of plastics in general and thermoplastics in particular. The influence of various chemical forces on the various properties of the thermo-plastics has been clearly elucidated. Chapter 2, deals with details concerning the chemistry, manufacture of resins, properties and uses of (*i*) polyolefins such as polyethylene, polypropylene, Ethylene/vinyl acetate copolymers (EVA) and ionomers (*ii*) Vinyl plastics such as polyvinyl chloride and copolymers, vinyl acetate, (*iii*) polystyrene and ethylene thermo plastics such as polyesters, Nylons, polycarbonates, poly acetals etc.. Chapter 3 gives details on the chemistry, properties and uses of thermosets such as Phenol-formaldehyde, urea-formaldehyde, melanine-formaldehyde; glass reinforced other setting polyester resins, epoxy resins, polyurethanes etc. In the next chapter the authors have dealt with the chemistry of natural polymers and their derivations such as celluose, cellulose acetate, ethyl cellulose etc., As against the above polymers chapter 5 is concernd with non plastic component used in manufacture of plastics. These include catalyst residues, processing aids such as anti-oxidants, anti-blocking agents, antistatics, heat stabilisers, plasticiser, lubricants, melt strength improvers, colourants, whiteners and a host of others. Awareness of these materials in plastics contacting food is a must as they play a major role in relation to effects on food quality and health hazards.

Chapter 6 on 'food hazards' presents brief details of food spoilage and food hazards relating to plastics. A basic model relating to interaction of food, plastic and environment has been discussed. Chapter 7 deals in detail with the 'toxicity hazards' to health and defines clearly the meanings of toxicity, toxic hazard and related terms in relation to plastics and food. Further, in this chapter dosage level of toxicity in the diet and levels such as LD_{50} which represents acute toxicity and interactions of plastics with mass have been dealt. In the next chapter 'Migration', the mass transfer of various ingredients of plastics into the food in contact has been discussed in detail making use of a basic model. Based on the mathematical model of diffusion of various components, practical applications of leaching of components into various types of foods have been elucidated.

Chapter 9 and 10 are earmarked for 'food quality' and organoleptic effects. Numerous aspects of food quality such as nutritive value appearance etc., and the effect of environment factors such as moisture, oxygen, light, micro-organisms etc., on the quality have been discussed and preservation by suitable methods and use of right type of plastics have been discussed. In chapter 10, origins of food tainting involving plastics, its practical assessment, organoleptic test procedure for assessment, causes and remedies of tainting have all been clearly described. Tainting is considered to be a function of packaging, handling conditions and food stuff and thus tainting problems of packaged food can only be solved by collaboration of raw material manufacture, converter and food packages.

Chapter 11, on 'plastic quality' deals with the effect of environmental changes such as physical, chemical and radiation, and microbiological as well as effect of foodstuff on the physico-chemical changes, of the plastics coming in contact with food, Chapter 12, deals about the 'Law and regulations' concerning the safe use of plastics in packaging of foods and describes in brief the legislature on food packaging materials in various nations of the world.

In chapters 13, 14, 15 and 16 details regarding various manufacturing processes used in manufacture of plastic films and bags and semirigid containers are discussed in great detail. Chapter 13 deals with blow, slit die extrusions of plastic films and orientation

methods. Lamination by coextrusion by adhesives and by coatings have also been dealt. Chapters 14 and 15 are earmarked for details regarding manufacturing of shrink wraps, sachet machines, transwraps, bags and woven plastic sacks and thermoforming containers. Chapter 16 presents plastic containers manufacturing methods such as compression moulding, injection moulding, blow moulding and sinter castings and discusses relative merits and demerits of these methods for various plastic containers. Chapter 17 deals with 'Integrated packaging' which may be described as complete or partial integration of the total packaging concept in commercial and technical sense.

Chapter 18 deals with manufacture of composites, laminates, cartons and their applications. Next chapter describes plastic closures and other ancillaries, their food contact hazards and general requirements for closures. Cap design consideration has also been dealt. The last chapter deals mostly on ornamentation on plastics where in details regarding labelling and printing are presented. Printing includes the pretreatment to be given to plastics, and describes various methods of printing such as offset printing, flexographic, Gravure, and electrostatic printing. Mention is also made of printing ink.

This book which is intended to be a technological book has successfully attempted to project sound fundamental basis for scientific and technical aspects of plastics used in food packaging. Illustrative examples and detailed description of individual systems processes etc., which are very useful to reader are presented. The book on 'plastic in contact with food' with its pronounced emphasis on the scientific and technical aspects of plastics, interaction of plastics with food and environment and its effect on the health problems is quite a useful addition to the growing library of books on food packaging.

N. BALASUBRAHMANYAM

Food Acid Manufacture: Recent Developments, by A. A. Lawrence, Noyes Data Corporation, Mill Road at Grand Avenue, New Jersey 07656, U.S.A. 1974, pp. 302, Price[2] $ 36.

Food acidulants constitute one of the most important groups of food additives. Their role in imparting characteristic taste and flavour to low pH foods is well known. Besides, their salts find a variety of uses in food and non-food industries. Most of them are of organic nature occuring in a number of plant products and till recently one of the main methods of their commercial manufacture has been by processing natural plant materials. Information on organic acids used commonly by the food industry is available from literature, the sources being widely scattered. However, some of the advances made in the technology of manufacture and application of these food acids are not readily available due to the same being patented. The efforts of M/s. Noyes Data Corporation in sifting through the patented literature in the U.S. on the subject to prepare a monograph authored by A.A. Lawrence are timely.

The author has considered most of the major food acids which include Citric, Fumaric, Adipic, Succinic, Malic, Tartaric and Lactic acids. Emphasis is on acidulants and hence those such as ascorbic, sorbic, propinic, glutamic and other acids which are not used for acidifying purposes are excluded from the purview of this publication.

Citric acid is commercially manufactured by mold fermentation. Great advances have been made in the U.S. on Citric acid fermentation so as to increase the yield and to overcome cell growth inhibition when different raw materials are used. Information covered by various patents has been collated and presented in the first Chapter. Also included in this section are fermentative production of citric acid using yeasts, bacteria and fungus. Synthesis from oxalacetic acid is another method by which citric acid can be made. Some of the recent uses of this acid in edible compositions have been discussed.

Fumaric acid is commercially made by isomerization of maleic acid. Being the most economical costwise and less hygroscopic by nature this acid is finding increasing uses lately, though its low solubility is a disadvantage. Some of the recent developments in fumaric acid manufacture are discussed in second Chapter. A number of patents have been filed on improving the techniques of isomerization as well as increasing the solubility of the acid. Other methods advocated include heating of a mixture of malic acid and maleic anhydride in a nitrogen atmosphere and fermentation of digested lignified cellulose using *Rhizopus nigricans*. A number of techniques have been developed to increase the cold water solubility of fumaric acid. Some of the uses of the acid are also enumerated in this Chapter.

Adipic acid is more renowned as an intermediate in the manufacture of nylon resins rather than as a food acid. Present in beet juice, adipic acid also is an important acidulant manufactured generally by a two stage oxidation process from cyclohexane. A number of modifications have been developed both for the first stage as well as for the second stage of oxidation. Improved techniques are available for

recovery and for crystallization of the acid from the reaction mixtures. Other methods reported for manufacture of adipic acid include one-step oxidation of cyclohexane, oxidation of olefines, cyclohexyl hydroxylamine, Nitroso cyclohexyl Dimer and methyl 1-hydroxy cyclohexane carboxylate, alkaline fusion of E-Hydroxy caproic acid and derivatives, reaction between acetylene and acetic acid, reaction between diolefine, carbon monoxide and water, hydro dimerisation of acrylic acid, three stage method from buta diene, liquid phase oxidation of xylenes, low temp nitric acid oxidation, and a two step hydrolyses of adipo mono nitrolic acid. Patented informations on improving the properties of adipic acid are also presented in third Chapter.

Succinic acid is used extensively in foods as a sequestrant, buffer and neutralizing agent. Fermentation of ammonium tartrate into succinic acid is one of the methods of its production. Chemical synthesis involves oxidation of paraffinic or isoparaffinic hydro carbons followed by separation of succinic acid from the mixture of dibasic and oxygenated contaminants. A number of patents have been taken suggesting modifications and improvements in some of the steps. These form the subject matter of fourth Chapter.

Chemical and Bio-chemical methods are available for preparing malic acid which is one of the most desirable food acidulants on account of its good solubility, smooth taste and flavour fixing qualities. Malic acid can be obtained either by chemical conversion of maleic acid or by catalytic oxidation of benzene. The Biochemical processes available include *Aspergillus* fermentation and isomerization of fumaric acid. Some of the recent developments in the above areas are discussed in fifth Chapter.

Tartaric acid finds extensive use in efferevescent beverages, baking and jelly powder formulations and fruit esters. New developments pertaining to fermentative production of tartaric acid and recovery techniques from wine sludges are presented in sixth Chapter.

Bacterial fermentation generating lactic acid is a well known phenomenon. This syrupy liquid is used as a food acidulant and a flavouring agent besides finding application in bakery preparations in the form stearoyl lactylate. Described in the last Chapter are some of the innovations claimed by several patents for the production of lactic acid by fermentation as well as chemical methods. Some of the newer applications of lactic acid are also discussed.

The publishers have given necessary indices for the companies as well as the inventors to which the patents have been assigned. Patent number index is also appended for easy reference. Though the informations presented have been collated by careful screening of a number of U.S. patents, the presentation is such that it provides interesting reading.

The publication is a very useful reference book for the food processing industry as well as research and development workers in the field.

V. H. POTTY

Dehydration of Natural and Simulated Dairy Products: by M. T. Gillies Noyes Data Corporation Park Ridge, New Jersey 07656, USA, 1974, pp. 327; Price: $ 36.

The Book under review is the 15th Food Technology Review published by the Noyes Data Corporation. Similar to the other reviews this volume has compiled the U.S. Patents on Dehydration of Natural and Simulated Dairy Products from 1960 to 1973.

The subject matter covered in 177 patents are classified under Eight Chapters: General Processes, Fat-Containing Milk; Skim Milk, Whey, Concentrated Milk, Eggs, Miscellaneous Dairy Products like egg products, dry dessert mixes, milk drinks, butter milk, sour cream, yogurt dried cheese, dry chocholate drink mixture; Simulated Dairy Products like coffee whitners, whipped toppings, cheese substitutes, etc., Three indexes covering Company Index, Inventor Index and U.S. Patent Numbers are provided at the end.

The Book has presented many useful technical information from the patent literature accompanied by sketches with some details of the equipment, as for example the various agglomeration and instantising technique. Though not a comprehensive review on the subjects covered since it covers only U.S. Patents, the book has certainly provided a number of facts and proposals in a concentrated form that could be useful for product and process development in Dairy Industry.

It will be a useful addition for any research and development laboratory and libraries in Food, Technology.

P. K. RAMANATHAN

ASSOCIATION NEWS

A Symposium on Fish Processing Industry in India organised by the Association of Food Scientists and Technologists (India) in collaboration with CFTRI at Mysore was held on 13th and 14th February 1975. Over 100 experts from the field participated in the deliberations conducted under six technical sessions spread over two days. Over 50 technical papers were presented during this period and Association hopes to compile and publish the proceedings in the near future.

Several recommendations emerged out of the Symposium which were summed up by Prof. P. C. George, Joint Commissioner of Fisheries, Government of India, New Delhi in the plenary Session. The recommendations are given below. It is hoped that these recommendations will be taken note of by all those who are involved in the planning and development of fisheries on a sound line in our country.

RECOMMENDATIONS

Raw Material Resources:

1. The Symposium having noted the potential of the resources of the pelagic fishes on the South-West Coast recommends that the exploratory and experimental fisheries organizations should pay their immediate attention to the development of methodologies for the exploitation of these resources and for solving problems in the handling for processing of pelagic fishes.

2. The Symposium recommends that for the quick development of methodologies in exploitation and utilization of the pelagic fishes, the government of India should permit import of requisite equipment and expertise as a short-term measure and further urges that the attention of the food technologists and engineers in India should be concentrated on the development of requisite equipment indigenously as a long term measure.

3. The Symposium further recommends that the large scale handling and processing of pelagic fishes should be in centralised places and not be in a disseminated way as has happened in the shrimp handling and processing sector.

4. The Symposium recognises the need for resources survey on a continuous basis for both pelagic and demersal fisheries and the timely dissemination of the results of such surveys to the industry. The setting up of suitable dissemination services to feed information on location and availability of fishes to the fishing industry as a permanent measure should be considered. Urgent steps should be taken to carry out the pelagic fishery surveys in other parts of our seas also.

5. The Symposium felt the need for urgent attention to be paid to detailed research on the bacterial flora of fresh water fsh. A crash programme on the development of improved methods of handling, and distribution of fresh water fish should be undertaken.

6. The Symposium noted with great interest the work on peeling of shrimp with the use of enzymes. This aspect should be explored further by continued studies at various research organizations.

7. The Symposium recognising the potential of tuna resources of Indian waters urges the Government to take urgent steps for the exploitation of these resources.

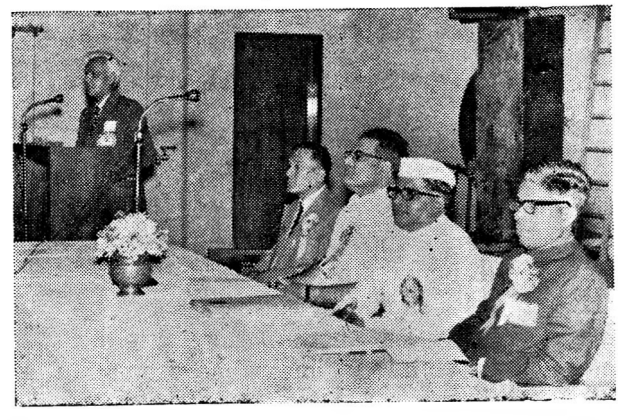
8. The Symposium having considered the various constraints faced by the industry appeals to the Government of India for arranging a liberal flow of requisite finance for the investments in exploiting the newly found resources.

Freezing of Fish:

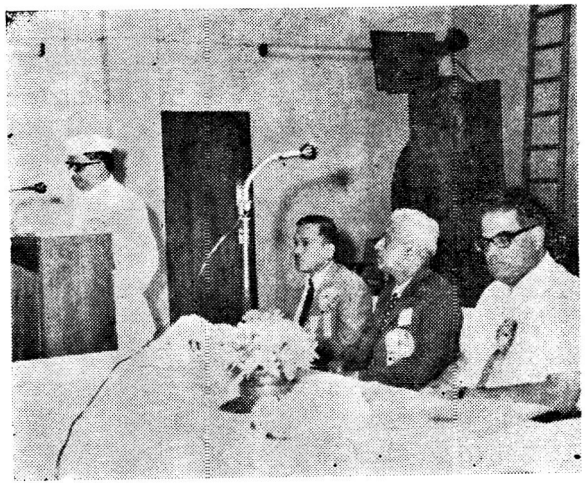
9. The Symposium noted with concern the frequent occurence of *Vibrio para haemolyticus* in frozen fish products. This being a pathogenic organism deserves attention for further investigation.

10. Possible use of liquid CO_2 and even air in the -80 to-100°F range may be explored for freezing of fish. Quality aspects of shrimp/fish frozen by different methods of freezing (plate freezing, cryogenic, immersion, etc) need extensive investigation.

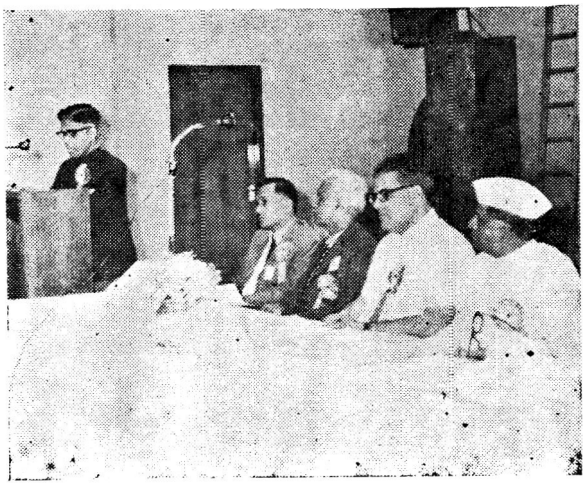
11. The Symposium felt that oil sardine and mackerel, being two important commercial varieties of fish deserve the utmost attention for freezing. The stored frozen product should have a minimum shelf life of six months. These fish contain highly un-



Dr. T. N. Ramachandra Rao, President, AFST, welcoming the gathering at the inaugural function of the Symposium.



Shri K. T. Rathod, Hon'ble Minister of State for Fisheries and Horticulture, Karnataka delivering the inaugural address.



Prof. P. C. George, Joint Commissioner of Fisheries, Govt., of India, New Delhi delivering the key-note address.

saturated fats and heme compounds and as such they are prone to easy oxidation and development of rancidity during frozen storage. An assessment of storage life of these products as obtained under commercial practice may be made. If necessary further Research and Development efforts may be directed in this direction.

12. It is recommended that greater attention should be bestowed upon freezing fish fillets and dressed fish both for internal consumption and export, if possible.

13. It is also recommended that the use of chilled sea water and ascrobic acid for the prevention of black spots on frozen prawns may further be investigated and procedural advice offered to the industry.

Processed Fish Products:

14. The Symposium notes that India has sizable resources of clams, mussels and oysters which have a good export market in the form of canned, frozen and other processed products. It is felt that MPEDA should identify the markets and advise the processing industries to undertake commercial production of these commodities. Technological problems, if any, relating to such production may be looked into by the Research and Development Laboratories.

15. With the introduction of subsidy for export of canned sardines, there is now an incentive to export this item. The medium of packing has to cater to the tastes of the importers. It is felt that necessary help should be given to the industry to import olive oil for this purpose. It is recommended that the canners may follow the procedures already evolved in the laboratories to reduce the free water in the pack. Since aluminium cans are more popular and are in greater demand in the world market and in view of the shortage of tin-plate, the symposium recommends that manufacture of this can should be immediately taken up in the country.

16. It is noted that fish sausage has found ready consumer acceptance in India. This provides an outlet for utilization of inexpensive varieties of fish. The two main constraints have been the non-availability of preservatives and the synthetic casing which require to be imported. The symposium strongly recommends the import of these materials or alternately their manufacture in the country with foreign collaboration if necessary.

17. The Symposium further recommends that the possibility of producing fish sausages in natural casings for distribution through cold-chain may also be explored. Canned Fish sausage may have an export market which requires examination.

18. Government and Government-aided-agencies may be advised to use low cost fish products of proven acceptability, developed at various research laboratories, in community feeding programme.

19. In view of the acute shortage of good quality protein and since other proteinaceous material available are not sufficient to meet the total requirement, it is felt that a concerted attempt should be made to supplement the diet of those who need it most with fish flour under government subsidy. Efforts to improve the functional properties of fish flour should continue to receive attention.

Traditional Fish Products and By-products:

20. For the guidance of actial users, the Symopsium stresses the need to draw up detailed reports in simple and understandable language on the traditional

methods of preservation of fish to improve the quality of the products cured in the country.

21. There is an urgent need to prepare complete project and feasibility reports for the utilization of waste from fish processing industry and the 'trash' fish either for the industrial or for edible purposes.

22. It is pointed out that clear and complete guidelines have to be drawn up for the production of fish meal of quality specified in the national and international standards.

23. The Symposium recommends that detailed guidelines may be worked out for the production of sardine oil of national standard so that it can be used for packing of fish and also for other sophisticated uses.

24. In view of the great potentialities of industrial products from hydrogenated sardine oil, project and feasibility reports for the production and manufacture of these products may be prepared and made available for industrial exploitation if necessary after pilot plant trials.

25. Research efforts should be directed on isolation of more unsaturated portions of sardine oil with a view to using them in hypocholesterolemic trials. Proper attention should be given to proper stabilisation of such fractions so that no health hazard is encountered.

26. The Symposium notes with anxiety that inspite of considerable and useful work carried out in India on traditional fish products, the industry is almost as primitive as it was decades ago. The matter may be examined in totality and ways and means found to improve the technology of traditional fish products which are generally consumed by the poorer section of our population.

Machinery and Equipment Needs:

27. The Symposium felt that there is a need for the design and development of suitable machinery in order to machanise certain operation in the handling and processing of fish so as to avoid loss in quality of the processed product.

28. Research Organizations should take up work on the development of know-how and machinery for the manufacture of products like partially hydrolized and de-odorised meal, sausage, sausage casings, IQF plants, knobbing machines, etc.

29. Development of Refrigeration machinery for medium sized boats is necessary if the industry is to make further progress.

30. The Symposium urges establishment of suitable machinery for assessing the claims of indigenous machinery manufacturers so that import can be restricted to a minimum.

31. Keeping in view self reliance, it is recommended that import of machinery, not available indigenously with proven efficiency, may be allowed for the intervening period. All encouragement should be given to develop indigenous expertise in fabrication of fish handling and processing equipment.

Quality Control and Marketing:

32. The Symposium calls for a periodical exchange of information between various parties concerned on the quality standards prescribed, procedures for fish sampling and analysis and various improvements worked out from time to time.

33. The efforts of ISI in laying down standards have been recognised. The Symposium requests ISI authorities that in prescribing standards they should take note of the practical and commercial applications for the products.

34. It was felt that before introduction of compulsory quality control, voluntary adoption to follow the standards would considerably assist in educating the processor and also help him to take note of the influence of the various factors affecting the marketing of the product in international markets.

35. The phased introduction of quality control on new products will help to meet the specific requirements of the product and also to assist in the marketing of institutional and retail packing requirements.

36. The Symposium stresses the need for drawing up definite codes of conduct and procedure, maintaining schedule for cleaning and sanitation, log books and registers, continuous recording etc. and the same should be improved upon periodically.

37. There should be frequent refresher training courses, organised by established research and development institutions connected with fishery science and technology with regard to sanitation and hygiene and also for processing of special products so that the industry is made quality conscious.

38. At present compulsory quality control is effective only for export products. Some efforts should be made for maintaining the quality of fish and fish products offered for sale in the internal market also.

Association of Food Scientists and Technologists Bangalore Chapter.

1. A meeting of the Bangalore Chapter was held on 21st January, 1975 at Food Crafts Institute, Bangalore. Sri I. J. Puri presided over the meeting. The Hon. Gl. Secretary placed before the meeting a resolution requesting the Association of Food Scientists and Technologists to start the Southern Regional Branch of the Association in Bangalore. The resolution was

approved and Secretary was asked to forward it to the Exec. Secretary of the Association at Mysore. The President then requested Sri. M. R. Chandrasekhara to present his talk on the proceedings of the Fourth International Congress of Food Science and Technology held at Madrid.

Sri Chandrasekhara said that he had an opportunity to tour the Continent and London and while doing so he attended the IVth International Congress of Food Science and Technology at Madrid and visited a few research laboratories in London and on the continent.

The IVth International Congress was sponsored by the International Union of Food Science and Technology of which the Association of Food Scientists and Technologists (India) is a member. The venue of the Congress was the Palace de Congress in Madrid. Before the congress was inaugurated there was a one day workshop organized by the League for International Food Education (LIFE) an organisation sponsored by the USAID. Developing countries like Indonesia, Malaya, Ceylon, Philippines, Central America and African Countries were represented. India was represented by Dr. Achaya, Executive Director of the Protein Foods and Nutritional Development Association of India. The object of the workshop was to bring about a discussion regarding the aid from developed countries for fostering food industries in developing countries. The state of art of food industries in each of the countries was presented and the possible areas in which further development could take place were discussed. Both Dr. Achaya and Chandrasekhara presented their views to the workshop on this important topic.

There were a host of papers presented at the International Congress of Food Science and Technology. The topics discussed ranged from protein isolates to flavours, gums and other food additives. Some of the papers presented on isolation of protein from peanut, coconut and rape seed were of considerable interest. There was much interest in composite flours for making of bread. It was reported that upto about 30% of wheat flour used in making bread could be substituted by starchy flours like tapioca, maize etc. It was also possible to use 3% of protein rich flours like soya or pulse flours along with 27% of starchy flours. A paper on the Nutrition Programmes in India with special reference to Balahar and Miltone was presented by M. R. Chandrasekhara. There was a very interesting round table conference on the transfer of technical aid to developing countries. Dr. H.A.B. Parpia as the Chairman gave a review of the need and objectives for transfer of technology. Dr. Achaya, spoke on the possible areas of technical help in the field of education and the problems of research institutions in India. Dr. Hulse was very critical of the so called technical aid from a developed country to a developing country. He felt that more often than not this failed or more important produced unpleasant results. It was no use exporting very sophisticated equipment to a developing country unless proper survey is made of the available raw materials for use in such equipment. He said that more often than not the mistakes which the technical experts from developed countries make are economically much more disasterous than the mistakes developing country might have made for lack of this expert advice,

Visit to Research Centres in London: Three research centres were visited. The first one was the British Food Manufacturing Industries Research Association at Leatherhead. Dr. A. W. Holmes is the Director of the Institute. The Institute is entirely financed by the industry and provided information which its members required regarding the standardisation, analysis or any other aspect of the foods they were manufacturing. The service is manitained secret for the use of the particular party which pays for it. The Laboratories are very well equipped and appear to be competent in solving the problems raised by the Industry. The Tropical Product Institute is a Government institute and undertakes research in tropical products from rubber, hides to coffee spices and other food products. The discovery of aflatoxin in groundnuts and its characterisation was done here and even now a very lively interest in this field is maintained at the Institute. The FAO project on the composition flours for bread making is also in operation in this laboratory. They have finalised a project for extraction of protein from coconut. M. R. Chandrasekhara addressed the Research Workers at TPI on the scope of work at CFTRI, Mysore. Dr. Carpenter's laboratory is working on the latest improvements in the available lysine method which was originally standardised by him. The method measures the loss in the nutritive value of foods during processing. On their invitation, the Speaker addressed the meeting of the scientists on some of the Social Welfare projects in India.

Visit to Wageningen (Nethertands): Wageningen in Neatherlands is a small town on the banks of Rhine. The remarkable point about this village is that it is surrounded by research institutes. The Agricultural University is located there as also the Institute for Processing & Storage of Agricultural Produce, the Department of Food Science and Technology, Institute of Cereals etc. A project of interest, which

the IBVL (Institute Vour Bewaring en Verwashing ver Landbouio Produken) has undertaken was on the extraction of protein from *Vicia faba* (broad bean). They grow a large quantity of this and they wanted to find out other uses for the material. The economics of this and the use for which the protein could be put, are factors still to be evaluated. Another project was the utilization of potato waste for starch and protein recovery. The potato waste is from the Chips Industry. Nearly 25% of the potato is wasted. Micro organisms are being used to enhance the protein content in the potato waste for feeding animals. The Institute of Cereals is also participating in the F.A.O. Project on the use of composite flours.

The Nestle Research Laboratories are situated in a village, Orbe near Geneva. The laboratories are exceedingly well equipped. They have basic food technology division, physics, chemistry and mathematics divisions, engineering food chemistry division and a division for operation research. In the basic divisions they are working on protein foods and, flavour stimulants. The Director Prof. Mauron, a very well known biochemist had under his direct control the biology service division. One of the projects they have undertaken is to find the affect of stimulants on the behaviour in rats. Dr. Buffa is the head of the Food Conservation section of UNICEF and is in-charge of several projects for production of protein foods in Algeria, Turkey and Morraco. The types of food they are producing are very similar to Balahar. Respective governments have taken very great interest in establishing units for the production of these.

The problems of production of weaning foods and other protein foods were discussed with officials of FAO, Rome. Later at Teheran, it was of interest to note a Government programme by which wheat is distributed to units under subsidy. They are asked to make "*Nan*" from the wheat and this is sold to all people under subsidised rates. But sale of wheat products other than "*Nan*" is prohibited and a very high penalty is imposed on those who break this law. It appears to be a very good method of providing the staple food to the population at a subsidised cost.

2. Feb. 4, 1975.

A luncheon was hosted by several food manufactures in the city like Kissan Products Ltd., Kwality Biscuits, Krishna Flour Mills and the Association of Food Scientists and Technologists in honour of the Joint Select Committee for the Prevention of Food Adulteration (Amendment Bill) which visited Bangalore on the 4th of February, 1975. This opportunity was taken to bring to the notice of the Committee the resolutions passed at the Symposium on Food Adulteration sponsored by the Bangalore Chapter. The Chairman of the Joint Select Committee was Sri Tirloki Singh and among the members were Sri Gulzarilal Nanda, Sri Krishnakant and others. The members present at the luncheon discussed the several modifications which are before the Parliament. The committee promised to study the recommendations and incorporate these in the Amendment wherever possible. Sri panduranga Setty, President of the Bangalore Chapter thanked all the members for accepting the inivitation of the food manufacturers and the Association of Food Scientists and Technologists and for having given them an opportunity to represent their views on the Amendments.

3. Meeting of the Bangalore Chapter held on 18th February, 1975 at Food Crafts Institute, Bangalore.

Mr. Bhavani Shanker Rao presided over the meeting. Mr. Jagadeesh Reddy spoke on "Seafood Processing" with particular reference to Indian fisheries development and exports.

The word "Seafoods" applied to the plants and animals harvested from the sea or cultivated waters for consumption. Several species of fish, shrimp or prawn, crabs, lobsters, squid, clams, seaweed etc., were few examples. Marketable products could be fresh, frozen, canned, dried, cured or pickled.

The world ocean which covered 70% of the surface of earth provided less than 2% of world food supplies. In many parts of the world specially in India the fishing is very primitive and very poorly exploited. Indian seafood is characterised by small coastal fishing vessels and traditional fishing methods. The present catch is within a narrow belt of about 15 miles to a depth of 180 feet. The annual production was 1.8 million tonnes which could be increased 10 fold. The per capita consumption in India is only 2.4 lbs./year as compared to 86 lbs. in Iceland and 7 lbs. in Japan. The Inland Fishery in India is 2000 years old but the production is only 500 lbs/acre which could be increased to 5000 lbs/acre. It was pointed out that one of the most significant developments in Indian Fisheries has been the unquestionable success of India's shrip industry dynamically moving into the worldmarkets. India exported 38 million valued at Rs. 79 crores to 94 different countries all over the world. The major importers were Japan, U.S.A., and U.K.

Association of Food Scientists and Technologists (Eastetn Regional Branch)

Short Term Course on Quality Control

A short term course of about 2 weeks on Quality Control in Fruit and Vegetable Processing Industry was organised from 7 to 19 December 1974 by the Eastern Regional Branch (AFST). This is the first time the Branchactivity has included a training programme. The venue of the course was the Food Technology and Bio-chemical Engineering Department of Jadavpur University and the course was inaugurated by the past President of AFST and Vice Chancellor, Jadavpur University, Dr. A. N. Bose. There were 7 participants, all from processing industries and the course content included both lectures and practicals.

As regards the faculty, Prof. Sunit Mukherjee, Mr. S. N. Mitra, formerly Director, Central Food Laboratory and Dr. P. K. Dutta of the All India Institute of Hygiene and Public Health were responsible for overall guidance.

Speaker	Organisation	Subject
Dr. S. K. Majumder, Professor	Department of Food Technology & Bio-Chemical Engineering, Jadavpur University.	Food Microbiology and Microbiological Methods
Dr. N. D. Banerji, Reader	–do–	–do–
Dr. D. K. Chattoraj, Reader	–do–	Food Chemistry and Food Analysis
Dr. S. Mukherjee, Lecturer	–do–	–do–
Dr. A. B. Som	All India Institute of Hygiene & Public Health, Calcutta	–do–
Mr. D. Raychaudhuri, Reader	Deptt of Food Technology & Bio-Chemical Engineering, Jadavpur University	Colour, Flavour & Texture of Food & Measurement
Mr. R. N.Ghosh, Deputy Director, Fruit & Vegetable Preservation	Department of Food, Ministry of Agriculture, Government of India	-do-
Mr. S. N. Mitra, Ex-Director,	Central Food Laboratory, Calcutta	Govt. and Trade Standards of quality and identity & Food additives
Dr. P. K. Dutta Associate Professor Nutrition & Bio-Chemistry	All India Institute of Hygiene & Public Health, Calcutta	–do–
Mr. L. G. Banerji Deputy Director	Indian Standards Institution Calcutta	Govt. and Trade Standards of Quality & Identity and Food additives
Dr. V. V. Karnik	Research Department Metal Box Co. (I) Ltd.	Quality Control in Canning of Fruits & Vegetables
Dr. S. C. Chakraborty	–do–	–do–
Mr. S. K. Sur Dy Technical Adviser	Deptt of Food, Ministry of Agriculture, Govt. of India	FPO Specification
Mr. R. N. Ghosh Deputy Director, Fruit & Vegetable Preservation	Department of Food, Ministry of Agriculture, Govt. of India	–do–
Dr. L. M. Banerji Mr. N. K. Pramanik	Central Combined Laboratory, Calcutta	Sanitation and Water Analysis
Dr. S. B. Chattoraj Director	–do–	–do–
Dr. B. R. Roy, Director,	Central Food Laboratory	Food Analysis

At the end of the course the participants took a written examination lasting an hour and a half. At a concluding session, discussion took place with the participants on the course content and whether the participants felt they had really benefitted from it. The response was very favourable and it is hoped that the Branch will organise further courses in future.

New Members of AFST

Mr. K. S. Narayana Iyer, Talayar Tea Company Limited Kottayam-686001.

Mr. Purnendu Ghosh, Fermentation Technology, CFTRI, Mysore-570013.

Mr. Vinod Bihari, Fementation Technology CFTRI, Mysore-570013.

Mr. N. S. Mahindrakar, MF & PT Discipline, CFTRI, Mysore-13.

Mr. A. Ram Susen, Union Carbide India Limited Vishakapatnam, AP.

Mr. O. P. Madan, M/s. Jagatjit Industries Ltd., Mameera, Punjab.

Mr. N. Kalyana Raman, Export Insepction Agency, Alleppey, Kerala.

Mr. Iyyadurai S, Export Inspection Agency, Madras, Tamil Nadu.

Mr. K. C. Balasubramanian, Projects Division, Amul Dairy, Anand, Gujarath.

Mr. A. V. Satish, Miltone Project, Bangalore Dairy, Bangalore-29.

Dr. M. A. Haleem, CFTRI, Mysore-570013.

Mr. R. N. Sharma, AFD Factory, Tundla, Agra.

Mr. P. Puttarajappa, MF & PT Discipline, CFTRI, Mysore-570013.

Mr. A. D. Ghadi, Cadbury Fry India Limited, Bombay.

Mr. K. Keshava Bhat, Lipid Technology, CFTRI, Mysore-570013.

Mr. T. V. George Vargheese, E.I.D. Parry Limited, Madras-600001.

Mr. Vitoon Danpak Dee, D-16, International Hostel, CFTRI, Mysore-570013.

Miss T. Emilia Verghese, FM & BT Discipline, CFTRI, Mysore-570013.

Miss Subarna Keshari Tuladhar, PP & FT Discipline, CFTRI, Mysore-570013.

Miss B. Shankary, PP & FT Discipline, CFTRI, Mysore-570013.

Mr. S. Narasimhan, PP & FT Discipline, CFTRI, Mysore-570013.

Mr. B. R. Roy, Director Central Food Laboratory, 3, Kyed Street, Calcutta-700016.

Dr. K. B. S. Murthy Reddy F & VT Discipline, CFTRI, Mysore-570013.

Mr. A. Padmanabha, Bongoh, Fruit Processing Industries, Manantoddy Road, Mysore-570008.

Mr. R. Jagannatha Rao, MF & PT Discipline, CFTRI, Mysore-570013.

Miss Thara Jayaram, MF & PT Discipline, CFTRI, Mysore-570013.

Mr. A. M. Shamsul Alam, C-18, FAO-IFTTC Hostel, CFTRI, Mysore-570013.

Dr. Richard Joseph, CFTRI, Mysore-570013.

Mr. K. V. Puranik Math, Deputy Director of Agriculture, Bidar P.O., Karnataka.

Mr. K. G. K. Menon, CFTRI Experiment Station, Hyderabad-500018.

Mr. S. Lingamurthy, Ampro Food Products Uppal, Hyderabad.

Mr. P. Ravi, Ministry of Agriculture, Department of Food, Hyderabad-500007.

Mr. C. K. Verma, 438, Anand Bhavan, 14th Road Khar, Bombay-52.

Mr. William Allen, 20077, B-Main Road, Jayanagar, Bangalore-11.

Mr. P. L. K. M. Rao, Agarwal Evening College of Science, Pattargatti, Hyderabad.

Smt. D. Sarojini, Govt. Junior College, Hyderabad.

Mr. A. Ranga Sai, N. B. Science College, Hyderabad.

Mr. T. K. Ananthachar, R & PT Discipline, CFTRI, Mysore-570013.

Mr. P. Kalyanaraman, Flavours and Essences Pvt. Ltd., Lalithamahal Road, Mysore-570010.

Mr. E. J. Coelho, Flavours and Essences Pvt. Ltd., Lalithamahal Road, Mysore-570010.

Mr. Subrata Basu, 8A, Picnic Garden, Ist Lane, Calcutta-39.

Mr. R. Ravi, Flavours and Essences Pvt. Ltd., Lalithamahal Road, Mysore-5700010.

Mr. Jagadish S. Pai, Dept. of Food Sci and Nutrition, Chenoweth, Univ. of Mass., Anherst, Mass 01002, USA.

Mr. Henry L. Mascarenhas, No. 20, I Floor, 7th Cross, Wilson Gardens, Bangalore-560027.

Mr. J. Sudarshelal Gupta, Royapettah High Road, Mylapore, Madras-4.

Mr. J. V. Parekh, National Dairy Research Institute, Karnal, Haryana.

Mr. Robert D. Sears Care Inc., 36, Lavelle Road, Bangalore.

Dr. L. V. Venkataraman, Protein Technology CFTRI, Mysore-570013.

Smt. M. P. Vaidehi, Shrirampuram, Bangalore-560021

Mr. C. Balachandran F & VT Discipline, CFTRI, Mysore-13.

Dr. N. N. Varshney, Indian Institute, of Technology, Kharagpur 721302

Dr. H. N. Asthana, Fermentation Technology Discipline, CFTRI, Mysore-570013.

Mr. V. B. Ganatra, Rang Mahal 212, Samuel Street, Vadgadi, Bombay-3.

Mr. P. Chatterjee, Ramesh Mitter Road, Calcutta-25

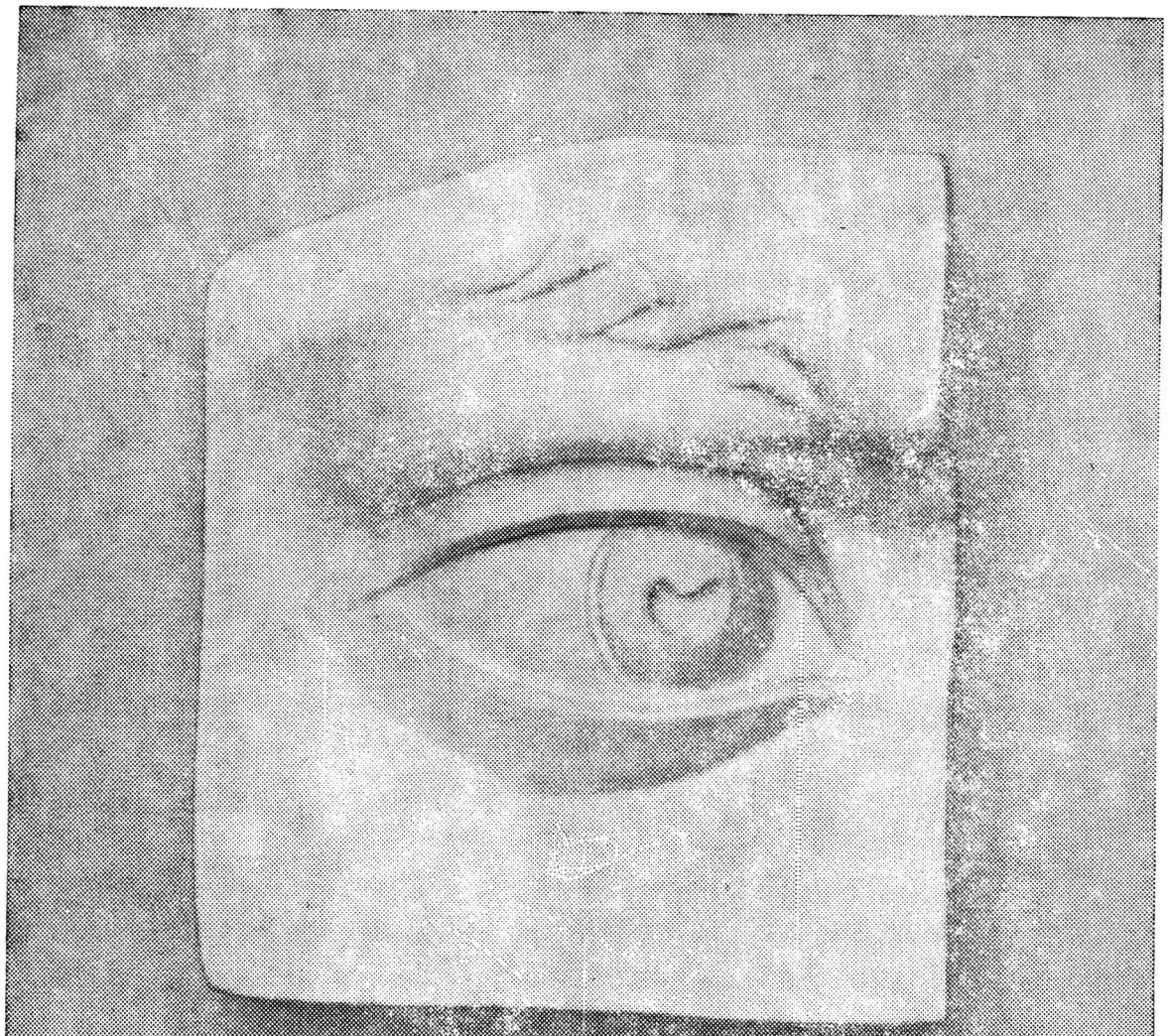
Today's Food Challenge

The 18th Annual Conference of the Canadian Institute of Food Science and Technology will be held at the Hotel Nova Scottan, Halifax, Nova Scotta, June 2nd to June 4th, 1975. Conference theme is "Today's Food Challenge". Ralph Davis—Registration Chairman, P.O. Box 550, Halifax, Nova Scotta, Canada.

ERRATA

Colorimetric Estimation of Tryptophan Content of Pulses, by M. V. Rama Rao, M. R. Tara and Chandra Kutty Krishnan, published in Journal of Food Science and Technology, September-October 1974, **11**(5).

Page No.	*Line No.*	*Reads*	*Corrected to read*
213	10	0.25	6.25
214	7	nitrate	nitrite
214	20	Interference in the amount of nitrite added	Interference in the amount of colour produced and the amount of nitrite added
214	35	0.78/16g	0.68g/16g
215	Table 4 last line	nitrate	nitrite



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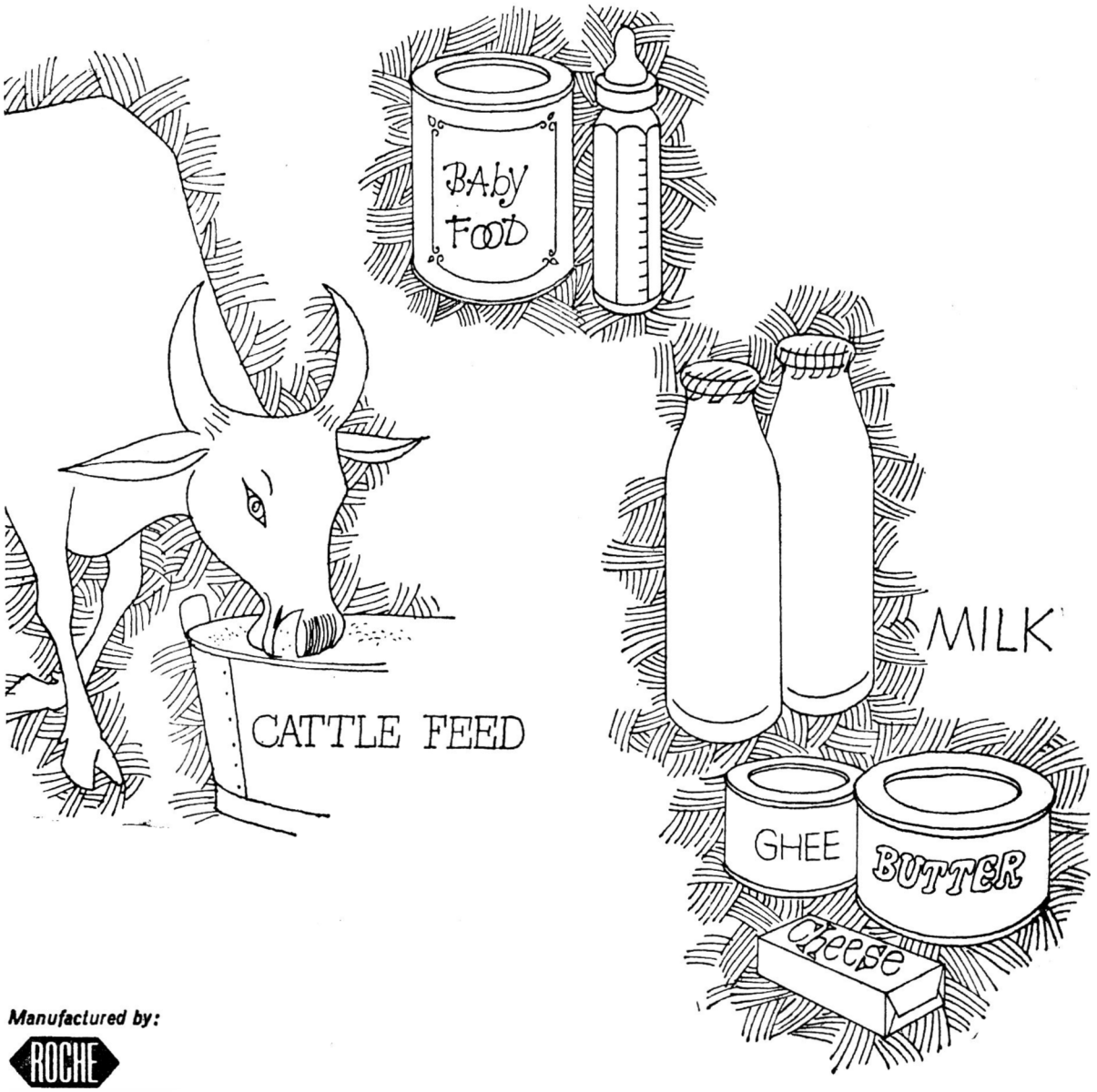

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2. Short communications in the nature of letters to the editor should clearly indicate the scope of the investigation and the salient features of the results.

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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