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ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA) MYSORE-570 013

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Methods for Estimation of Aflatoxins: A Critical Appraisal

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Carcinogenic and hepatotoxic nature of aflatoxins, their prevalence in the commodities meant for human and animal consumption, and the resultant stringent measures adopted by the importing countries for accepting the commodities, have collectively imparted extreme importance to the methods of estimation of aflatoxins. Consequently, each sample needs to be subjected to reliable estimation of aflatoxin before exporting. There are innumerable methods; both most sophisticated and the old ones, while the latter are still reliable and are commonly used. This review deals with the advantages and the shortcomings of each of these techniques so as to facilitate selection of the most dependable and practicable methods by the analysts of the developing countries, which often are the producers of the commodities meant for export.

Keywords : Aflatoxin estimation methods, Sampling, Extraction, Purification, Detection, Estimation, Thin layer chromatography, High pressure liquid chromatography, Fluorodensitometry, Instrumental methods, Enzyme-linked immuno sorbent assay, Radio-immuno assay, Comparison of assay methods.

Importance of analytical methods

Carcinogenic nature (Van Rensberg et al. 1985; Hsieh 1986) and the involvement of aflatoxins in the diseases of animals and humans (Newberne and Butler 1969; Gopalan et al. 1972; Krishnamachari et al. 1975) have collectively urged major public health organizations to stipulate maximum levels of aflatoxins that could be permitted in food and feeds (Schuller et al. 1983; Egmond 1989). These levels are based on the amounts that do not cause adverse effects on health and productivity of animals, when toxin is ingested through feed, and also the amount of toxic metabolites usually present in animal-derived foods (Park et al. 1989). Initially, Food and Drug Administration (FDA) focussed on limiting aflatoxin in animal feed to the lowest practical analyzable level of 30 µg/kg (FDA 1970, 1974). As analytical capabilities for determining aflatoxin improved, the permitted level was reduced to 20 µg/kg. On the basis of data that the aflatoxin contamination in feed above 20 ppb could not result in significant aflatoxin residues in foods, nor adversely affect animal health, the tolerance limits for aflatoxins in animal feeds were fixed in the range of 10 to 600 µg/kg in 1982 (FDA 1982; Park and Pohland 1986). The tolerance limit appears to be a result of national needs (Park et al. 1988). Countries which import the susceptible commodities, especially for human consumption, have lower permissible limits, than those producing the products. Whatever the limit may be, it is emphasized that the method, by which the level of aflatoxins is estimated, plays a very important role, either from the point of view of the effect on the health of humans and livestock or to make the product acceptable in the international market.

Principles of estimation

Methods of aflatoxin analysis are mainly based on the properties of aflatoxins. (Pons and Goldblatt 1965). The solubility of aflatoxins in organic solvents, like chloroform, methanol, ethanol, acetone, acetonitrile, benzene, etc., helps their quantitative extraction from the commodities. Their insoluble character in diethyl ether, petroleum ether, and hexane affords a method to separate them from certain interfering pigments. Characteristic fluorescence (Sargeant et al. 1961a) and absorption under long wave ultraviolet light (Vander Merwe and Fourie 1963) aid detection and estimation.

Steps in methodology

The steps involved in the methods of detection and estimation of aflatoxin are (1) sampling and sample preparation, (2) extraction, (3) purification, and (4) detection or estimation.

Sampling : Mycotoxin contamination of particular products such as grains and nuts is likely to occur in pockets of high concentration, and may not be randomly distributed (AOAC 1990). Within shelled peanuts, aflatoxin may be concentrated in less than 5% of the peanuts (Cucullu et al. 1966). Those peanuts containing aflatoxin might have concentrations upto 1,000,000 µg/per kg (Whitaker and Wiser 1969). Cucullu et al (1966) found that only 12 kernels from a sample of approximately 5000 peanuts contained detectable levels of aflatoxin. Furthermore, the quantities of aflatoxin in the

contaminated kernels varied from approximately 0.1 to 220 µg. They have also found that the topographical distribution of aflatoxin within single kernel is not uniform, even when the kernels contain high levels of toxin. If the toxin content is high in the heart and low in the outer portion of some kernels, it is concentrated on the outer portion and almost absent in the centre. The variation in the distribution of aflatoxin occurs in shelled groundnuts, peanut butter, roasted peanuts (Coker 1989) and cotton seed (Whitaker and Wiser 1969). Sixty five per cent aflatoxin is reported to be present in the sediment of groundnut oil (Basappa and Sreenivasa Murthy 1977). It is needless to say that the sampling error is less in uniformly mixable sample like liquids and semi-solids than in solids.

These above facts indicate that the quality control protocols for raw groundnut kernels must include statistically-based sampling methods, which can accommodate the hot spots of aflatoxin and provide samples which are truly the representative of the batch (Coker 1984). The Tropical Products Institute's (TPI 1965) plan is claimed to be a simple low cost one that can be used to determine whether a batch of groundnut kernels has a total aflatoxin content exceeding 30 µg/kg. It requires a collection of a representative 10.5 kg sample (15 to 20 batches preferably), which is randomly divided into three of 3.5 kg each portions. It is imperative that the whole of the 3.5 kg sub-sample is analysed. The plan developed by the United States Department of Agriculture (USDA) requires an initial sample of approximately 66 kg (Coker 1989). However, for a population of low quality (where, perhaps, 30% of the batches carry more than 30 ppb aflatoxin), the consumer risk is high with the use of TPI plan. Elsewhere, accumulation of a sample of at least 15 lb size is recommended (Pons and Goldblatt 1969). But, the major hurdle in these sampling methods is to collect samples from small farmers, who can supply only a few quintals and not in lots of tons.

It is, thus, apparent that adequate sampling is extremely important and also difficult. It is felt that there is no method available for error-free sampling. In the absence of a suitable method of sampling, it is suggested that whatever the quantity available, the sample is coned and aliquots are drawn from as many points as possible, using a probe or sampler, if necessary. The entire sample should be ground to pass through a 10 mesh screen, again coned and quartered to provide a 100 g analytical sample (Pons and Goldblatt 1969). Once ground, it is immaterial whether a 5, 10 or 25 g sample is analysed. Francis et al (1988) analysed 5, 10 and 25 test portions of finely ground 15 lb samples of contaminated corn by densitometry and statistically compared with 50 g test portions of the same composites for aflatoxin concentration variance. The results showed good agreement for all weights of samples analysed.

Extraction: Unlike sampling, there are a number of methods which quantitatively extract aflatoxin from the commodities. The analyst should select the solvent system which selectively extracts aflatoxin, leaving behind most of the interfering compounds. Sargeant et al (1961a,b) were the first to extract aflatoxin from mouldy peanut meal using methanol. Following this observation, other workers succeeded in complete extraction of aflatoxin from contaminated groundnuts and groundnut products. For example, Coomes and Sanders (1953) extracted aflatoxin using Soxhlet apparatus in 6 h, whereas Broadbent et al (1963) recommended 4 h extraction. Later, other workers, who followed this method, observed 1-2% loss of aflatoxin/h (Trager et al. 1964; Coomes et al. 1965; Nabney and Nesbitt 1965) of extraction and noticed that methanol also extracts other lipids, pigments and carbohydrates, along with toxin at elevated temperatures and that these interfere in the subsequent analytical steps (Pons and Goldblatt 1969). The extraction method was further improved later on, for shortening the time and avoiding the interfering substances. For example, Nesheim (1964a), comminuted peanuts with a methanol : water (55:45) solvent or hexane for 3.5 min in a high speed blender to effect simultaneous defatting and extraction. In case of the low fat peanut meal, hexane was omitted (Nesheim 1964 a,b).

The method is slightly modified by including sodium chloride, which helps in breaking of emulsion and adopted as official method II or BF method (AOAC 1990). This method is undoubtedly simple and quantitatively extracts aflatoxins from groundnuts. But, the shortcoming is that the aqueous methanol portion, which contains entire amount of aflatoxin, forms the middle layer with the use of hexane. Many times, mixing of some amount of hexane layer with the solid particles of the lowest sediment is unavoidable. The phase separation, either by centrifuging or by just standing takes long time, thereby delaying the analysis (Unpublished data). Among the equilibrium extraction methods, the solvent system of

acetone:water (70:30 or 65:35), to extract toxin from cottonseed, peanuts and other agricultural commodities, as suggested by Pons et al (1966a) or Stoloff et al (1966), is advantageous, because neutral and polar lipids are not soluble in acetone water mixture and their interference during subsequent analytical steps, is thus avoided.

Later, Pons et al (1980) modified the solvent system to 85:15 ratio of acetone:water. Aflatoxins can also be extracted after prewetting the materials. Lee (1965) slurried defatted peanuts and peanut meal with ten-fold excess water and extracted with chloroform by shaking for 30 min on a shaker. The method appears simple, but involves defatting step. There will be problem of emulsion, resulting in delayed phase separation. No purification step of the extract is included. Eppley (1966a) used the basic concept of Lee (1965), i.e., extraction with chloroform of prewetted material, which was later adopted as official method under the heading contaminants branch (CB) method (AOAC 1990). The essential steps of this method are as follows: The material is prewetted with water at the ratio of 50:25 w/v, shaken with 5 times chloroform (w/v), mixed with good amount of celite, filtered and subjected to silica gel column purification. Although CB method has many advantages, it has a few disadvantages too, as observed in our laboratory (Unpublished data). The advantages are (a) the method provides almost pure aflatoxin extract and (b) quantitative recovery from the test materials (Chang et al. 1979). The disadvantage is the extraction of fat along with aflatoxin, in case of full fat materials like groundnuts (AOAC 1990). This fat, although will be removed during the subsequent column purification, can introduce an error due to residual volume of fat (e.g., groundnut seeds). Mehan et al (1985) recovered less toxin by CB method than by other methods. Perhaps, during column chromatography, using silica gel column, there could be a few occasions, where aflatoxin is lost. There were occasions, when aflatoxin was eluted along with diethyl ether or destroyed by diethyl ether solvent (Unpublished data). Hence, the analyst must be extremely careful about the purity of the solvents, especially diethyl ether. It is better to ensure the stability and recovery of toxin by first working with standard aflatoxin.

Extract purification: The primary methanolic extract can be purified by liquid-liquid partition system (Coomes and Sanders 1963). de Iongh et al (1964) suggested partition between methanol:water:chloroform in separating funnel, while the use of acetone:hexane:salt solution (Broadbent et al. 1963; Genest and Smith 1963; TPI 1965; Coomes et al. 1965; Robertson et al. 1965) or liquid-liquid extraction with chloroform (Trager et al. 1964; Nabney and Nesbitt 1965) was also advocated. Wherever methanol is used, it undoubtedly extracts quantitatively, but along with other substances. It is a good solvent for fat and pigment also (Pons and Goldblatt 1969). Wherever chloroform is used, there is a likelihood of a problem of emulsion during phase separation of the solvent phase. By using salt solution and hexane, the interference by the fat and to some extent, by the pigment can be avoided.

All the solvent systems mentioned above are suitable for the samples containing fairly high amounts of toxin (Nesheim 1964a). The solvent system to extract aflatoxin can be selected by the analyst depending on the sample to be analysed and the availability of the solvent. For samples causing emulsion problem (for example, wheat flour and egg), the system containing salt solution may be chosen. For low fat samples, direct chloroform extraction of the water slurry (Lee 1965) or aqueous methanol extraction can be used.

Pons and Goldblatt (1965) and Pons et al (1966a) suggested extraction of aflatoxins from cottonseed, peanuts and other agricultural materials, using aqueous acetone (70:30 acetone:water). As neutral and polar lipids are essentially insoluble in the acetone:water solvent, efficient defatting and aflatoxin extraction are conducted simultaneously. Stoloff et al (1966) used acetone:water (65:35) for extraction of aflatoxins from cottonseed products, using a blender. The interfering pigments from a variety of agricultural products can be removed as insoluble lead-salt by treating the extracts with neutral lead acetate (Pons et al. 1966a). There appears to be only two methods available for extraction of aflatoxin from oil (Pons et al. 1966a; Parker and Melnick 1966). Method of Parker and Melnik (1966) includes extraction of the oil with 55% methanol (aqueous) by thoroughly shaking (thrice) for 1.5 min and extraction of methanolic extracts with hexane, to remove traces of all contamination with hexane. Aqueous methanolic extracts are, then, evaporated to reduce the volume and extracted by liquid/liquid extraction with chloroform for 3 h.

The method described by Pons et al (1966a) is extremely simple, as compared to that of Parker and Melnick (1966). The oil is dissolved in hexane and the hexane-oil mixture is extracted by swirling

with 70% acetone (aqueous). This solvent removes aflatoxin quantitatively, with little oil residue. Aqueous acetone is boiled with lead acetate to precipitate any pigment as lead salts and to reduce acetone content. Finally, aflatoxin is taken into chloroform by liquid/liquid extraction. This method is undoubtedly superior to that of Parker and Melnick (1966). Aqueous acetone extract is almost pigment-and fat-free (Pons and Goldblatt 1969). Often, the treatment with lead acetate does not appear to be needed. Phase separation is fast and the whole operation can be finished within 45 min. So far, there is no other better method published. Pons et al (1980) have modified their method of extraction and purification for use with agricultural commodities. The solvent used is acetone:water (85:15) for primary extraction. The acetone extract is treated with lead acetate by keeping at room temperature for 5 min, instead of boiling it on water bath, thus saving time, which otherwise is required for evaporation of acetone. After lead acetate treatment, the extract is filtered through a Buchner funnel over a bed of hyflosupercel or celite filter aid.

Although the methods described above are suitable for most of the materials, analytical problems are encountered in certain cases. Coffee, cocoa and tea are difficult to analyse, since they contain alkaloids like caffeine and theobromine, which have similar solubility characteristics, as those of aflatoxins and the solvent extraction systems remove both aflatoxins and alkaloids simultaneously (Campbell 1968). The problem, thus, is the separation of a small amount of aflatoxins from a relatively large amounts of alkaloids (Campbell 1968). Levi and Borker (1968) and Scott (1968) have reported the methods for coffee, in which florisil is employed in the cleanup step of the procedure. Campbell (1968) found batch to batch variation in the capacity of florisil for binding aflatoxins, thereby resulting in losses upto 100%. Still, the method has been described as the only sensitive method available so far. Campbell (1968) was able to detect as little as 2 µg of added toxin in green coffee. Scott (1969) has published a method for analysis of cocoa beans, which uses silver nitrate precipitation step to separate aflatoxins from theobromines. The method is a modification of (CB) procedure for peanuts, except that it requires defatting with hexane and treatment with $AgNO_3$, before and after extraction with chloroform. The method has been adopted as official first action method (AOAC 1990). Tea presents one of the most challenging aflatoxin analytical problems encountered to date, because of some interfering compounds, in addition to alkaloids (Campbell 1968). Efficient method for aflatoxin analysis in tea is yet to be developed. Shantha and Rati (Unpublished data) could not recover 200 ppb added aflatoxin from black tea or roasted coffee by the method of Levi and Borker (1968).

Apart from precipitation with lead acetate (Pons et al. 1966a), silver nitrate (Scott 1969) and silica gel column chromatography (Eppley 1966a), several other workers adopted aluminium oxide (Trager et al. 1964), silicic acid, sephadex, florisil, celite, liquid column chromatography, aluminium oxide and silica gel thin layer chromatography (Pons et al. 1968; Manabe et al. 1968; Genest and Smith 1963). Coomes and Sanders (1963) were the first to propose a neutral aluminium oxide column to purify the primary extract of peanut products, Manabe et al (1968) found that the aflatoxin G_1 and G_2 are absorbed in the column and only aflatoxins B_1 and B_2 are eluted selectively, when a crude aflatoxin mixture is added to an aluminium oxide column and eluted with a chloroform solvent, containing 1% methanol. Mayura and Sreenivasa Murthy (1969) eluted all the four aflatoxins from 1 x 1 neutral alumina column using chloroform: acetone : methyl alcohol (6:3:1). They were also able to estimate all the four aflatoxins separately by selective absorption and destruction of aflatoxins B_1 and G_1. Aflatoxins B_1 and B_2 were eluted with chloroform: methanol:acetone (1:1:8) and aflatoxins G_1 and G_2 were absorbed on the column. In the mixture of aflatoxins B_1, B_2, G_1, and G_2, only aflatoxins B_1 and G_1 were destroyed by nitric acid or NO_2. Similarly, when a mixture of B_1 and G_1 aflatoxins is passed through alumina column, only aflatoxin B_1 is eluted with chloroform:methanol: acetone (1:1:8) system. By determining the spectrophotometric absorption of different elutes, values of each component could be obtained.

Distinct methods are used, when the estimation is done by HPLC. For example, in the method of Tutelyan et al (1989), a finely ground sample (25 g) was extracted with 125 ml acetone containing 10% aqueous NaCl solution (4:1, v/v) for 30 min on a wrist action shaker. After filtration, 50 ml of 10% aqueous lead acetate solution was added to 50 ml filtrate. After 10 min, the extract was filtered through paper, the aqueous layer was defatted with hexane and aflatoxin was extracted from the aqueous layer with chloroform. The chloroform extract was dried over anhydrous Na_2SO_4 and

evaporated to dryness under vacuo. The residue dissolved in a small volume of chloroform was cleaned up using a small silica gel column, packed with 2 g silica gel (100-160 µm) in between two layers of anhydrous sodium sulphate and chloroform-acetone (5:1) as the eluant. The eluate was evaporated to dryness and the residue was dissolved in 0.5-1.0 ml of toluene:acetonitrile (97:3, v/v).

The sample can also be extracted and purified by the method described by the Hetmanski and Scudumore (1989). The steps involved are (i) extraction of the sample with dichloromethane in the presence of water and hyflosupercel and (ii) purification by gel permeation chromatography (GPC) using glass column packed with Bio-beads ($S\text{-}X_3$) and use of dichloromethane:hexane (3:1) as the eluant. The purified extract was dissolved in acetonitrile: water or toluene: acetonitrile, injected into the chromatograph and eluted with diethyl-ether:methanol:water (95:4:1) mobile phase, at a flow rate 1.0 ml/min. Identification of aflatoxin was effected by comparing the retention time with that of an aflatoxin standard. The retention time is 8-14 min and the order of elution is aflatoxins B_1, B_2, G_1 and G_2 at 8, 9, 10.5 and 12.5 min, respectively.

Detection and estimation methods

As mentioned earlier, the flourescence exhibited by the aflatoxins under long wave UV is the basis for detection and estimation of aflatoxins (Pons and Goldblatt 1969) by visual and instrumental techniques. Modern analytical techniques like enzyme - linked immuno sorbent assay (ELISA) and radio immuno assay (RIA) are based on antigen and antibody reactions, wherein the aflatoxins present in the sample compete for antibody in proportion to its concentrations. These methods are examined critically in the following sections:

Estimation by thin layer chromatography : The first chromatographic separation of aflatoxins was proposed simultaneously by Coomes and Sanders (1963) and Broadbent et al (1963). Coomes and Sanders (1963) used paper chromatography and benzene:toluene: cyclohexane:ethanol:water (3:3:5:8:5 v/v, upper layer saturated with acetic acid) as developing solvent. The sensitivity of detection is only 0.2 µg. Consequently, this method is almost given up and is replaced by the more rapid and sensitive thin layer chromatography. Broadbent et al (1963) used neutral alumina plates and chloroform:methanol:water (98:0.5:1.5) as developing solvent. Manabe et al (1968) used aluminium oxide, G, containing about 15% calcium sulphate. The sensitivity of detection was 6×10^{-3} µg, i.e., 30-fold more sensitive than paper chromatography. The problem with alumina plates is the poor resolution of aflatoxins and maintenance of activity of the coated plates (Manabe et al. 1968).

The use of silica gel (kieselgel G) coated TLC plates for the resolution of the aflatoxins B_1, B_2, G_1 and G_2 was introduced by de Iongh et al (1964). Subsequently, numerous procedures incorporated the use of TLC plates coated with silica gel G at wet thicknesses ranging from 300 to 500 µ and various chloroform methanol development solvents (Coomes et al. 1965; Nabney and Nesbitt 1965; TPI 1965). Trager et al. (1964) introduced the use of silica gel GHR for improved resolution of aflatoxins B_1, B_2, G_1, and G_2, using chloroform: methanol (95:5) for development. A distinct improvement in the resolution of individual aflatoxins on silica gel - coated plates was reported by Engebrecht et al (1965), using chloroform: acetone (9:1) as developing solvent. Better resolution of the toxins was observed by Eppley (1966b), when the same plates were developed in unlined and unequilibrated developing tanks. Manabe et al (1968) compared the resolution of different components of aflatoxins on silica gel G, containing 13% calcium sulphate and silica gel HR, not containing any calcium sulphate, in order to find out the effect of calcium sulphate. Good separation was obtained with 5% acetone in chloroform in case of silica gel G and with 3% methanol in chloroform in case of silica gel HR.

The developed plate is observed under long wave ultraviolet (UV) light in a dark place in a viewing cabinet to conduct visual estimation. Early methods for aflatoxin B_1 estimation used serial dilutions of unknown extracts to extinction of fluorescence and the quantification was achieved based on prior estimation of the amount of B_1 producing the least observable fluorescence under the TLC development conditions (Coomes et al. 1965). Later, as authentic aflatoxin B_1 became more available, the fluorescence intensities of aflatoxin B_1 in unknown extracts and in standards chromatographed under the same conditions were compared directly to allow a better semi-quantitative estimation (de Iongh et al. 1964). The intensity of the fluorescence of aflatoxins makes possible the visual detection of as little as $3\text{-}4 \times 10^{-6}$ µg (0.3 to 0.6 µg) of aflatoxin B_1 or G_1 on a TLC plate (Goldblatt 1968; Heusinkveld et al. 1965).

Objective estimation using spectrophotometer : The first effort to improve the accuracy and precision of aflatoxin measurements by the use of objective methods was reported by Nabney and Nesbitt (1965). Concentrated chloroform solutions of partially purified extracts from peanut meals were streaked across silica gel plates and the plates were developed successively with diethyl ether and then with chloroform: methanol (9:1) to separate aflatoxin bands. The bands were removed, eluted with methanol and aflatoxins B_1 and B_2 were estimated by absorption spectrophotometry at 363 nm. Since absorption is much less sensitive than fluorescence, by a factor of 1000 or more (Goldblatt 1968), some 3-10 µg of isolated aflatoxins were required for accurate estimation. Mayura and Sreenivasa Murthy (1969) found that it was necessary to have 17 µg of isolated aflatoxin for measurement of absorption by spectrometer.

Ayres and Sinhuber (1966) suggested, for the first time, the fluorodensitometric measurement of aflatoxin B_1 directly on silica gel plates. The basic system (Goldblatt 1968) is shown in Fig. 1.

A developed TLC plate is placed, gel layer down, on a motor driven stage, over a low intensity long wave 320-390 nm ultraviolet source. A search unit

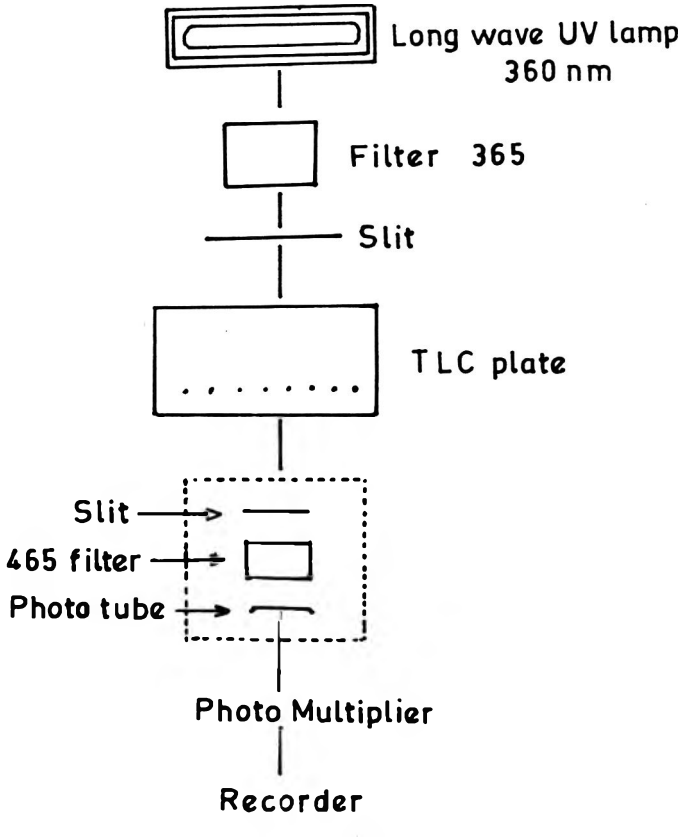

Fig. 1. Schematic diagram of densitometer.
Source : Pons and Goldblatt (1969)

containing a narrow exit slit, a secondary long-wave ultraviolet screening filter and a photomultiplier tube, is lowered to about 1 mm above the plate surface. As the separated aflatoxin spots pass across the ultraviolet source, these are excited to fluorescence, and the emitted fluorescence radiation (420-450 nm) as well as a portion of the long wave UV excitation radiation passes successively through the exit slit. The screening filter is used to remove long wave UV excitation radiation and impinge on the photomultiplier tube. The tube output is amplified and fed to a millivolt recorder, where the fluorescence emission of the aflatoxin spots are recorded as symmetrical curves. Ayres and Sinhuber (1966) found a logarithmic relationship between emitted fluorescence energy and concentration, which was linear over a range of about 2.5 to 15 x 10^{-4} µg of aflatoxin B_1 per spot, while Pons and Goldblatt (1965) demonstrated a linear relationship between emitted fluorescence, as measured by peak areas and concentration over a range of 2 to 105 x 10^{-5} µg per spot.

Comparison of visual, spectrophotometric and fluorodensitometric estimation of aflatoxin: An experienced human eye can detect the fluorescence of 0.001 µg of aflatoxin B_1 and less amount of aflatoxins B_2, G_1 and G_2 under 8-10 W ultraviolet lamp, whereas spectrophotometer requires very high amounts, some 3-10 µg, since absorption is much less sensitive than fluorescence by a factor of 1000 or more (Pons and Goldblatt 1969).

As far as fluorodensitometric estimation is concerned, the coefficient of variation reported by various workers is different (Pons et al. 1966 b, 1968). With an aflatoxin standard containing B_1, B_2, G_1 and G_2 resolved on eight plates, precision estimates ranged from ±4–10% for individual aflatoxin and ± 6% for total aflatoxins (Pons 1968). Beckwith and Stoloff (1968) reported that the coefficient of variation of a single estimation was ± 12%. However, it could be reduced to ± 5% for the average of five measurement.

Undoubtedly, the instrumental measurement is superior to visual estimation, since it eliminates the subjective error, provided the conditions of the thin layer chromatography are scrupulously followed so as to eliminate all the impurities coming at the R_f of aflatoxin. The sample must be perfectly purified. The spots should be in a perfect linear position, for which the silica gel layer should be uniform. It has been observed

TABLE 1. COLLABORATIVE STUDY OF AFLATOXIN DETERMINATION IN COTTONSEED PRODUCTS

Samples analysed	Aflatoxins added (B_1+B_2) µg/kg	Aflatoxin estimation method: Visual analysis[a] Mean recovery, %	Visual analysis[a] Standard deviation, µg/kg	Visual analysis[a] Coefficient of variation, %	Fluorodensitometric analysis[b] Mean recovery, %	Fluorodensitometric analysis[b] Standard deviation, µg/kg	Fluorodensitometric analysis[b] Coefficient of variation, %
Spiked samples							
Meal	31	101	± 6.2	20	100	± 5.2	17
Meats	56	84	± 8.8	19	99	± 5.0	9
Meal	56	77	± 8.6	20	74	± 7.1	17
Meal	100	82	±16.3	20	87	±11.4	13
Meats	112	82	±19.0	21	91	±16.7	16
Naturally contaminated samples							
Meal	34[c]	-	± 5.6	17	-	± 3.8	11
Meal	133[c]	-	±12.7	10	-	± 6.0	4
Meal	170[c]	-	±28.8	18	-	±13.0	7
Mean, spiked		85	-	20	90	-	14
Mean, natural		-	-	15	-	-	8

[a] 12 laboratories; [b] 8 laboratories; [c] Mean values from visual and fluorodensitometric analysis; Data from Pons (1969); Pons and Goldblatt (1969).

many times that the spots from an arc than a line (unpublished data). Slight difference in the R_f value will alter the peaks, thereby reducing the accuracy of measurement. Since the equipment is much more expensive than the UV lamp, it may not be available readily in all the analytical laboratories. On the other hand, a well experienced analyst can detect the same amount, as detected by the instrument. He/she can identify the aflatoxin spots, in spite of the difference in R_f value. He/she can also distinguish between the aflatoxin and the spurious compounds coming at the same R_f by the difference in the nature of fluorescence. Beckwith and Stoloff (1968) established the precision limits of visual estimation at ± 20-28% under ideal conditions. Eppley (1966a) indicated a possible measurement of ±15-25%, when the unknown is interpolated between two standards.

Comparative data on visual and fluorodensitometric aflatoxin estimates, as reported by several investigators, (Pons and Goldblatt 1969) are summarized in Table 1. It may be noted that estimates by either measurement system are of the same order of magnitude. A collaborative study in which aflatoxins in cottonseed products were determined, both visually by twelve laboratories and by fluorodensitometry on thin layer plates by eight laboratories also showed a good correlation between aflatoxin estimates by the two measurement techniques (Pons and Goldblatt 1969). It is our experience that aflatoxins can be estimated visually within precision of 15% by following the dilution to extinction method of Coomes et al (1965) and Rati et al (1987).

Estimation of aflatoxins by HPLC : High pressure liquid chromatography (HPLC) offers promise as an objective instrumental system for improving accuracy and precision of aflatoxin determination in a variety of agricultural commodities. (Selber and Hsieh 1973; Seitz 1975, Buchanan et al. 1975; Thorpe and Stoloff 1975; Pons 1976; Pons and Franz 1977; Takahashi 1977). Aflatoxins are completely resolved on a micro particulate (10-20 µm) porous silica gel column, viz., µ-Bondapak C_{18} 4 mm inner diam x 30 cm or 5 µm ultrasphere-Si silica gel column 250 x 4.6 mm inner diam (Pons and Franz 1978; Tutelyan et al. 1989). Initially, Pons and Franz (1978) detected aflatoxins B_1 and B_2 by ultraviolet absorbance at 360-365 nm and aflatoxins G_1 and G_2 by fluorescence, since aflatoxins B_1 and B_2 show very little solution fluorescence, whereas aflatoxins G_1 and G_2 are highly fluorescent in the solvents used for normal phase HPLC. Since the fluorescence detection method is more sensitive and less subject to interference by artifacts and the hemiacetal derivatives allow sensitive solution fluorescence, Pons (1979) suggested reverse phase HPLC by fluorescence detection. Preparation of derivatives of both the samples and standards as well as the possibilities of variability in the completeness of the derivatization mitigates against wider application of derivatization technique. Tutelyan et al (1989) modified the HPLC method, wherein they used a normal phase HPLC method, which allows both aflatoxins B_1, B_2, G_1, and G_2 as well as the aflatoxin metabolites M_1, B_{2a} and G_{2a} to be

determined with low detection limits (0.1 μg/kg), without preliminary derivatization.

Although HPLC method is extremely sensitive and allows the detection of aflatoxins at less than ppb level, the availability, the cost of the instrument, the need to use only special grade solvents (which are also expensive) and the fact that only one sample can be analysed at a time are the factors which preclude it from being adopted for routine analysis of the samples. Pons (1969) has illustrated good correlation between HPLC and TLC methods of analysis, which again show that there are good alternative methods to HPLC.

Estimation of aflatoxins by immuno assay techniques : Lawellin et al (1977) are, perhaps, the first to report enzyme linked immuno sorbent assay (ELISA) for analysis of aflatoxin B_1. Subsequently, Chu and co-workers have done a voluminous work on the immuno assay technique for analysing mycotoxins (Chu and Ueno 1977; Chu et al. 1982). These assays permit the detection of aflatoxins at pg levels.

The enzyme immuno assay techniques (EIA) have been classified as either competitive or non-competitive assays (Engvall 1980). In the competitive assay, labelled and unlabelled antigens compete for a limited number of antibody sites. In the non-competitive assay, the antigen to be measured is first allowed to react with antibody on a solid phase, followed by measurement of the binding of enzyme labelled immune reactant (Engvall 1980).

Since aflatoxins are compounds of lower molecular weights (haptens), they do not elicit production of antibodies in an animal system (Kabar 1980). Hence, these must be conjugated to a protein or a polypeptide (Chu 1984). Since aflatoxins do not possess a reactive group, they cannot be conjugated directly to a protein (Chu 1992). Aflatoxins are either halogenated or treated with carboxymethoxylamine to obtain the oxime (Chu et al. 1977). The halogenated aflatoxin B_1 is allowed to react directly (Sizaret and Malaveille 1983; Groopman et al. 1984). Lawellin et al (1977) converted aflatoxin B_1 into aflatoxin B_{2a}, before coupling to bovine serum albumin (BSA). The carboxymethyl oxime is conjugated to protein by mixed anhydride method (Shashidar and Narasinga Rao 1988), which involves activation to the corresponding anhydride by isobutyl chloroformate and then direct reaction with the protein. Fig. 2 gives the schematic diagram of the reactions involved.

Thus, immuno assay of aflatoxins involves (i) preparation of aflatoxin B_1 oxime, (ii) conjugation of the oxime to a protein, (iii) production of antibodies, and (iv) establishment of immuno assay protocols.

Preparation of aflatoxin B_1 oxime : Aflatoxin B_1 and carboxymethyl amine at 1:2 molar ratio are refluxed at 110°C for 2.5 h in water:pyridine:MeOH (1:1:4) medium and left overnight (Chu et al. 1977). The oxime is purified by silica gel TLC, developed in 9:1 chloroform:acetone, followed by 9:1 benzene: acetic acid (Lau et al. 1981; Chu et al. 1982; Shashidhar and Narasinga Rao 1988).

Conjugation of the oxime to protein by mixed anhydride : Conjugation of aflatoxin B_1 protein carrier is achieved either through the cyclopentenone ring by conversion of the carbonyl group of aflatoxins to carboxymethyl oxime (Fig. 2) derivatives or through the dihydrofuran ring (Shashidhar and Narasinga Rao 1988). Aflatoxin B_1 oxime is stirred in dioxane + tri-n-butylamine + isobutyl chloroformate (10+0.1+0.1) solvent mixture at 4-6°C. Bovine serum albumin (BSA) dissolved in dioxane + water + 0.1 N NaOH (10+47+3) is added in the proportion of 1:50 (protein:mycotoxin) and stirred for 4 h at 4-6°C. The un-reacted toxin is removed by dialysis against distilled water or buffer or by Sephadex gel filtration. Although BSA has been commonly used as carrier protein (Sizaret and Malaveille 1983) for preparation of immunogens, other proteins such as γ-globulin, human serum albumin, ovalbumin lysozyme, polylysine and keyhole limpet hemocyanin can also be used (Chu 1991).

For conjugation, water-soluble carbodiimide (Chu and Ueno 1977) or n-hydroxy succinimide (Chu 1990, 1991; Pestka 1988) is also used, instead of mixed anhydride. Aflatoxin or aflatoxin derivative is generally reacted with a protein carrier at a molar ratio of 10:1 to 100:1 (mycotoxin: protein) and pH 5-8 in the presence of a coupling reagent such as EDPC 1-(3 dimethyl amino propyl)-3-ethyl carbodiimide (Chu et al. 1977). Alternatively, the mycotoxin or its derivative is first activated with N-OH-succinimide in the presence of carbodiimide and then conjugated to protein directly (Chu 1991; Pestka 1988).

Production of antibodies against aflatoxins : Polyclonal antibodies against aflatoxin B_1 are produced by subcutaneous injection of the immunogen, mixed with the Freund's adjuvant at the multiple sites on the back of the rabbit (Lawellin et al. 1977). Goats and pigs have also

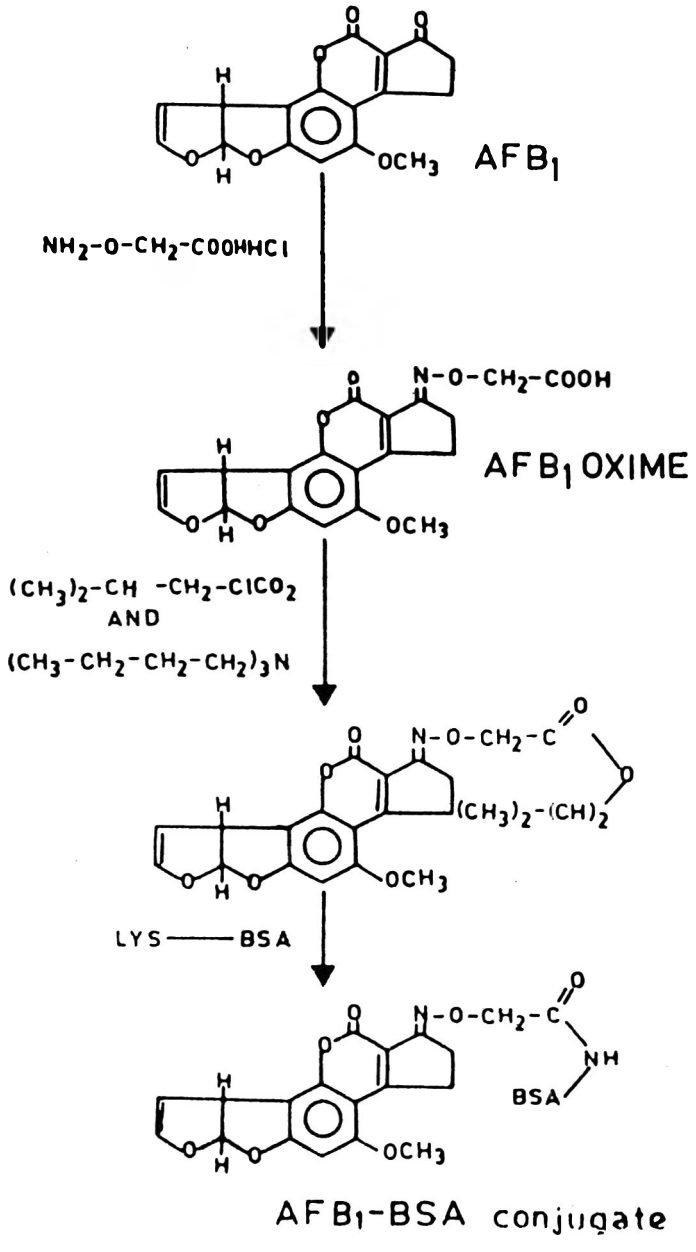

Fig. 2. Reaction sequence of AFB_1-BSA conjugation
Source : Shashidhar and Rao (1988)

been used for the purpose (Bierman and Terplan 1980; Chu and Ueno 1977; Langone and Vunakis 1976). Subsequent booster injections are given once each month. Antibodies are produced from 5th week onwards (Unpublished data).

Monoclonal antibodies are preferred for their specificity and the unlimited production of antibodies (Chu 1992). For production of monoclonal antibodies, spleen cells are obtained from mice that have been immunized with mycotoxin protein conjugates and are, subsequently, fused with myeloma cells. The hybrid cells are propagated to produce antibodies either in tissue culture or in the ascites fluids of mice (Pestka 1988).

Polyclonal antibodies, produced using carboxymethoxylamine, generally recognize the dihydrofuran moiety and, hence, are highly specific towards aflatoxin B_1, and there will be some cross reactivity with aflatoxin G_1 (Chu 1992). It is simple and easy to produce polyclonal antibodies for the specific purposes of detection and estimation of aflatoxins, as compared to the production of monoclonal antibodies, the procedure for which is more tedious and requires specialized techniques to produce hybridoma cells and the antibodies (Tijssen 1985).

Development of immuno assay protocol : The immuno assay of aflatoxins is based on the competition between the toxin under test and the aflatoxin marker (either an enzyme bound or radio-labelled) for the specific antibody sites. The extent of binding to the antibody depends on the concentration of the toxin (Lawellin et al. 1977). Depending on the marker used, the assay has been divided into (i) radio immuno assay, if labelled, aflatoxin is used and (ii) enzyme linked immuno sorbent assay (ELISA), if enzyme is used.

Radio immuno assay : The radio immuno assay (RIA) procedure includes the incubation of specific antibody with a solution of unknown sample or known standard and a constant amount of labelled toxin (either tritiated or I^{-125} tyramine- AFB_1-1-0 carboxymethyl oxime) with high specific activity (Chu 1986). The bound and free toxins are separated by any one of the methods such as ammonium sulphate precipitation (Chu and Ueno 1977), double antibody technique (Langone and Vunakis 1976), a solid phase RIA method (Sun and Chu 1977), dextran coated charcoal column (Fukal et al. 1986), albumin-coated charcoal and polyethylene glycol 6000 (Fukal et al. 1986; Rauch et al. 1987). The labels in the bound and free fractions are determined. The toxin concentration of the unknown sample is determined by using the standard curve, obtained by plotting the ratio of the radio activities in the bound fraction and the free fraction versus the logarithm of the concentration of unlabelled standard toxin. It is reported that RIA can detect 0.2 to 0.5 µg (2-5 ppb) toxin and the sensitivity is improved to 0.05 to 0.5 µg (1-5 ppb), if purified (Sun and Chu 1977).

Although RIA is simple and highly sensitive, it suffers from the risk involved in the use of radio-active material and the difficulty in the availability of the expensive instrument to measure radio-

activity (Engvall and Carlsson 1976).

Enzyme linked immuno sorbent assay : Two types of ELISA have been used for the analysis of aflatoxin. (i) direct competitive ELISA, and (ii) indirect competitive ELISA (Engvall 1980).

In the direct competitive ELISA, specific antibodies are initially coated on a solid phase like polystyrene microtitre plates (Chu 1986, 1990, 1991), nylon beads, Terasaki plates (Pestka and Chu 1984) or polystyrene tubes (Biermann and Terplan 1980). However, use of microtitre plates are more popular. A sample or standard toxin solution is incubated with aflatoxin-enzyme conjugate and then added to the microtitre plate wetted with the antibody. The sample or standard and the enzyme can also be added separately in two steps (Chu 1991). After incubation, the plates are washed with washing buffer containing Tween-20 and the bound enzyme is determined by incubating with suitable substrate. The resulting colour, which is inversely proportional to the concentration of aflatoxin, is measured by a spectrophotometer or ELISA reader (Shashidhar and Narasinga Rao 1988; Chu 1991).

In the indirect competitive ELISA, aflatoxin-protein conjugate (Chu et al. 1987; Chu 1990, 1991; Morgan 1985; Pestka 1988), in which the protein used is different from that used to raise the antibodies, is coated to the microtitre plate. The plate is incubated with specific antibody against aflatoxin, in the presence of aflatoxin to be estimated (Pestka 1988), which is added either in one or two steps. The amount of antibody bound to the plate is, then, determined by the reaction with a second antibody-enzyme complex, such as goat anti-rabbit IgG-enzyme complex or goat anti mouse IgG (IgM)-enzyme complex, which are commercially available. Toxin in the sample and in solid phase competes for the same binding sites with the specific antibody in solution (Chu 1992).

Indirect ELISA offers a method, which requires very much less antibody, than that in the direct assay, but suffers from the disadvantage that an additional step is involved, prior to the addition of second antibody (Chu 1992).

Dot binding of antigens to nitro cellulose : Antigen may be bound in small dots to nitro cellulose using, micropipetting devices (Tijssen 1985). Other binding sites can be saturated with an unrelated inert protein or other blocking agents (Tijssen 1985). The subsequent immuno enzymatic revelation of the antigen produces an insoluble coloured product, which precipitates at the site of the enzyme and is viewed against a white background. The attractiveness of these membranes resides in the fact that very small amounts of immuno reactants are required and, hence, applicable for monitoring of samples (Tijssen 1985). The discriminatory power is claimed to be greater than that obtained in microtitre plates (Tijssen 1985).

Quick screening test : In addition, there are quick screening tests based on ELISA (Park et al. 1987, 1989). Instead of coating the microtitre plate with the antibody, paper discs or membranes, the plastic cups are coated with the antibody. A few drops of the sample are applied on the paper, reacted with the aflatoxin enzyme conjugate, washed with buffer saline and finally reacted with the substrate (Park et al. 1987, 1989). ELISA kits are available commercially and allow the estimation of aflatoxins in a few minutes.

Advantages and limitations of immuno assays: The ELISA technique is the most sensitive method which allows the detection of pico gram levels of toxin (Chu et al. 1987). The sample extract does not require the elaborate purification step (Chu 1992), unlike in HPLC. On the other hand, the disadvantages are many. ELISA needs special infrastructure facility. For production of antigens, it requires fine chemicals, which often are not readily available. Production of antibody requires a minimum of 1 month time (Chu and Ueno 1977). Microtitre plates should be of high quality so that the variation among different wells is minimised (Shekarchi et al. 1984). It requires skill to develop the protocol. Although imported commercial kits are available, their shelf-life is limited and these may not be available, when the analyses are to be carried out urgently. There is always a chance for cross-reaction owing to the complexity of the biological sample and hence additional purification step is needed (Tijssen 1985).

Overviews

Analytical methods followed for the qualitative and quantitative estimation are by TLC, (visual and fluorodensitometric), HPLC, ELISA and RIA. Visual TLC is the method of choice in the countries, where other expensive instruments and the infrastructure for immuno assays are not available, though it is criticised for high degree of variation due to individual's acuity. But, high degree of variation

can be alleviated by multiple observers (Pons 1969). Visual TLC estimation is simple and reliable, so long as the analyst ensures the validity of the method by acceptable recovery experiment. Background interference should be considered, whenever fluorodensitometer is used. Immuno assays and HPLC methods, although sensitive, are not readily applicable. As Egmond and Wagstaffe (1989) opined, it is more important to apply rigorous quality assurance to the measurement procedure than to rely blindly upon standardized and often archaic methods.

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Interrelationship Between Some Structural Features of Paddy and Indices of Technological Quality of Rice

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Data on structural features of 25 varieties of paddy revealed that grain breadth was positively correlated with white belly (chalkiness on ventral side) and negatively with the tightness of husk interlocking. The latter could be indexed inversely by the ease of shelling of paddy; but shellability was influenced positively by grain breadth also. Rice hardness was adversely affected by grain chalkiness, but hardness also differed among varieties due to other reasons. Grain cracking was correlated positively with grain chalkiness and negatively with grain hardness and husk interlocking score. A maximum brown rice breadth of 2.3 mm, a maximum shellability of 75% and a minimum popping expansion of 12 times could be useful as simple screening indices for acceptable technological quality of rice and for rapid screening of breeding lines.

Keywords : Paddy, Rice, Structure, Physical property, Morphology, Technological quality.

Various physical and structural features of 25 varieties of paddy had been measured, while studying the varietal differences in popping expansion of rice (Murugesan and Bhattacharya 1991). Statistical scrutiny of these data revealed many interesting interrelations. It became apparent that many structural features of paddy were interrelated. These relations are of interest in understanding the morphology of the grain. Apart from this, specific values of some of these features could be considered as bench marks for technological properties of rice. Hence, these values could be used for screening of new lines during breeding programmes. These results are reported in this paper.

Materials and Methods

The samples (25 varieties), the experimental methods and data were the same as reported earlier (Murugesan and Bhattacharya 1991), except for those specified.

Husk interlocking score, determined in transverse cross sections of paddy under a stereomicroscope, expressed the strength of lemma-palea closing, the minimum score being 1 for very loose and maximum 24 for very tight interlocking (Murugesan and Bhattacharya 1991). Husk thickness and husk-to-kernel gap were measured in the same samples with an ocular micrometer. Shellability of paddy (per cent, by number of grains dehusked in a pass under standard conditions) was determined by passing 100 paddy grains one by one with the help of a vibratory feeder through a Satake testing dehusker (type THU), the gap between the two rolls of which was set at five-eighths the mean paddy thickness (Murugesan and Bhattachar;ya 1991). It could also be determined with a hand rice polisher, the two data being correlated ($r = 0.836$, for $n = 25$). White belly (grains with ventral-side chalky area) and chalky grains, in general, (viz., white belly, white core and white back) were counted in 100 brown rice kernels under transmitted light in a microbiological colony counter (Indudhara Swamy and Bhattacharya 1982). Cracked grains were similarly counted in paddy under a paddy crack detector (Srinivas and Desikachar 1973). Brown rice breadth was measured in 20 grains using a vernier scale and thickness with a dial thickness gauge. Hardness, i.e., the breaking strength, of brown rice kernels was determined with a Kiya hardness tester (Webb et al. 1986) after complete drying at 105°C (Wingfield 1985). Paddy (25 g), preadjusted to 14% moisture, was popped with 225°C air and the expansion measured (Murugesan and Bhattacharya 1986).

Correlation coefficients among the different properties were calculated by standard methods once for all the varieties ($n = 25$) and again after excluding the two waxy samples ($n= 23$) which, being entirely opaque, could not be evaluated for grain chalkiness.

Results and Discussion

Data on various structural features of the samples and their popping expansion ratio are summarized in Table 1. The matrix of correlation is presented in Table 2.

The score for husk interlocking could be

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TABLE 1. SOME STRUCTURAL AND MORPHOLOGICAL FEATURES OF PADDY

Property and unit	Range and Mean ± S.D.	
	n = 25	n = 23*
Husk interlocking score	5.6 - 13.3	5.6 - 13.3
	(8.6 ± 2.2)	(8.6 ± 2.2)
Shellability, %	60 - 93	60 - 93
	(80 ± 10)	(79 ± 10)
White belly grains, %	-	0 - 85
	-	(31 ± 31)
Chalky grains, %	-	18 - 87
	-	(47 ± 23)
Cracked grains, %	0 - 75	0 - 75
	(24 ± 19)	(25 ± 19)
Grain hardness, kg/kernel	5.7 - 10.2	5.7 - 10.2
	(7.2 ± 1.2)	(7.3 ± 1.2)
Brown rice breadth, mm	1.8 - 3.1	1.8 - 3.1
	(2.5 ± 0.3)	(2.5 ± 0.3)
Husk content, %	16.7 - 24.8	16.7 - 24.8
	(20.2 ± 2.1)	(20.0 ± 2.0)
Husk thickness, µm	82 - 115	82 - 115
	(96 ± 7)	(96 ± 7)
Husk-to-kernel gap, µm	55 - 174	55 - 123
	(82 ± 26)	(77 ± 17)
Popping expansion ratio	2.0 - 16.2	2.0 - 16.2
	(10.2 ± 4.1)	(10.1 ± 4.1)

* Excluding two waxy varieties which were opaque and hence could not be tested for chalkiness.

White belly was positively related to grain breadth (Table 2). Bhashyam and Srinivas (1981) had noted that varieties having brown rice width of 2.8 mm and above invariably showed white belly in each grain. In contrast, varieties less than 2.0 mm wide never had any white belly, while those of intermediate width had mixed grains. Srinivas et al (1985) later explained that nutrients travelled from the dorsal towards the ventral edge in the developing grain. Consequently, grains that were too wide had some portion left relatively unfilled at the ventral end, i.e., showed white belly. The present results agree well with these observations. It can be seen that the 25 varieties could be divided into two groups, with 2.3-2.4 mm brown-rice breadth as the dividing line (Fig.2). Varieties narrower in width had few white belly grains, but wider ones had many.

Two properties of rice grain mentioned in the literature are of interest in the context of the dividing line as shown in Fig. 2. First, all dimensions of paddy other than breadth (length, thickness,

TABLE 2. CORRELATION COEFFICIENTS (X 1000) AMONG MORPHOLOGICAL FEATURES OF PADDY*

	Shellability	White belly	Chalky grains	Grain breadth	Grain hardness	Husk content	Husk thickness	Husk-to-kernel gap	Cracked grains, %
Husk	-755[a]	-	-	-489[c]	+574[b]	+393	-416[c]	-236	-640[a]
interlocking score	-778[a]	-567[b]	-553[b]	-578[b]	+603[b]	+394	-479[c]	-239	-638[b]
Shellability, %		-	-	+772[a]	-578[b]	-518[c]	+389	+463[c]	+357
		+703[a]	+582[b]	+790[a]	-549[b]	-663[a]	+336	+436[c]	+407
White belly			-	-	-	-	-	-	-
grains, %			+920[a]	+795[a]	-529[b]	-510[c]	+573[b]	+648[a]	+514[c]
Chalky grain, %				-	-	-	-	-	-
				+655[a]	-527[b]	-383	+546[b]	+554[b]	+520[c]
Grain breadth, mm					-484[c]	-465[c]	+571[b]	+558[b]	+299
					-468[c]	-656[a]	+538[b]	+644[a]	+401
Grain hardness,						+118	-263	-317	-446[c]
kg/grain						+221	-195	-205	-516[c]
Husk content, %							+55	+78	-301
							+117	+316	-216
Husk thickness, µm								+586[b]	+412[c]
								+483[c]	+565[b]
Husk-to-kernel gap, µm									-35
									+152

* First value under each property refers to n = 25; second value to n = 23 (i.e., excluding 2 waxy varieties which, being opaque, could not be tested for chalkiness).
[a, b, c] Significant at 0.1, 1.0 and 5.0% level, respectively. The rest are not significant.

reasonably well predicted from the shellability of paddy (Table 2). The scatter in the values (Fig. 1A) was due to the influence of grain breadth on shellability (Fig. 1B). Apparently, the grain was gripped at its two convex faces by the two rubber rolls, and then sheared in the direction of its breadth, the grip and hence, the shelling improved as the grain breadth increased.

weight, length:breadth ratio, length:thickness ratio) were related to the corresponding dimension of milled rice by a single relationship each. In contrast, breadths of paddy and milled rice were related by two relationships. One equation was for slender grains, with milled-rice breadth < 2.3 mm; another equation was for round grains, with milled-rice breadth > 2.3 mm (Bhattacharya et al. 1972).

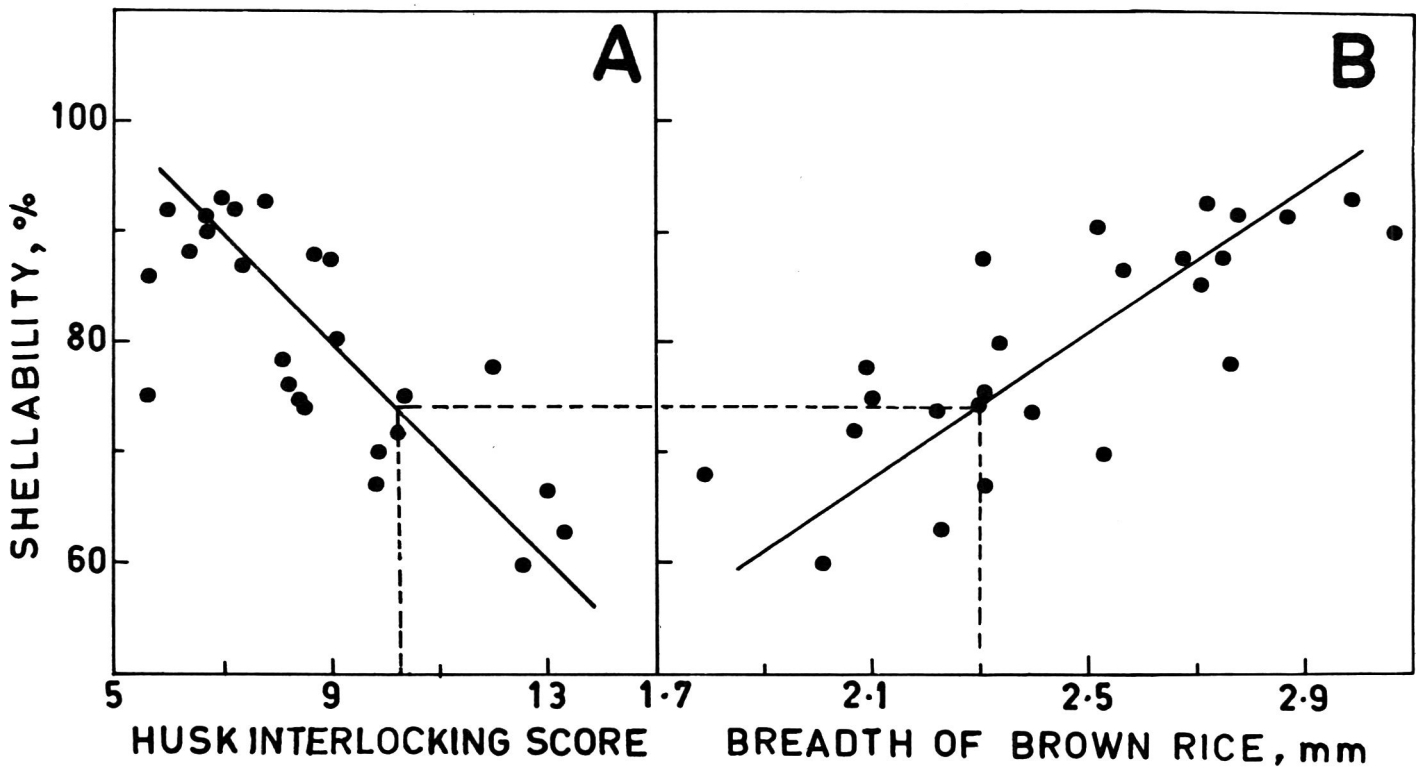

Fig. 1. Influence of (A) husk interlocking score and (B) breadth of brown rice on shellability of paddy

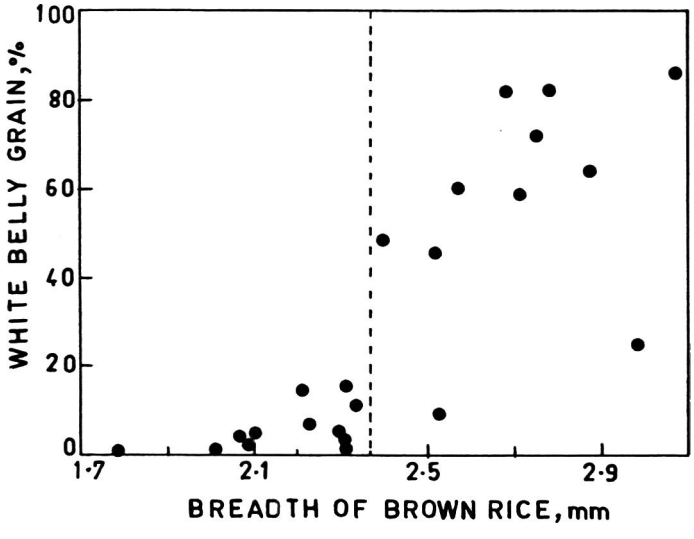

Fig. 2. Dependence of white belly on breadth of brown rice.

Second, Sowbhagya et al (1984) used 2.26 mm milled-rice breadth, based on certain considerations, as a dividing line for classifying rice varieties into 'fine' and 'coarse' classes. As all these three independent studies of three different aspects showed a nearly identical dividing line (2.25-2.3 mm breadth for milled rice should be equivalent to 2.3-2.4 mm for brown rice), a brown-rice breadth of 2.3-2.4 mm could be considered as an intrinsic quality index for rice.

White core (central chalkiness) and white back (dorsal chalkiness) grains were not separately counted in the present study. But, as the coefficient of correlation of grain breadth to white belly was greater than that to all chalky gains together (Table 2), it can be said that the effect of breadth was on white belly and not on white core or white back.

Grain hardness of brown rice showed a negative correlation to white belly, and hence to grain breadth as well. It was also adversely affected by cracked grains for obvious reasons (Table 2). Varietal differences in grain hardness and effect of kernel chalkiness (viz., white belly, white core and white back together) on grain hardness were studied in 10 varieties (Table 3). The data showed that hardness was adversely affected not only by white belly, but also by kernel chalkiness in general. Hardness also decreased as the area of chalkiness increased. In maize (Robutti et al. 1974; Hoseney et al. 1983; Pomeranz et al. 1986) and sorghum (Maxson et al. 1971; Hoseney et al. 1974) too, grains with floury or mealy endosperm have been shown to be softer than those with vitreous endosperm. But, the results also showed that grain hardness independently differed among varieties even in vitreous grains. What factors caused this difference is not known. Chemical factors tested in 10 varieties (total and hot-water-insoluble amylose, protein, non-starchy polysaccharides, pentosans, uronic acids, gelatinization temperature) had no influence on grain hardness (hence data not reported).

TABLE 3. EFFECT OF CHALKINESS ON THE HARDNESS OF BROWN RICE*

Variety	Hardness of grain having chalky area											
	Aggregate			0%			< 20%			> 20%		
	A	B	C	A	B	C	A	B	C	A	B	C
'GEB24'	7.3	3.6	4.4	8.5	4.2	5.2	6.7	3.3	4.1	5.0	2.5	3.0
SD	0.8	0.4	0.5	1.3	0.6	0.8	0.8	0.4	0.5	0.7	0.3	0.4
'Bengwan'	7.8	3.3	4.3	8.8	3.8	4.8	5.5	2.4	3.0	3.9	1.7	2.1
SD	1.2	0.5	0.6	1.3	0.6	0.7	0.8	0.3	0.4	0.6	0.2	0.3
'Blue bonnet'	8.1	3.2	4.2	8.4	3.3	4.3	6.6	2.6	3.4	5.4	2.1	2.8
SD	1.6	0.6	0.8	1.3	0.5	0.6	0.8	0.3	0.4	0.7	0.3	0.4
'Syntha'	8.9	3.9	4.9	9.6	4.2	5.2	7.1	3.1	3.9	4.8	2.1	2.6
SD	1.8	0.8	1.0	1.9	0.8	1.0	1.0	0.4	0.6	0.7	0.3	0.4
'Madhu'	6.0	2.6	3.7	7.7	3.4	4.8	5.4	2.4	3.3	4.4	1.9	2.7
SD	1.0	0.4	0.6	1.1	0.5	0.7	0.7	0.3	0.4	0.6	0.3	0.4
'T141'	7.0	3.0	4.1	8.1	3.5	4.7	5.8	2.5	2.4	4.6	2.0	2.7
SD	1.4	0.6	0.8	1.2	0.5	0.7	0.9	0.4	0.5	0.8	0.4	0.5
'Intan'	8.9	4.3	5.2	9.0	4.3	5.3	6.8	3.2	4.0	4.5	2.2	2.6
SD	2.1	1.0	1.2	1.9	0.9	1.1	1.0	0.5	0.6	0.8	0.4	0.5
'Jaya'	6.1	2.3	3.4	7.1	2.6	3.9	5.8	2.2	3.2	4.9	1.8	2.7
SD	1.6	0.6	0.9	1.4	0.5	0.8	0.8	0.3	0.4	0.7	0.3	0.4
'SR26B'	6.9	2.8	3.8	8.5	3.4	4.7	6.2	2.5	3.4	5.5	2.2	3.0
SD	1.6	0.6	0.9	1.8	0.7	1.0	1.3	0.5	0.7	1.0	0.4	0.6
'Br 9'	5.7	2.7	3.7	6.6	3.1	4.3	4.5	2.1	2.9	3.2	1.5	2.1
SD	1.2	0.6	0.8	1.3	0.6	0.8	0.6	0.3	0.4	0.4	0.2	0.3

*Hardness values are expressed as A , kg/kernel; B, Kg/mm grain breadth; C, kg/mm grain thickness.

The husk-to-kernel gap seemed to be related to the grain breadth (Table 2). The wider the brown rice, the more was the gap due to reasons not clear. It was earlier reported (Bhattacharya et al. 1972) that although milled rice had a constant density of 1.45 g/ml among varieties, the density of paddy showed two mean values: 1.22 g/ml for slender varieties and 1.18 g/ml for round varieties. In as much as round grains were also broad (Bhattacharya et al. 1972; Sowbhagya et al. 1984), it would, therefore, imply that broad grains generally had a high air space, i.e., a high husk-to-kernel gap. Thus, these two independent studies with different objectives gave identical results.

Cracked grains are undesirable in rice (Bhattacharya 1980), and hence its possible correlation with other features was examined. The percentage of cracked grains was found to be influenced positively by white belly and grain chalkiness (Table 2). This is in agreement with earlier work (Indudhara Swamy and Bhattacharya 1982; Bhashyam et al. 1985). Again, husk interlocking score was negatively correlated with cracked grains. Thus, a well closed husk probably protects the grain from rapid exchange of moisture with the external surroundings, for instance during drying and grain maturation. It is well known that cracks in rice arise from rapid absorption or desorption of moisture (Bhattacharya 1980). Further, cracked grains negatively correlated with grain hardness, and positively with husk thickness. There was no other significant relationship. Multiple correlation analysis revealed that the husk score, white belly (or general chalkiness), kernel hardness and husk thickness together accounted for about 55% of the variation in cracked grains among different samples ($R = 0.734$, $R^2 = 0.539$). What other factors influenced grain cracking is not known. Chemical factors (amylose, protein, gelatinization temperature) studied in 10 varieties seemed to have no influence on grain cracking (hence data not reported).

Husk thickness was negatively related to husk interlocking score (Table 2), possibly indicating a lower flexibility of thick husk to bend and hook around the edge. Broad grains too had loose husk locking, an undesirable feature, possibly because broad grains had thick husk, and *vice versa*. Thick husk also seemed to favour a larger husk-to-kernel gap, again probably due to the same reason of a thick husk being not flexible enough to bend tightly around the edge. Thick husk is, thus, by and large an undesirable characteristic. Unfortunately, it could not be predicted from the husk content, the two being unrelated.

Practical significance : Four rice grain characters, viz., broad grains, soft grains, white belly and loose husk can be considered as technologically undesirable for the following reasons. Broad grains promote white belly, which, in turn, lowers grain hardness and promotes grain cracking. Consequently, it indirectly affects milling quality of

rice adversely. Broad grains also affect cooking rate of rice, for they have less surface area per unit weight and hence need more time and energy to cook (Bhattacharya and Sowbhagya 1971). Loose closure of husk appears to be correlated with easier grain cracking, which promotes milling breakage. It may also facilitate entry of insects and pathogens into the grain and adversely affect the storage quality (Bechtel and Pomeranz 1980). Soft rice grains may be susceptible to insect attack (Pomeranz and Webb 1985). Indices of rice quality can be derived from these characteristics from the relations derived above.

Grains with more than 2.3 mm brown rice width are likely to have poor milling, storage and cooking qualities and should be rejected during screening of lines in varietal development. Alternatively, all lines with grain chalkiness, especially white belly, should be rejected. Shellability could be another useful test. High shellability would indicate a loose husk closure or too broad kernel, both of which are technologically undesirable. A simple test with either a laboratory rubber-roll sheller or even a hand sheller could be standardized for use with just 100 paddy grains as a screening test in rice breeding. Lines having a shellability of over 75%, being associated with > 2.3 mm grain breadth and poor husk closure (Fig. 1), should be rejected during breeding.

Since all the four undesirable characteristics mentioned above are also detrimental to popping of rice (Murugesan and Bhattacharya 1991), popping quality may be yet another excellent test for the technological qualities of rice. A small commercial home popper could be used with just 5-10 g paddy for screening during breeding. It can be seen (Fig. 3) that a shellability of 75%, grain breadth of 2.3 mm and husk interlocking score of 10-11 (which corresponds to shellability of 75% : Fig. 1A) correspond to a popping expansion ratio of 12-14. An expansion ratio of 12 can, thus, be considered a minimum quality marker of paddy.

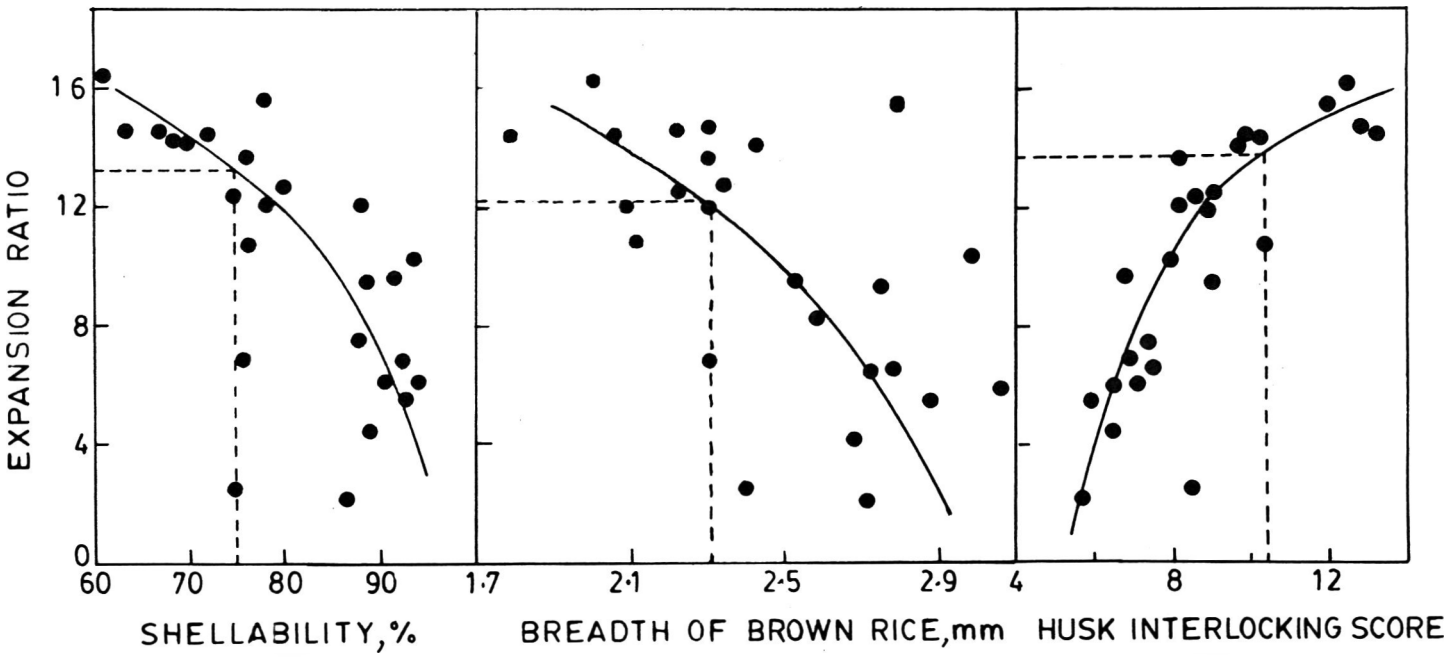

Fig. 3. Relationship of popping expansion of rice to various grain characteristics of paddy.

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Microscopic Structure and Carbohydrate Digestibility of Ready-to-eat Puffed Rice Products

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In vitro carbohydrate digestibility of ready-to-eat puffed products from rice, such as *lahi* (puffed paddy) , *Poha* (beaten rice) , *murmura* (puffed rice), and *chiwdi* (puffed *poha*), was determined using pancreatic and salivary amylases, separately. It was found that puffing enhanced the digestibility, as against the decrease in case of flaking. The data on the extent of starch gelatinization in these products indicated that gelatinization was a pre-requisite for puffing and also for increase in digestibility. However, gelatinization was not the only factor in enhancing digestibility of puffed products. Microscopic studies on the structure of these products gave interesting trends.

Keywords : Starch, Rice products, Carbohydrate digestibility, Microscopic structure, *Lahi, Poha, Murmura, Chiwdi*

All over the world, several ready-to-eat cereal products are prepared conventionally by flaking or puffing. Being crisp and friable in texture, as well as readily consumable, such products enjoy wide popularity among consumers of all ages. The puffed cereals can also be incorporated in several snacks and confectionery products. In addition to the organoleptic advantage, such products are believed to be easily digested, and utilized more efficiently, as indicated by several *in vitro* and *in vivo* studies. (McNeil et al. 1971; Modi and Kulkarni 1976; Walker et al. 1970; Borgida 1976; Galyean et al. 1976; Calet 1975; Prajwala Mouliswar et al. 1993; Ngo Som et al. 1992). During processing, several physico-chemical and structural changes take place in the kernel. Endosperm, a compact reservoir of food for developing embryo, is converted into an expanded, porous, friable mass, and the cell integrity is almost lost. Mechanism of expansion of grain during puffing is explained as rapid volatilization of super-heated moisture from within the wet, hot and plastic mass, thereby leading to enormous increase in volume. Puffing as well as flaking is reported to bring about 30-50% gelatinization of starch, thus indirectly effecting the digestibility and feed efficiency (Antonio and Julliano 1973).

In India, ready-to-eat puffed products are commonly prepared from rice and millets. Each product has distinct appearance, texture and flavour of its own. The information available on such products, however, is scarce. The present paper reports the changes in structure and *in vitro* starch digestibility of these products, as compared with those of rice starch.

Materials and Methods

The study was confined to 'Luchai' variety of rice *(Oryza sativa)* from which four of the puffed products i.e., *Lahi* (puffed paddy) , *Murmura* or *Mudi* (puffed rice), *Poha* (beaten rice) and *Chiwdi* (puffed beaten rice) were prepared. The samples were purchased from the local market, and stored in air-tight containers. Total starch content was estimated by anthrone method (Plummer 1972). Extent of gelatinization was calculated by the method of Birch et al (1970), by comparing the blue value of the samples with a set of blue values for standard mixtures of raw and gelatinized rice starch. *In vitro* digestibility with salivary and pancreatic amylase was determined individually, since salivary amylase is reported to act on gelatinized starch, in contrast to pancreatic amylase, which acts on both gelatinized and native starches. Suitably diluted human saliva, standardized against gelatinized corn starch, constituted the source of salivary amylase. Pancreatin (from porcine pancreas, grade II, Sigma Chemical Co. USA) served as the source of pancreatic amylase. Finely ground sample to contain 0.05 g starch was suspended in 5 ml of phosphate buffer (pH 6.8 for salivary amylase and 7.2 for pancreatic amylase) and incubated with suitably diluted amylase preparation. Reducing sugar, liberated at appropriate time interval, was estimated by the method of Nelson-Somogyi (Plummer 1971). *In vitro* digestibility was expressed as µg reducing sugar liberated/min/g sample. Results were compared with corresponding gelatinized starch control.

Microscopic studies were performed on free hand sections of the products. *Poha* was rubbed on a rough surface till paper thin. These sections were treated with 70% ethanol (15 min), followed by dehydration with 90% ethanol (15 min). The sections were stained to localize native starch,

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gelatinized starch, and celluloses by standard stains (MacMaster 1964) prepared in alcoholic solvent.

Results and Discussion

The 'Luchai'variety of rice is used commonly for commercial production of all the four puffed products selected in the present studies. It is worth mentioning that traditional processing steps were employed commercially. For the processing of rice to *lahi*, the paddy is soaked in water to about 30% moisture. Excess water is drained, equilibrated for 2-3 h, and puffed in sand bath at 220°-250°C. The parboiled rice is processed as above to make *murmura*. In case of *poha* manufacture, the parboiled paddy is pressed in between the rollers, dried and then slightly roasted. The parboiled paddy is pressed and puffed in sand bath to obtain *chiwdi*.

Starch contents in the samples ranged between 64 and 69%. All puffed products showed enhanced *in vitro* starch digestibility (Table 1), with salivary as well as pancreatic amylase, *lahi* showing maximum increase (38%), while *poha* showed 10% decrease in digestibility. No significant difference was noticed in the action of salivary and pancreatic amylases, in spite of the differences in the extent of gelatinization (Table 1), which exceeded 60% in all puffed rice products. Extent of gelatinization is considered as one of the factors which determines the digestibility of puffed or flaked products. Amongst the processed rice products, minimum

TABLE 1. EFFECT OF PUFFING ON *IN VITRO* DIGESTIBILITY OF CEREALS.

Products	Starch %	% digestibility with pancreatic amylase, relative to original grain	% digestibility with salivary amylase, relative to original grain	Extent of gelatinization, %
Rice	68.8	100.0	100.0	-
Lahi	65.9	138.8	135.0	60
Murmura	67.5	105.0	103.0	100
Chiwdi	64.3	121.3	122.2	100
Poha	65.1	90.0	79.6	70

gelatinization occurred in *lahi* (60%), followed by *poha* (70%), while the gelatinization in *murmura* and *chiwdi* was almost complete. Borgida et al (1975) have reported that the expansion or puffing of corn improved both *in vitro* and *in vivo* digestibilities, compared to the flaking of the corn. Depressed feed efficiency on flaking of corn, as compared to control or popped sample, has also been reported by these workers. Improved growth rates were also observed on expansion and extrusion of corn. Starch granules in the endosperm of the rice grains are embedded in a matrix of protein and cellulose, and are not easily accessible to the action of amylolytic enzymes, as against the isolated starch. During the puffing process, starch absorbs large portions of available moisture, becomes thermoplastic and develops a distinct structure of its own (Mercier and Fillet 1975). It, thus, explains the improved digestibility of the puffed rice samples.

McNeill et al (1975) conducted experiments to ascertain physical properties of carbohydrates from processed sorghum grain. They reported that gelatinization was not the only factor involved in increasing the efficiency of *in vitro* carbohydrate utilization and suggested that it might not be necessary to destroy starch granule integrity completely, as it occurred in the process of starch gelatinization. Thus, any processing method, which alters kernel structure to enhance the release of individual starch granules from protein matrix and also increases their accessibility or susceptibility to enzyme activity, should increase carbohydrate digestibility. It is, therefore, possible that other temperature-induced changes in protein matrix and pyrolysis of starch to form dextrins might have caused lower *in vitro* digestibility of starch in *chiwdi* and *murmura*.

The studies on the microscopic structure and localization of starch in puffed and flaked products gave interesting results. For example, a transverse section of parboiled rice indicated that the starch granules were partially gelatinized and migrated towards the periphery during the process of parboiling (Fig. 1A). The transverse section of raw rice (Fig. 1B) showed a regular smooth outline, a compact structure of endosperm cells and raw starch particles, which were distributed uniformly throughout the kernel. These were not easily stained. Aleurone layer was intact and it consisted of rectangular flattened cells devoid of starch, but rich in proteins. The longitudinal section of *poha* (Fig. 1C) showed that the outline and the internal cell structure were disrupted and irregular. Moreover, partially gelatinized starch granules were displaced towards the periphery. Very few vacuoles were seen in the structure and deep irregular furrows extended from the periphery to the centre of the kernel. Most of the cells were ruptured, starch was displaced towards periphery and large air cells were randomly distributed in *murmura* (Fig. 1D). The outer surface of *murmura* was smooth and showed fragments of aleurone layer at places.

In *chiwdi*, the coagulated starch granules were

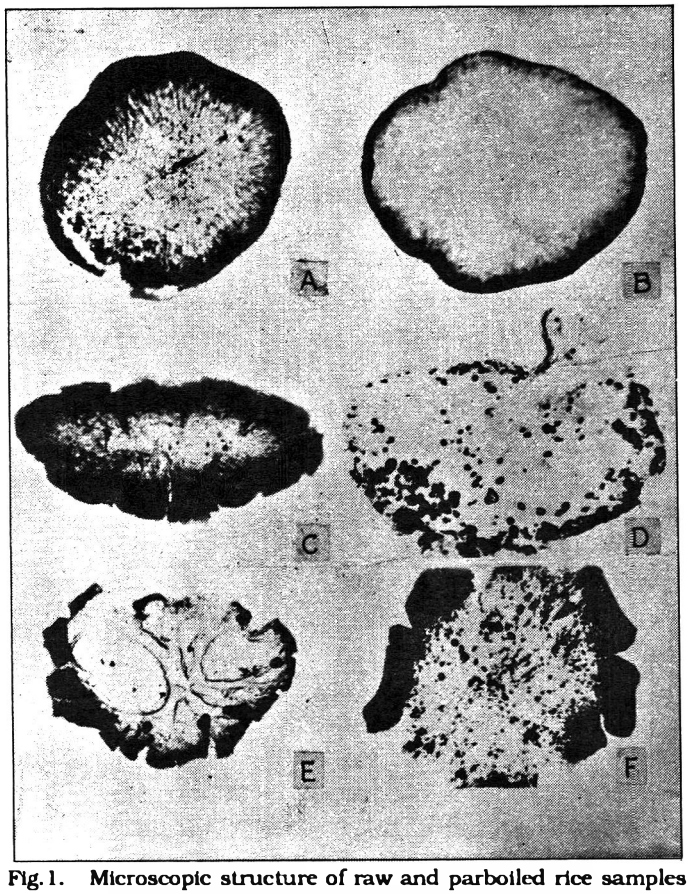

Fig.1. Microscopic structure of raw and parboiled rice samples and the puffed products. (Magnification : 100 x) stained with iodine. A: Transverse section of parboiled rice, B: Transverse section of raw rice, C: Longitudinal section of *poha*, D: Longitudinal section of *murmura*, E: Transverse section of *chiwdi*, F: Transverse section of *lahi*.

displaced towards the periphery (Fig. 1E). The compact structure found in *poha* (Fig. 1C) appeared expanded extensively in *chiwdi* (Fig. 1E) and air gaps of various sizes were found. Starch gave brown stain due to dextrinization. Furrows present in *poha* structure were further expanded and the whole structure had a very delicate fragile appearance in *chiwdi* (Fig. 1E). The outline of *lahi* showed bilateral symmetry, to some extent, with a central air cell and porous mass (Fig. 1F). Most of the gelatinized starch granules were displaced towards the periphery, while some granules gathered near the central air cell and a few were distributed in the complete cross section. The cellular structure of *lahi* (Fig. 1F) appeared much more compact than *murmura* (Fig. 1D) and *chiwdi* (Fig. 1E). The breakdown of cell structure was also relatively less in *lahi*. During soaking, a moisture gradient, from periphery to centre of the kernel, was set up, which might be the reason for the relatively less destruction of the compact kernel structure during puffing. Pressure of husk at the time of puffing caused an uneven expansion, and formation of ridges and furrows on the surface of *lahi*. Protein-rich aleurone layer was identified on the ridges. This fact might be related to the increased accessibility of starch to amylase activity, thereby increasing *in vitro* digestibility.

From the above observations, it is evident that the puffing causes an expansion in volume due to

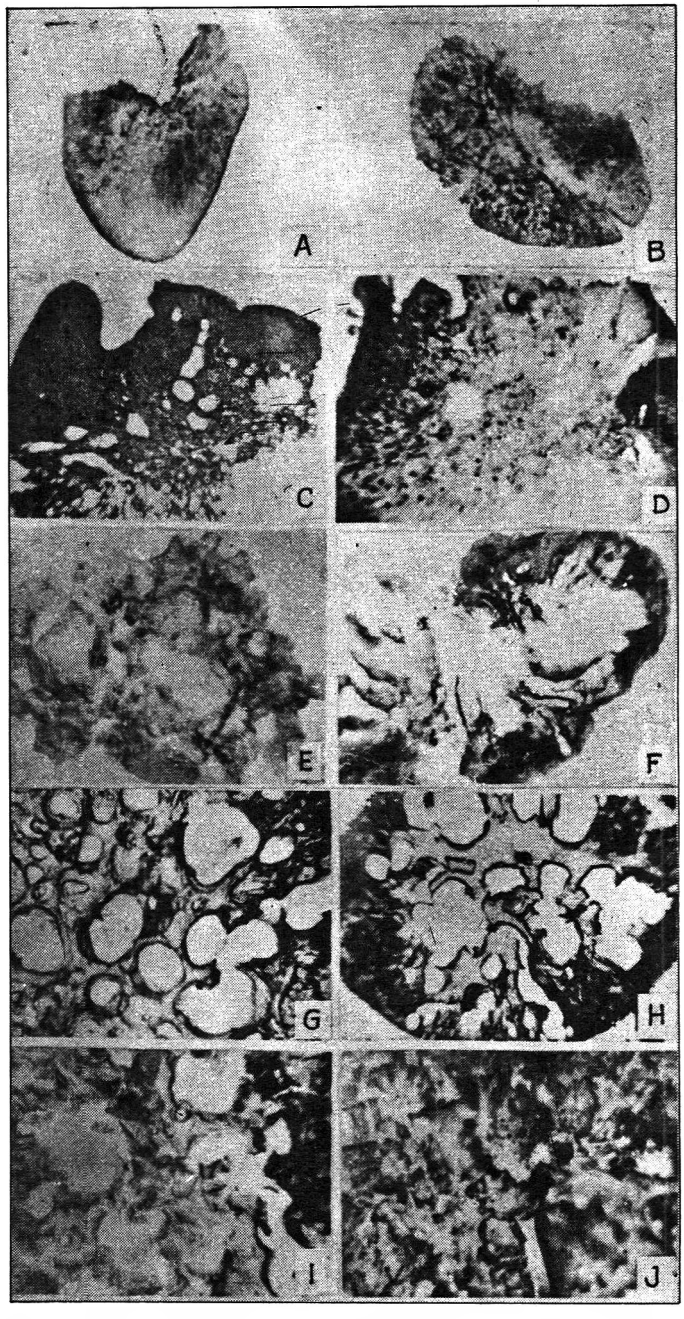

Fig.2. Effect of addition of a drop of pancreatin on the section of the rice and its puffed products stained with iodine. (Magnification : 240 X). A: Transverse section of parboiled rice (colour-brown), B: As A, after action of pancreatin (colour-blue), C: Transverse section of *lahi* (colour-brown), D: As C, after action of pancreatin (colour-white), E: Longitudinal section of *poha* (colour-reddish brown), F: As E, after action of pancreatin (colour-dark brown with blue spots), G: Longitudinal section of *chiwdi* (colour-brown), H: As G, after action of pancreatin (colour-violet blue), I: Transverse section of *murmura* (colour-brown), J: As I, after action of pancreatin (colour-violet blue).

instantaneous expansion of moisture. It brings about starch galatinization and improves the starch digestibility. However, treatments prior to puffing influence the final structure, and *in vitro* digestibility to some extent. Thus, various puffed products, prepared from rice appear to possess specific structural features. Marginal differences with salivary and pancreatic amylases indicate that digestibility is not directly correlated to the extent of gelatinization. There is a possibility that the pretreatments influence the changes in starch and proteins of the kernel in different ways, thereby leading to altered accessibility of starch to enzymatic reaction. Babichenko and Sorochinskeya (1972) tried to explain the changes in the microstructure of starch in popcorn. They observed that the protein network was somewhat disturbed and size of starch grains increased greatly, thereby forming a netlike structure in popped popcorn kernel. Formation of porous structures in burst kernels was best at 235°C. In waxy corn,in which amylose was almost fully absent, none of the kernels opened. The observations on the processed rice products are in agreement with these results.

The action of pancreatic enzymes on the sections of these puffed products was also studied under the microscope (Fig. 2 A-J). When the sections were stained with iodine solution, these gave reddish brown to violet colouration due to the presence of gelatinized starch and dextrin.

However, soon after a drop of pancreatin solution was added, the structure of the section was disrupted and the colour also changed from brown to blue violet. This might be due to degradation of dextrins into lower molecular weight products. In addition, the protein matrix may also get degraded, thereby exposing the undegraded starch to the action of iodine.

Above observations indicate that the treatments prior to puffing of rice affect the *in vitro* carbohydrate digestibility and kernel structure in a characteristic manner.

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Effect of Soy-fortification on Quality Characteristics of *Chapatis*

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Chapatis (unleavened flat bread), prepared from whole wheat flour and wheat flour-defatted soy flour (90:10) blend were evaluated for their quality characteristics. Soy-fortified *chapatis* contained 28.8 and 19.0% higher protein and available lysine, than the whole wheat *chapatis*. The former also contained higher amounts of calcium, phosphorus and iron, than the latter. Soy-fortification increased protein efficiency ratio of *chapatis* from 1.3 to 1.7 and *in vitro* protein digestibility from 71.3 to 73.1%. Weights of liver and heart of the rats, fed on wheat *chapatis* diet, were significantly lower, than those observed in case of casein and soy-fortified *chapati* diets.However, the weights of testis of rats fed on soy-fortified *chapatis* were significantly higher than those with other diets. Soy-fortified *chapatis* were softer than whole wheat *chapatis*, but retained 13% of the trypsin inhibitor activity, originally present in defatted soy flour.

Keywords : *Chapatis*, Soy-fortification, Chemical composition, *In vitro* digestibility, Nutritional quality, Textural characteristics.

Chapati (unleavened flat bread) is a common item in the diets of people of Indian sub-continent and parts of Africa, Mongolia and China. In India, about 85-90% of wheat is used in the form of *chapati* and its culinary variations (Austin and Ram 1971). Traditionally, *chapati* is prepared from whole wheat flour., Wheat contains about 8-12% proteins and its limiting amino acid is lysine (Kesarda et al. 1976). In contrast, soybean and its protein products contain higher amounts of quality proteins and are abundantly rich in lysine (Snyder and Kwon 1987). Wheat-soya blends are also used for breadmaking and their rheological and baking qualities have been studied (Pratima Mishra et al. 1991). Therefore, fortification of wheat flour with soybean products, such as defatted soy flour (50-50% protein) for *chapati* preparation will not only enhance the protein content of the diet but also raise its nutritional value, thereby helping in combating malnutrition. Defatted soy flour is widely used for various extruded rice products (Chauhan and Bains 1985); bread (Sahni et al. 1976).

Incorporation of defatted soy flour in wheat flour has been found to balance the amino acid pattern of *chapati*, without affecting its acceptability characteristics (Ebler and Walker 1983; Lindell and Walker 1984). Although optimum levels of defatted soy flour incorporation for producing an acceptable *chapati* have been worked out (Rathod and Williams 1973; Bhat and Vivian 1980), the information on quality characteristics of *chapati* made from wheat-soy blend is limited. The objectives of this study were to determine the effects of fortifying wheat flour with defatted soy flour on chemical, nutritional and textural characteristics of *chapati*.

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Materials and Methods

Whole wheat flour ('Hari Bhog') was from local market, while defatted soy flour ('Vital') was procured from Britannia Industries Ltd. *Chapatis* were prepared from whole wheat flour and wheat flour-defatted soy flour (90:10) blend, using conventional method (Rawat 1990).

Nutritional evaluation : Protein quality of *chapati* was evaluated by means of protein efficiency ratio (PER) according to AOAC (1984) procedure. Twenty four male albino rats ('Wistar' strain, 21 ± 1 day old), obtained from Indian Veterinary Research Institute, Izatnagar, India, were divided into 3 groups of 8 animals each, in such a way that the average weight of all the groups was more or less same. The animals were housed in wire screen cages individually and held at 26.6 ± 2.2°C (46.6 ± 7.9% relative humidity) under a 12 h light dark cycle. One group was given a diet of basal ration consisting of (g/100 g) groundnut oil 8, vitaminized oil 1, mineral premix 4, vitamin premix 1, casein 12.3, and starch to make up to 100 g. Second and third groups were fed on *chapatis* made from whole wheat flour and wheat flour-soy flour blend, respectively. Water and food were provided *ad libitum*. At the end of 4 weeks, 5 rats from each group were anaesthetized for collecting heart, liver, testis and kidneys, after recording their fresh weights. Data of animal experiments were statistically analyzed by the method of Raghuramulu et al (1983).

Analytical methods : Protein contents of flours and *chapatis* were determined by Kjeldahl method

(AOAC 1984), using a nitrogen to protein conversion factor of 5.71 for wheat and 6.25 for soybeans. Moisture, fat, ash, crude fibre, reducing and non-reducing sugars in the samples were estimated according to AACC (1976) procedures. Phosphorus and iron were determined by colorimetric methods as described by Ranganna (1986), while calcium was determined by AACC (1976) method, after precipitating it as calcium oxalate. The available lysine contents in flours and *chapatis* were determined by Carpenter's method as modified by Booth (1971). The trypsin inhibitor activities of soy flour and soy fortified *chapatis* were measured by the method of Kakade et al (1974). *In vitro* protein digestibilities of *chapatis* were determined using pepsin and pancreatin enzymes according to the procedure of Akeson and Stahman (1964).

Texture analysis : The texture of *chapatis* was determined using an Instron Universal Testing Machine (Model 1111, Instron Ltd., UK). The tensile strength was measured as the force required to push a flat cylindrical punch of 3 mm dia through the *chapati* and expressed as Newtons. It was calculated by measuring the height of peak of compression curve recorded under following conditions : load range 200 N, cross head speed 20 mm per min, cycles 2 and range 0-2. The tensile strength of fresh *chapatis* was measured within 30 min after puffing. For storage studies, the *chapatis* were wrapped in polyethylene bags to prevent drying and stored at 21±1°C.

Results and Discussion

Whole wheat flour used in the investigation contained 12.4% proteins, whereas the protein content in defatted soy flour was 57.1% (Table 1). Defatted soy flour also contained higher amounts of ash, crude fibre, calcium, phosphorus, iron, sugars and available lysine than wheat flour. These observations are in agreement with earlier reports (Kellor 1974; Kaur and Hira 1988). Soy flour exhibited trypsin inhibitor activity, indicating that it was mildly toasted following solvent extraction.

Chemical composition of *chapatis* indicated that fortification of wheat flour with defatted soy flour at 10% level enhanced their protein contents by 28.8% and available lysine contents by 19%. Such fortification also resulted in increasing the calcium, phosphorus and iron contents of *chapatis* by 25.4, 45.7 and 38.2%, respectively. Increases in contents of these nutrients are attributed to their higher contents in defatted soy flour. Bhat and Vivian (1980) have reported an increase of 17% in protein content of *chapatis* as compared to 28.8% increase observed in this investigation for similar level (10%) of soy fortification in wheat flour. It may be attributed to lower protein content (47.9%) of soy flour used by them.

Baking resulted in reduction in available lysine in whole and soy fortified *chapatis* by 4.5 and 3.8%, respectively (Table 1). Bhat and Vivian (1980) also observed losses of 4.7 and 5.7% in available lysine in whole wheat and soy-fortified *chapatis* upon baking, respectively. Results of this investigation also showed that baking of *chapatis* reduced trypsin inhibitor activity by 87%. Kaur and Hira (1988) have also observed trypsin inhibitor activity in soy-fortified *chapatis*, thereby suggesting that trypsin inhibitor was not completely inactivated during *chapati* making process.

TABLE 1. COMPOSITION OF WHOLE WHEAT FLOUR, DEFATTED SOY FLOUR AND *CHAPATIS*

Constituents	Whole wheat flour	*Chapatis*	Defatted Soy flour	fortified *chapatis*
Protein %	12.4	11.8	57.1	15.2
Fat, %	1.5	1.8	0.7	1.4
Ash, %	1.0	1.7	7.4	2.5
Crude fibre, %	0.7	1.5	3.4	2.4
Carbohydrates (by difference), %	84.4	83.2	31.4	78.5
Calcium, mg/100g	120.0	166.7	311.4	209.1
Phosphorus, mg/100g	150.0	194.2	698.6	283.1
Iron, mg/100g	2.6	3.4	16.0	4.7
Reducing sugars, %	0.6	0.5	1.2	0.5
Non-reducing sugars,%	1.7	1.7	3.3	1.9
Available lysine, g/16g N	2.2	2.1	6.0	2.5
Trypsin inhibitor activity, TUI/mg	-	-	0.3	0.04

Whole wheat and soy-fortified *chapatis* contained 31.5 and 33.5% moisture, respectively.

Results of animal experiments (Table 2) showed that various diets differed significantly ($P<0.05$) in their PER values. The corrected PER values of whole wheat *chapatis* (1.3) and soy-fortified *chapatis* (1.7) were significantly different. The higher PER of soy-fortified *chapatis* may be attributed to their higher protein and available lysine contents.

No significant differences in the weights of liver, kidney and heart of rats fed on casein and soy-fortified *chapati* diet were observed. In contrast, the weights of heart and liver of rats fed on whole wheat *chapati* diet were significantly lower ($P < 0.05$). However, the weights of kidneys of rats on all the three diets were more or less same, whereas the weights of testis of rats fed on soy-fortified *chapati* diet were significantly higher ($P < 0.05$) than the weights of testis of rats fed on casein or whole wheat *chapati* diets.

The *in vitro* digestibility of soy-fortified *chapatis* (73.1%) was slightly greater than that observed for whole wheat *chapatis* (71.3%). However, *in vitro*

TABLE 2. PROTEIN EFFICIENCY RATIO, INTERNAL BODY ORGAN WEIGHTS AND TENSILE STRENGTH OF WHOLE WHEAT AND SOY-FORTIFIED *CHAPATIS*

Attributes	Diets		
	Casein	Whole wheat flour *chapatis*	Soy-fortified *chapatis*
Total gain in body weight after 4 weeks, g	46.9	23.0	43.9
Total protein intake, g	19.8	18.8	27.4
Observed PER	2.4	1.2	1.6
Corrected PER	2.5[c]	1.3[a]	1.7[b]
Internal body organ weights			
Liver weight, g	4.3[b]	3.1[a]	4.7[a]
Kidney weight, g	1.0	0.8	1.1
Heart weight, g	0.5[b]	0.3[a]	0.5[b]
Testis weight, g	0.5[a]	0.4[a]	0.7[b]
Tensile strength, Newtons			
Storage period, hr.			
0	-	52.9	45.0
2	-	86.3	65.1
4	-	111.3	90.3
6	-	190.9	158.1

Means followed by different superscripts in a row differ significantly at 5% level.

digestibility of both was much less than casein diet (86.5%). Lindell and Walker (1984) have reported somewhat higher values for *in vitro* digestibility of whole wheat flour (77.2%) and wheat-soy blend (80.0%).

Fortification of wheat flour with defatted soy flour yielded *chapatis* with softer texture (Table 2). This may be ascribed to greater moisture content (33.5%) of soy-fortified *chapatis* than whole wheat *chapatis* (31.5%). Bhat and Vivian (1980) have also made similar observations. The tensile strength of *chapatis* increased with storage time. However, at any time of storage, soy-fortified *chapatis* exhibited lesser tensile strength than whole wheat *chapatis*, which is attributed to greater retention of moisture by defatted soy flour, than that by the whole wheat flour (Kinsella, 1979). Gandhi and Bourne (1988) also noted that soy-fortified *chapatis* were softer than whole wheat *chapatis*.

It may be concluded from the results of the present investigation that fortification of wheat flour with defatted soy flour would enhance nutrient contents of *chapatis*. Further, it made the *chapatis* softer and enhanced their PER, but only marginally improved their digestibility. However, soy-fortified *chapatis* retained 13% of the trypsin inhibitor activity, originally present in defatted soy flour.

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Effects of Defatting, Coagulants and Coagulation pHs on Soy Protein Isolates

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Defatted soy flours obtained from 4 different sources were analysed for various chemical characteristics and used to prepare soy protein isolates by using different coagulants and at coagulation pHs. Defatted soy flour from source B showed the highest protein and ash contents. Comparatively lower values of soluble nitrogen, urease activity and trypsin inhibitor activity, in contrast to higher value of non-enzymatic browning, indicated that the defatted soy flour obtained from source C received more heat treatment than the others. The isolates made at lower pHs were whitish in colour, but softer in consistency, as compared to those made at higher pHs. Defatted soy flour from different manufacturers exhibited wide variations in physico-chemical properties of protein isolates. Amongst coagulants and coagulation pHs, HCl and pH of 4.5 produced better quality protein isolates.

Keywords : Defatted soy flour, Protein isolates, Nitrogen solubility index, Urease activity, Trypsin inhibitors.

Among oilseeds, soybean is considered as a major source of edible oil (Smith and Circle 1978). In India, the number of soybean oil extraction industries have increased rapidly (Singh 1989). Consequently, the production of defatted meal, a protein rich by-product, has increased in recent years. Of the total meal produced, only about 3% is being directly used as human food (Singh 1989). Soy flour is widely used for making soy *idli* (Aholkar and Parekh 1983), *chapati* (Bhat and Vivian 1980) and bread (Sahni and Krishnamurthy 1975), and also for extruded soy rice products (Chauhan and Bains, 1985). Soy protein isolates, being rich source of lysine (Kolb 1974), are used for developing products, such as high protein beverage baby food formulations and breakfast foods (Singh and Chauhan 1989). To alleviate protein calorie malnutrition, the development of such products is useful, particularly in developing countries, where cereal-based diets are consumed (Smith and Circle 1978).

The isolate of good quality soy protein depends on several factors, such as raw material, pH of coagulation and type of coagulant used (Van Megan 1974; Hutton and Campbell 1977; Miller 1981). The information available on the effects of type of defatted soybean, coagulants and pH of coagulation on the quality of protein isolate is scanty, in India. Therefore, the objective of this study was to evaluate commercial defatted soy flours from different sources for making protein isolates and to investigate the effects of coagulants as well as of coagulation pH on the quality of isolates.

Materials and Methods

Samples of defatted soy flour were obtained from four different manufacturers, i.e. A, B, C and D. The manufacturers have not disclosed the methodology of defatting for the sake of maintaining commercial secrecy.

Analysis of defatted soy flour : Moisture, total ash, total fat and crude protein contents were determined by using standard AOAC (1984) methods. Nitrogen solubility was estimated by AOCS (1973) method. Urease activity was determined by using the modified method of Caskey and Knapp (1944). Non-enzymatic browning was determined by the method as described by Ranganna (1986). For trypsin inhibitor activity, modified method of Kakade et al (1974) was used.

Preparation of soy protein isolate : Each of the defatted soy flour was dispersed in water (1:12) and mixed in a mechanical stirrer (Gansons India Ltd.). The pH of the dispersion was adjusted to 9.5 with sodium hydroxide (20%) and stirred for 30 min to solubilize proteins. The dispersion was, then centrifuged (MG Research centrifuge) at 6000 rpm for 30 min. Proteins in the supernatant were precipitated by using 20% of each coagulant (HCl, H_2SO_4, acetic acid, lactic acid, citric acid, $CaCl_2$ and $CaSO_4$ + $MgSO_4$) at 3.5, 4.0 and 4.5 pHs. The precipitated proteins were centrifuged (6000 rpm for 15 min), washed with water and neutralized with 20% sodium hydroxide.

Different isolates were compared visually for

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colour and consistency. Nitrogen solubility and protein contents of isolates were estimated by the procedures described for defatted soy flour. All analyses were performed in duplicates and the average values are reported.

Results and Discussion

The proximate composition of defatted soy flour procured from different sources showed that protein and ash contents were minimum and maximum in defatted soy flour from sources D and B, respectively (Table 1). The fat contents in all the samples ranged from 1.13 to 1.4%. Proximate compositions of defatted soy flours obtained in the present investigation are in accordance with the earlier reports (Chauhan 1982; Dublish 1984; Kaushik 1985; Madan Mohan 1985; Singh et al. 1989).

From the results in Table 1, it is evident that the defatted soy flour obtained from source C showed the lowest value for nitrogen solubility, urease activity and trypsin inhibitor activity, in contrast to the highest value for non-enzymatic browning, as compared to the flours from other sources, thereby indicating comparatively higher degree of heat treatment to this flour (Kellor 1974). On the basis of these characteristics, defatted soy flour from source B had comparatively better quality (Table 1).

TABLE 1. PROXIMATE COMPOSITION (%) NITROGEN SOLUBILITY, UREASE ACTIVITY (UA), TRYPSIN UNITS INHIBITED (TUI) AND NON-ENZYMATIC BROWNING (NEB) OF DIFFERENT DEFATTED SOY FLOURS

Defatted soy flour source	Proximate composition %					Nitrogen solubility, %	Urease activity*	Trypsin unit inhibited, %	Non-enzymatic browning, OD
	Moisture	Protein	Fat	Ash	Carbohydrates				
A	4.1	55.1	1.1	6.8	32.9	9.5	2.5	41.0	0.18
B	2.8	57.3	1.3	7.8	30.8	91.7	2.0	43.0	0.17
C	2.9	57.2	1.2	6.6	32.1	47.6	0.4	6.5	0.21
D	4.2	54.9	1.4	6.3	33.2	87.5	1.9	41.5	0.16
SEM ±	0.08	0.14	0.07	0.18	0.44	0.74	0.07	0.48	0.01

All values are average of two replicates. * Expressed as an increase in pH over control.

Colour and consistency of isolates : Soy protein isolates, when precipitated by different coagulants at pH 3.5, showed whitish colour as compared to light whitish colour at other pHs (Table 2). However,

TABLE 2. COLOUR AND CONSISTENCY OF SOY PROTEIN ISOLATE MADE FROM DEFATTED SOY FLOUR OF DIFFERENT SOURCES USING DIFFERENT COAGULANTS AND COAGULATION PH

Coagulants	Coagulation pH	Defatted soy flour							
		A		B		C		D	
		Colour	Consistency	Colour	Consistency	Colour	Consistency	Colour	Consistency
HCl	4.5	1	3	1	3	1	3	1	3
	4.0	2	2	3	3	1	2	1	2
	3.5	3	1	3	2	1	1	1	1
H_2SO_4	4.5	1	3	1	3	1	3	1	3
	4.0	2	2	1	3	1	2	1	2
	3.5	3	1	3	2	3	2	3	2
Citric acid	4.5	1	3	1	3	1	3	1	3
	4.0	1	2	1	2	1	2	1	2
	3.5	3	1	3	1	3	1	3	1
Acetic acid	4.5	1	3	1	3	1	3	1	3
	4.0	1	2	1	1	1	3	1	2
	3.5	3	1	3	1	3	1	3	1
Lactic acid	4.5	1	3	1	3	1	3	1	3
	4.0	1	2	1	2	1	2	1	2
	3.5	3	1	3	1	3	1	3	1
$CaSO_4$ + $MgSO_4$	7.6	1	2	-1	3	-1	2	-1	2
$CaCl_2$	7.6	-1	2	-1	2	-1	2	-1	2

All values are average of those recorded by 10 panel members
1 = Whitish yellow, Semi-solid (Slurry type); 2 = Whitish light yellow, Semi-solid; 3 = Whitish solid; -1 = negative, dull whitish.

the soy protein isolates made from the defatted flour from source C was more yellowish as compared to other samples of soy protein isolates. This difference in colour may be attributed to the variation in heat treatment during the processing of defatted soy flours. Protein isolates, obtained by precipitation at pH 7.6 with $CaSO_4$+ $MgSO_4$ and $CaCl_2$, were dull whitish in colour. Mattil (1974) also reported similar range of variation in the colour of commercial soy protein isolates.

Soy protein isolates, precipitated at pH 4.5 by different coagulants, were of harder consistency, in comparison to those precipitated at other pH values, irrespective of defatted soy flour source, thereby indicating that pH 4.5 would be an iso-electric pH for majority of the soy proteins. However, the soy protein isolates precipitated at neutral pH, by $CaSO_4$ + $MgSO_4$ and $CaCl_2$ coagulants, were granular in texture. Protein isolate obtained by using HCl at pH 4.5 was found to be comparatively better with respect to colour and consistency.

4.5, with the use of HCl as coagulant. In contrast, the isolate prepared from the defatted soy flour from source C, with the use of acetic acid at pH 3.5, gave the minimum yields on flour weight basis. The yield of isolate decreased with the decrease in coagulation pH from 4.5 to 3.5, irrespective of defatted soy flour source and coagulants. These observations with respect to yield are in accordance with those reported earlier (Horan 1974; Meyar 1971; Lawhon et al. 1981). The yield of isolate on protein weight basis was also maximum for the sample which showed similar trend as on defatted soy flour weight basis and it ranged from 37.2 to 70.4% (Table 3). With the same coagulants and coagulation pH, the flour from source C gave the minimum yield of isolate, thereby indicating that the flour C was produced under higher degree of heat treatment (Cogan et al. 1967). It is interesting to observe that recoveries of isolates were much lower with the use of salt coagulation, as compared to acid coagulation. Similar results with respect to

TABLE 3. YIELD (d.b) OF PROTEIN ISOLATE MADE FROM DEFATTED SOY FLOUR OF DIFFERENT SOURCES USING DIFFERENT COAGULANTS AND COAGULATION pH

Coagulants	Coagulation pH	Defatted soy flour A		B		C		D	
		Yield %		Yield %		Yield %		Yield %	
		On defatted soy flour basis	On protein basis	On defatted soy flour basis	On protein basis	On defatted soy flour basis	On protein basis	On defatted soy flour basis	On protein basis
HCl	4.5	39.0	68.0	41.5	70.4	34.6	57.2	38.5	66.5
	4.0	31.0	52.6	32.5	53.1	31.5	56.9	31.0	52.6
	3.5	26.1	42.5	27.3	43.3	27.0	41.4	25.2	41.1
H_2SO_4	4.5	37.5	54.8	39.5	65.9	33.8	55.7	36.6	63.2
	4.0	29.5	48.9	31.8	51.1	29.1	45.9	29.5	48.9
	3.5	25.1	41.0	26.0	41.1	26.0	39.8	25.8	40.7
Citric acid	4.5	36.5	60.5	39.0	62.9	33.3	52.3	36.0	59.6
	4.0	30.1	49.8	30.5	49.1	30.3	47.7	30.0	49.6
	3.5	25.1	40.8	25.8	41.2	26.1	39.6	25.0	40.6
Acetic acid	4.5	36.5	60.5	39.0	62.9	39.3	52.3	36.0	59.6
	4.0	29.1	47.8	30.1	47.8	29.0	44.4	29.1	46.8
	3.5	25.0	39.7	25.0	49.3	24.3	37.2	25.0	39.5
Lactic acid	4.5	36.5	62.5	39.5	65.5	33.0	55.0	36.4	62.3
	4.0	30.1	49.9	32.0	51.8	31.5	49.7	30.0	49.8
	3.5	25.0	40.2	27.5	43.8	26.1	40.0	25.0	40.4
$CaSO_4$ + $MgSO_4$	7.6	28.0	42.1	31.5	46.6	27.0	38.0	27.4	40.5
$CaCl_2$	7.6	28.5	43.7	32.0	48.4	27.0	39.2	27.5	41.8
Standard error (SE±)		0.85	4.05	0.74	4.08	0.94	3.60	0.92	4.05

All values are means of two replications

Yield of isolates: From the results presented in Table 3, it is evident that the yield of protein isolates ranged from 24.3 to 41.5%, on defatted flour weight basis. Maximum yield was obtained from the defatted soy flour from source B at pH

protein recovery from defatted soy flour were reported by Meyar (1971).

Protein and nitrogen solubilities of isolates : The isolate made from the defatted soy flour from source B showed maximum values for protein

TABLE 4. PROTEIN (d.b) AND NITROGEN SOLUBILITY OF SOY PROTEIN ISOLATE MADE FROM DEFATTED SOY FLOUR OF DIFFERENT SOURCES USING DIFFERENT COAGULANTS AND COAGULATION pH

Coagulants	Coagulation pH	Defatted soy flour							
		A		B		C		D	
		Protein, %	Nitrogen solubility, %	Protein, %	Nitrogen solubility, %	Protein, %	Nitrogen solubility, %	Protein, %	Nitrogen solubility, %
HCl	4.5	96.1	76.8	97.1	78.6	94.5	59.4	95.3	74.9
	4.0	93.5	74.3	93.5	96.5	92.5	62.0	93.2	71.8
	3.5	89.7	70.7	90.8	74.4	87.7	62.4	89.5	70.9
H_2SO_4	4.5	95.2	74.9	95.6	77.4	94.3	66.9	94.8	73.9
	4.0	91.0	72.2	92.0	74.1	90.2	75.2	91.0	71.4
	3.5	90.0	71.0	90.5	73.3	87.5	62.1	89.4	70.06
Citric acid	4.5	94.1	73.7	94.5	76.5	93.7	65.5	94.1	73.4
	4.0	91.2	71.2	92.1	72.0	90.0	64.7	90.9	70.2
	3.5	89.5	69.2	91.8	70.1	86.8	62.4	89.2	69.1
Acetic acid	4.5	91.3	71.1	92.2	71.5	89.7	63.7	90.9	70.0
	4.0	90.5	70.2	91.0	70.7	87.5	62.5	88.4	68.7
	3.5	87.6	67.7	90.0	68.0	85.6	60.8	86.8	65.6
Lactic acid	4.5	94.3	73.8	95.0	77.2	92.7	65.5	94.0	73.3
	4.0	91.1	71.2	92.8	73.0	90.3	63.8	91.1	71.1
	3.5	88.7	69.1	91.2	59.1	87.6	61.9	88.8	68.4
$CaSO_4$ + $MgSO_4$	7.6	82.9	21.2	84.7	21.9	80.5	16.3	81.2	20.2
$CaCl_2$	7.6	84.5	22.9	86.6	26.3	83.1	21.2	83.5	22.2
Standard error (SE±)	0.85	1.18	2.19	1.31	2.42	0.82	1.78	1.15	2.33

All values are means of two replications

content and soluble nitrogen, while that from source C had the minimum values for these attributes (Table 4). The higher values of soluble nitrogen for the isolate from the flour from source B again indicated that this flour had undergone comparatively lesser degree of heat treatment during processing. The solubility of nitrogen decreased with the decrease in isolation pH, irrespective of flour and coagulants. The values of protein and soluble nitrogen in the present study fall in the range of 80.5-97.1%; and 20.2-96.5%, respectively, as reported by earlier workers (Mattil 1974; Manak et al. 1980). It also depends upon the extent of heat treatment and method of isolation.

On the basis of these observations with respect to colour consistency, yield and soluble nitrogen, the protein isolate prepared from defatted soy flour from source B by using HCl as coagulant at pH 4.5 was of comparatively better quality. This showed that the defatted soy flour from different sources can yield a product of different characteristics and, therefore, one should be cautious, while purchasing defatted soy flour for isolating proteins.

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In vitro and *In vivo* Availability of Iron from Home Processed Supplementary Foods

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Four supplements, containing 16.47 ± 0.48 to 17.67 ± 0.55 mg iron/100 g and consisting of whole wheat, pearl millet, *Bengal*gram, greengram, groundnuts, amaranth leaves *(Amaranthus gangeticus)* and jaggery, were developed, employing home processing methods of roasting and malting. Domestic processing increased the *in vitro* iron availability, due to lower levels of anti-nutrients. Pearl millet *(Bajra)*-based supplemented groups(III and IV) of rats showed significantly ($P < 0.05$) higher haemoglobin levels in blood than those fed wheat-based supplements (I and II) and similar levels to those of group fed on commercial food. The apparent absorption, balance and retention of iron in rats fed on pearl millet supplements was also significantly higher than those of wheat-based ones and commercial product fed group. The data showed that home processed supplement mixes of cereals, pulses, nuts and leafy vegetables being good sources of iron could improve iron status of young children of low income groups.

Keywords : *In vitro* and *In vivo* bioavailability, Iron, Home processing, Supplementary foods.

Iron deficiency anaemia is a serious public health problem facing the vulnerable sections of the population in India and ranks as a major factor in the current high rate of prenatal mortality (ICMR 1989). This is more prominent in growing infants, children, adolescents and the pregnant women, accounting for 60-70% mortality (Narasinga Rao 1983). It has been reported that anaemic lacto-vegetarian *Punjabi* girls have low haemoglobin levels when compared to anaemic non-vegetarians and non-anaemic groups (Nagi and Mann 1991). Iron deficiency can arise either due to inadequate intake or poor absorption of dietary iron or due to excessive losses of iron from the body. Children (30-35%) of low income groups in rural areas had anaemia due to iron deficiency (ICMR 1989). Dietary surveys conducted by National Nutrition Monitoring Bureau (NNMB 1981) in rural areas of India revealed that 35 to 50% of pre-school children had iron deficiency anaemia. Weaning foods in developing countries are normally prepared into thick or soft porridge using local cereals. The iron contents of such staples from endogenous origin and contamination from soil particles, are generally high (Bothwell et al. 1979), but it has a low bioavailability (Hallberg 1981; Gilloly et al. 1983; Gupta and Singh 1988). The low bioavailability of iron from cereal diets is also due to inhibitors such as fibre components, phytates, tannin (Latunde-data 1991). Traditional household level food processing such as dehulling, soaking, germination and fermentation could be utilized to reduce the amount, of these anti-nutritional factors.

Keeping these facts in view, the present study was conducted to develop supplementary foods from different food ingredients, employing simple domestic processing methods, and to study *in vitro* and *in vivo* iron availability from these foods.

Materials and Methods

A number of combinations of commonly consumed foodstuffs such as wheat, pearl millet, greengram and *Bengal*gram were theoretically calculated for their protein contents, essential amino acid profile and chemical scores. These combinations were enriched with groundnut (a rich source of energy and protein, also available easily in rural areas of Haryana), jaggery (to increase the energy density and iron content) and amaranth *(Amaranthus gangeticus)* leaves (as a source of minerals). The grains of wheat 'WH-283', pearl millet 'CJ-104', greengram 'K-851' and *Bengal*gram 'C-130' were obtained from the Directorate of Farms of the University, in a single lot. Jaggery, groundnuts and amaranth leaves were procured in one lot from local market. The grains were cleaned and stored in plastic containers at room temperature.

According to PAG (1977) guidelines, the essential amino acid content of the supplements should be similar to that of egg protein and the supplements should satisfy fully the supplementary feeding guidelines of providing at least one third of recommended dietary allowances of nutrients to young children. Accordingly, the proportions of cereal, pulse, oilseed, amaranth and jaggery in the ratio of 4:1:1:1:4 were selected. In all the cases, the cereals and groundnuts were roasted, the

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pulses were malted and the amaranth leaves were sun-dried. Combinations I and II contained whole wheat, which was replaced by whole pearl millet *(Bajra)* in combinations III and IV. Combinations I and III contained *Bengal*gram, which was substituted by greengram in combinations II and IV. As per local market prices, the costs of 110 g combination work out to be Rs. 0.66, 0.72, 0.67 and 0.70 for combinations I, II, III and IV, respectively.

Cleaned and picked grains of *Bengal*gram and greengram were steeped in double the amount of water at ambient temperature (25-30°C) and 70% relative humidity for 12 h. The soaked grains were wrapped in damp muslin cloth for germination. The sprouts were dried overnight at room temperature by keeping under a fan and roasted in oven at 70°C for 2 h to develop characteristic malt aroma. Wheat, pearl millet and groundnuts were separately roasted in a skillet for 2, 1 and 2 h, respectively, till a uniformly light brown colour developed. Amaranth leaves were cleaned, washed and sun-dried. The malted and the roasted ingredients were ground in a Cyclone mill (mesh size 0.5 mm) individually. The flour, thus obtained, powdered jaggery and sun-dried amaranth leaves were blended thoroughly.

Nutritional evaluation: Fresh samples of supplement mixes were analysed for iron content using atomic absorption spectrophotometer (Lindsey and Norwell 1969). *In vitro* iron availability (dialyzable iron) was determined by the method described by Miller and Schricker (1982). The data were processed for the analysis of variance, according to standard methods of statistical analysis (Snedecor and Cochran 1967).

In vivo *iron bioavailability* : Twenty one day old albino rats ('Wistar' strain), weighing 30 ± 5 g. were obtained from disease-free animal house of the University. The rats were randomly divided into different groups, each consisting of 8 rats. The rats were housed individually in polypropylene cages in air-conditioned room, maintained at 21-22°C, with 12 h light and dark cycle. Food and distilled water were given *ad libitum*. The basal diet was prepared according to the recommendations of National Academy of Science (NAS 1972). Six types of diets (4 supplement mixtures, 1 commercial product diet and 1 synthetic diet containing casein) were prepared at a time in quantities sufficient for one week feeding and kept in the refrigerator (Table 1). All the diets contained 10% protein.

After 28 days of feeding, the rats were transferred singly to metabolic cages for estimating iron bioavailability and weighed diets were given to rats daily during the experimental period. The experiment was conducted for 9 days. Rats got acclimatized during the first four days. Faeces and urine were collected separately from each rat during the last 5 days of the experiment. The amount of food consumed during this period was also recorded. The iron contents in food, faeces and urine were determined, after acid digestion (Lindsey and Norwell 1969). For acid digestion, 1 g ground sample or 5 ml of urine was taken in conical flask and 25 ml of diacid mixture HNO_3:$HClO_4$ 5:1, v/v) was added and kept overnight. At the end of experiment, the rats were anaesthetized with diethyl ether and blood was collected from dorsal aorta. Haemoglobin level in blood was estimated by the method of Dacie and Lewis (1975). From these data, the apparent retention, absorption and balance of iron were calculated. The overall availability of iron was judged from chemical parameters, iron retention and haemoglobin content.

Results and Discussion

Iron content of supplements: Iron contents of supplements I, II, III and IV as well as the

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS (g/100 g DIET)

Components	Diet					
	Casein as protein source	Supplement I	Supplement II	Supplement III	Supplement IV	Commercial product
Casein	11.76	-	-	-	-	-
Supplement I	-	80.25	-	-	-	-
Supplement II	-	-	78.30	-	-	-
Supplement III	-	-	-	85.47	-	-
Supplement IV	-	-	-	-	84.74	-
Commercial product	-	-	-	-	-	64.51
Groundnut oil	10.00	4.70	4.90	4.00	4.25	1.00
Corn starch	57.74	9.85	11.60	5.33	5.81	29.29

Each diet contained 4, 1 and 0.20 g mineral mixture 1, vitamin mixture 2 and choline bitartarate/100 g diet, respectively. Casein diet contained 10, 5 and 0.3 g sucrose, cellulose and DL-methionine/100 g diet, respectively. Each diet contained 10% protein.

commercial product were 16.66 ±0.30, 16.47 ± 0.48, 17.67 ± 0.55, 17.38 ±0.62 and 7.5 mg/100 g, with the highest value in supplement III and the lowest in supplement II. The iron contents of wheat-based supplements were found to be significantly (P < 0.05) lower than those of *bajra*-based supplements. Shulk et al (1986) reported 14 mg iron in 100 g 'Soylac' weaning food. On the contrary, lower amount of iron (7.6 mg) has been found in another mixture (Chandrasekhar et al. 1988). Higher contents of iron in present supplements may be due to the addition of jaggery. The recommended daily intake of iron for children of 1-3 years of age is 20-25 mg/day and thus the supplements would meet more than one-third requirement of iron for pre-school children.

In vitro *iron availability :* The *in vitro* iron availability of raw (unprocessed) as well as processed (developed) pearl millet-based supplements III and IV was significantly (P < 0.05) higher than that of the wheat-based supplements I and II (Table 2). On processing, the *In vitro* iron availability increased in all the supplements and varied from 31.01 to 35.48%, it being the highest in supplement IV and lowest in supplement I. Improved availability of iron in soybean sprouts has also been reported earlier (Latunde-data 1991).

Iron bioavailability (in vivo) : Iron intakes of synthetic diet group was significantly (P<0.05) higher than those which were fed on commercial product diet (Table 2). Faecal excretion of all the dietary groups varied from 77.85 to 86.79% of iron intake. This shows that iron is excreted mainly through faeces. Groups fed on pearl millet-based supplements showed significantly (P<0.05) lower faecal excretion of iron than those fed on wheat-based supplements.

Apparent absorption of iron in all the groups ranged from 13.20 ±1.98 (supplement II) to 20.14 ± 2.10 (synthetic diet). Groups III and IV fed on pearl millet supplements showed significantly (P<0.05) lower apparent absorption of iron than that of synthetic diet group (Table 2). All the groups were on positive iron balance. *Bajra*-supplemented groups showed higher iron balance status as compared to that of wheat supplemented groups.

Apparent retention of iron varied from 8.62 ± 2.60 to 17.05±1.98% in all the dietary groups. Pearl millet-based supplemented groups showed significantly (P<0.05) higher apparent retention of iron than those by the wheat-based ones. The lower values in wheat-based supplements might be due to higher levels of copper and phytic acid in these supplements, which reduce the absorption of iron. Chaudhary and Kapoor (1985), Mason (1979) and Kakker and Kapoor (1989) also reported that high levels of copper resulted in lower iron absorption and retention.

Haemoglobin content : Haemoglobin values of

TABLE 2. *IN VIVO* BIOAVAILABILITY OF IRON IN RATS FED ON DIFFERENT SUPPLEMENTS, COMMERCIAL PRODUCT AND SYNTHETIC DIET, CONVERSION OF IRON INTAKE INTO HAEMOGLOBIN IRON AND EFFECT OF PROCESSING ON *IN VITRO* IRON AVAILABILITY.

Attributes	Dietary group				Commercial product	Synthetic diet	(SEm)	CD (P<0.05)
	I	II	III	IV				
Iron intake	5.2	5.3	5.2	5.2	5.6	5.5	0.90	2.70
Faecal excretion	4.5	4.6	4.4	4.3	4.7	4.4	0.03	0.10
Urinary excretion	0.2	0.2	0.2	0.2	0.2	0.1	0.04	0.12
Apparent absorption	13.4	13.2	16.1	16.3	16.7	20.1	0.86	2.60
Balance	0.4	0.4	0.6	0.6	0.7	0.9	0.03	0.10
Apparent retention	8.6	9.9	11.8	11.9	13.2	17.0	0.90	2.70
Iron intake	27.0	27.8	28.1	28.9	28.7	30.0	1.41	4.25
Body weight								
Initial	30.2	31.2	31.5	30.5	30.6	30.3	3.57	10.71
Final	68.4	71.3	77.5	79.2	86.7	86.5	2.70	8.15
Haemoglobin level	10.6	10.7	11.8	11.9	11.7	12.2	0.32	0.98
Iron intake converted into haemoglobin iron	21.6	21.3	22.4	22.5	22.5	23.2	1.45	4.46
Dialysable iron								
Raw	7.9	8.0	8.1	8.1	-	-	0.02	0.08
Developed	10.3	10.6	10.9	11.0	-	-	0.08	0.25

In vitro values are mean ± SD of three replicates. Values for *in vitro* iron availability are mean ± SD of eight replicates.

TABLE 3. CORRELATION COEFFICIENT (r) OF GROUP MEANS OF APPARENT ABSORPTION AND APPARENT RETENTION OF IRON IN RATS WITH *IN VITRO* AVAILABILITY OF IRON

Variable pair tested	Correlation coefficients of supplements			
	I	II	III	IV
In vitro availability of iron vs apparent absorption	0.893*	0.896*	0.942*	0.952*
In vitro availability of iron vs apparent retention	0.879*	0.877*	0.945*	0.956*

Based on 6 degrees of freedom. * Significant at 5% level of significance.

different groups ranged from 10.68 ± 0.52 (supplement I) to 12.25±0.87 g/100 ml blood (synthetic diet) (Table 2). The initial haemoglobin content in blood was 9.52 g/100 ml. Groups fed on pearl millet-based supplements showed significantly ($P<0.05$) higher haemoglobin contents than those fed on wheat-based ones. This may be due to higher copper concentration in pearl millet-based supplements. Weisenberg et al (1980) also reported higher haemoglobin values with increased copper intake. Commercial product group had significantly higher haemoglobin levels (11.78± 0.42 g/100 g). when compared to those fed on wheat-based supplements. Conversion efficiency of dietary iron into haemoglobin was the highest in rats fed on pearl millet-based supplements and the values were slightly higher than those for wheat-based products. Percentage of iron intake converted into haemoglobin iron in group fed on synthetic diet, containing optimum levels of trace elements, was found to be in accordance with Kapil (1982), who reported that 23% of iron consumed was converted into haemoglobin iron in rats fed on a diet containing optimum levels of trace elements.

Correlation coefficient analysis data show that there is a positive and significant ($P<0.05$) correlation between *in vitro* bioavailability of iron and apparent absorption in all the four supplements (Table 3). This shows that *in vitro* method, which is easy, rapid and does not involve animal experiments, can be used with reliability for estimating the availability of iron from food materials.

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Studies on the Physico-chemical Characteristics and Processing Quality of Two IIHR Tomato Varieties in Relation to Commercial Cultivars

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'Arka Ashish' and 'Arka Ahuti', two tomato varieties developed at this Institute (IIHR) were compared with 6 commercial cultivars of different regions, in order to determine their physico-chemical characteristics and test their suitability for processing into puree. Both the varieties had better fruit weight than 'Pusa Ruby', 'Punjab Chhuhara' and 'Lerica', in addition to the greater fruit firmness than all other varieties (except 'Lerica'). The juice yield was maximum in 'Arka Ashish', while it was comparable to 'Roopali' and 'Lerica' in case of 'Arka Ahuti'. The total soluble solids of IIHR varieties were better than 'Pusa Ruby' and 'Roma', while the acidity and ascorbic acid content were similar to other cultivars. Lycopene content was maximum in 'Arka Ashish' followed by 'Pusa Gaurav' and 'Arka Ahuti'. In viscosity, the IIHR varieties were better than 'Pusa Ruby', 'Roma' and 'Pusa Gaurav'. The puree prepared from 'Arka Ashish' and 'Arka Ahuti' possessed significantly higher amount of lycopene and better viscosity than all other varieties and performed better than all the rest in sensory evaluation.'Lerica' and 'Pusa Gaurav' were the next best varieties.

Keywords : Tomato, Physico-chemical characteristics, Processing quality, Puree, IIHR varieties, Commercial cultivars, Sensory evaluation.

Tomato *(Lycopersicon esculentum* Mill) constitutes one of the major vegetable crops in India and its products rank first among all processed vegetables (Sethi and Anand 1986). Tomatoes are used for salads, in various daily food preparations and canned or processed into juice, soup, puree, ketchup and *chutney*. Quality and flavour of the processed products depend upon chemical composition of the tomato, which has been reported to vary greatly with variety (Winser 1979). The most desirable qualities for processing of tomatoes have been considered as high total solids, acidity between 0.3 and 0.4%, uniform red colour, smooth surface, free from wrinkles, small core, firm texture and uniform ripening (Premchandra et al. 1976). Physico-chemical characteristics of different varieties of tomatoes (Kaur et al. 1976), seasonal composition of different varieties, varietal suitability for canning (Beerth and Rane 1976) as well as manufacture of ketchup (Nanjundaswamy et al. 1960; Pruthi et al. 1980), juice (Adsule et al. 1980) and puree (Sethi and Anand 1986) and nutritional aspects (Theymoli Balasubramanian 1984) have been reported.

The intensive research work, carried out during the eighties at this Institute for evolving tomato varieties suitable for processing, led to the identification of two promising varieties 'Arka Ahuti' ('Sel-11') and 'Arka Ashish' ('IIHR 674 SB') (Anon 1992). 'Arka Ahuti' is a pure line selection from 'Ottawa-60'. The semi-determinate plants of 'Arka Ahuti' bear oblong and firm fruits with thick flesh, bilocular, uniform ripening and a TSS of 5.2 °Brix with a yield potential of 450 q./ha. in 135 days, whereas 'Arka Ashish' is an improvement over 'UC 82B' from California. It is a determinate type plant producing uniform ripening, thick fleshed, very firm, bilocular, oval fruits and concentrated fruit maturity. The fruit colour is excellent with a TSS of 4.8 °Brix and a yield potential of 350-400 q/ha. in 135 days (Anon 1992). In this study, these two new varieties of IIHR were compared with six commercial cultivars of different regions, in order to determine their physico-chemical composition and to test their suitability for processing. Since addition of spices, salt and sugar during the preparation of ketchup or soup alters the natural flavour, it was decided to select puree as a test product. Further, puree is an intermediary product which can be used for making ketchup, sauce, soup, juice, etc. Use of puree as a test product for testing tomato hybrids has also been adopted by other workers (Sethi and Anand 1986).

Materials and Methods

Raw materials: Ripe, firm, uniform tomatoes of 'Pusa Ruby' 'Roma', 'Punjab Chhuhara', 'Roopali' (F_1 hybrid from IAHS, Bangalore), 'Lerica' (F_1 hybrid),'Arka Ahuti' (IIHR), 'Arka Ashish' (IIHR) and 'Pusa Gaurav' (S-152 of IARI), grown at the experimental farm of the Institute during 1987 to 1991, were used.

Physico-chemical parameters: Ten fruits of each variety/hybrid were randomly selected and

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average fruit weight and firmness were noted using a Tulaman balance and penetrometer/fruit pressure tester (Effegi, Italy, model FT011), respectively. Green portion, pomace, juice yield and chemical constituents were recorded, after juice extraction from 5 kg lot. Total soluble solids (TSS) in the juice was determined by a hand refractometer (0-32° Brix) at room temperature and necessary temperature corrections were applied. The pH, acidity, ascorbic acid and total solids in juice were determined by AOAC (1984) method, while the lycopene content was determined by the method of Ranganna (1986). Viscosity was recorded using a digital viscometer (Brookfield, USA, model LVTD) at a constant temperature of 20°C.

Preparation of puree : Fruits (5.0 kg), from each cultivar/variety/hybrid, were washed, cored and cut into small pieces. These were boiled individually in their own juice for 10 min and passed through 30 mesh stainless steel sieves to obtain juice. The juice was concentrated in a pan by heating to get a TSS of 14° Brix and filled hot into a clean sterilized 200 ml bottle, crown corked and heat processed for 15 min in boiling water. The puree was analysed for various chemical constituents. The sensory quality of the puree was assessed by a panel of 10 judges using Hedonic scale having 30, 30 and 40 marks for colour, consistency and flavour, respectively. All the data were analysed statistically using randomised block design (RBD) with four replications and means were compared either at a probability of 1% or 5% level (Sundararaj et al. 1972).

Results and Discussion

Physical parameters: Weights of fruits varied significantly in different varieties. Fruit weights of 'Roma', 'Pusa Gaurav', 'Arka Ahuti' 'Arka Ashish', were significantly higher than 'Pusa Ruby', 'Punjab Chhuhara' and 'Lerica' at a p value of 1% (Table 1). 'Lerica' recorded the highest fruit firmness, followed by 'Arka Ashish', while fruits of 'Pusa Ruby' and 'Roma' were very soft. Green portion (stem end and green shoulder) was the highest in 'Pusa Ruby' followed by 'Roopali', while it was the lowest in 'Lerica', 'Arka Ahuti' and 'Arka Ashish'. It is interesting to note that some varieties will have greenish patches even at fully ripe condition. The green portions reported in the present case, consist of green patches, stem end and also core, which were removed at the time of cutting of fruits to avoid the browning of the product. The sum of values for green portion, pomace, juice yield was less than 100 due to preparatory losses. The pomace content (seed and skin left over after juice extraction) was the lowest in 'Lerica' and highest in 'Roma',while juice yield was maximum in 'Arka Ashish' and minimum in 'Pusa Ruby'. However, the differences in pomace content and juice yield were statistically not significant.

Chemical composition of juice: The total soluble solids content was maximum in 'Lerica' followed by 'Roopali' and 'Arka Ashish', while it was the lowest in 'Pusa Ruby'. The acidity varied from 0.44% to 0.68%, but the differences were statistically not significant. Large variations were observed with respect to ascorbic acid contents, the lowest being in 'Roma' and the highest in 'Roopali'. All other

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF DIFFERENT TOMATO VARIETIES

Variety	Fruit weight, g	Firmness, lbs/ sq. inch	Green portion, %	Pomace, %	Juice yield, %	TSS, °B	pH	Acidity, %	Ascorbic acid, mg/100g	Lycopene, mg/100g	Total solids, %	Viscosity, cps	Puree yield, %
'Pusa Ruby'	41	4.0	5.5	7.6	67	4.8	4.2	0.59	10.9	4.3	6.7	93	29
'Roma'	60	5.5	3.6	8.0	69	5.4	4.3	0.57	2.7	4.6	7.4	112	32
'P.Chhuhara'	43	5.7	3.0	6.7	69	5.0	4.3	0.49	8.6	5.1	6.4	184	29
'Roopali'	52	5.9	5.2	6.1	60	6.7	4.2	0.49	24.3	4.0	7.6	173	31
'Lerica'	45	8.4	1.8	4.7	64	8.6	4.0	0.68	9.1	5.0	10.0	1033	40
'Arka Ahuti'	56	6.0	2.0	5.5	65	5.2	4.3	0.55	5.2	5.2	7.0	270	30
'Arka Ashish'	56	7.1	2.2	7.0	71	5.6	4.3	0.52	6.7	7.0	7.0	188	30
'Pusa Gaurav'	60	5.9	3.8	5.7	63	5.6	4.4	0.44	7.9	6.0	7.0	132	28
SEm ±	2.32	0.29	0.61	0.8	2.6	0.18	0.05	0.06	1.7	1.0	0.3	6.44	1.77
CD 5%	7.05	0.88	1.84	NS	NS	0.53	0.15	NS	5.1	NS	0.9	19.52	5.37
CD 1%	9.78	1.22	2.55	-	-	0.74	0.20	-	7.1	-	1.3	27.10	7.46

varieties possessed significantly higher ascorbic acid contents than 'Roma' even at a probability level of 1%. The lycopene content was the highest in 'Arka Ashish' followed by 'Pusa Gaurav'. The total solids content was maximum in 'Lerica', mainly due to its higher TSS content. It is stressed that the differences between TSS value and total solids are dependent on insoluble solids content of the puree, which will be very low, when fully ripe fruits are used. Very large variations were observed in viscosity of different varieties. The viscosity was maximum in 'Lerica' and minimum in 'Pusa Ruby'. All other varieties possessed significantly higher viscosity than 'Pusa Ruby', but lower than 'Lerica' at a p value of 1%. 'Arka Ahuti' had significantly higher viscosity than all other varieties except 'Lerica', while the viscosity of 'Arka Ashish', 'Punjab Chhuhara' and 'Roopali' were comparable with each other. The yield of puree was significantly higher in 'Lerica', than in all other varieties, which could be attributed to its higher TSS and total solid contents. The rest had a very narrow difference in puree yield.

Chemical composition of puree: The data on chemical composition of puree, prepared from different varieties, are presented in Table 2. No significant differences were observed in TSS, acidity, total solids and total sugars content of puree from different varieties/hybrids, since the TSS of puree was adjusted to 14° Brix. Ascorbic acid content of the puree from 'Roopali' was significantly higher than any other variety, which was attributed mainly to its higher ascorbic acid content in the juice. The lycopene content of the puree was maximum in 'Arka Ashish', which was significantly higher than that in 'Roma' at 1% probability value. 'Pusa Gaurav' and 'Arka Ahuti' possessed significantly higher lycopene at a p value of 5%

TABLE 2. PHYSICO-CHEMICAL COMPOSITION OF TOMATO PUREE PREPARED FROM DIFFERENT VARIETIES AT 0 AND 6 MONTHS OF STORAGE

Variety	TSS, °B	pH	Acidity, %	Total solids, %	Ascorbic acid, mg/100g	Lycopene, mg/100g %	Reducing sugars, %	Total sugars, %	Viscosity, cps
Fresh, 0 month storage									
'Pusa Ruby'	14.0	4.2	1.08	14.4	30.7	11.0	10.1	11.1	1520
'Roma'	14.3	4.2	1.00	14.6	24.0	10.0	10.4	11.2	2073
'P.Chhuhara'	14.1	4.3	1.03	15.2	28.0	12.2	9.2	10.4	3276
'Roopali'	15.1	4.2	0.96	16.0	47.0	8.4	9.6	9.8	1740
'Lerica'	14.1	4.2	0.94	14.6	31.0	10.4	9.5	9.7	2900
'A.Ahuti'	14.7	4.3	0.96	15.2	26.7	14.7	9.8	9.9	3840
'A.Ashish'	14.2	4.3	0.99	14.8	20.0	19.3	9.5	9.9	4000
'Pusa Gaurav'	14.3	4.4	1.02	15.3	28.3	16.7	8.5	10.0	2033
SEm ±	0.51	0.04	0.10	0.37	4.4	1.5	0.3	0.6	139
CD 5%	NS	0.13	NS	NS	13.4	4.5	0.8	NS	423
CD 1%	-	NS	-	-	NS	6.3	NS	-	587
6 months storage									
'Pusa Ruby'	13.9	4.1	1.33	14.9	29.7	9.6	11.6	11.8	1627
'Roma'	14.2	4.3	1.01	15.1	27.7	9.4	11.2	11.3	1920
'P.Chhuhara'	14.0	4.3	1.05	16.0	23.7	11.6	10.4	10.7	3726
'Roopali'	13.6	4.1	0.99	14.5	47.0	8.4	9.7	10.1	1880
'Lerica'	13.4	4.2	0.95	14.4	25.7	10.2	9.5	9.9	2867
'A.Ahuti'	14.4	4.3	0.95	16.0	18.7	12.3	9.7	9.9	3743
'A.Ashish'	14.2	4.2	0.96	15.7	16.0	18.7	11.5	11.8	3360
'Pusa Gaurav'	13.8	4.3	1.16	16.1	23.7	16.5	10.6	11.3	2143
SEm ±	0.37	0.06	0.11	0.46	4.4	1.4	0.1	0.2	152
CD 5%	NS	NS	NS	NS	13.2	4.3	0.4	0.5	462
CD 1%	-	-	-	-	18.3	6.0	0.6	0.6	641

TABLE 3. SENSORY QUALITY OF TOMATO PUREE PREPARED FROM DIFFERENT VARIETIES AT 0 AND 6 MONTHS OF STORAGE

Variety	Sensory score			
	Colour, 30	Consistency, 30	Flavour, 40	Total, 100
Fresh, 0 month storage				
'Pusa Ruby'	18	18	21	57
'Roma'	20	20	24	64
'P.Chhuhara'	21	21	23	65
'Roopali'	17	20	21	58
'Lerica'	22	22	25	69
'A.Ahuti'	21	22	27	70
'A.Ashish'	24	23	26	73
'Pusa Gaurav'	21	22	24	67
SEm ±	0.58	0.99	0.97	1.89
CD 5%	1.77	3.01	2.94	5.73
CD 1%	2.46	4.10	4.08	7.95
6 months storage				
'Pusa Ruby'	18	19	20	57
'Roma'	20	21	25	66
'P.Chhuhara'	19	19	24	62
'Roopali'	19	19	24	62
'Lerica'	21	24	22	67
'A.Ahuti'	21	23	27	70
'A.Ashish'	24	23	27	74
'Pusa Gaurav'	22	21	24	67
SEm ±	0.89	0.84	1.06	1.60
CD 5%	2.71	2.56	3.21	4.87
CD 1%	3.76	3.56	NS	6.76

only. The variations in lycopene content of puree from other tomatoes was not significant. The viscosity was lowest in 'Pusa Ruby' and it was maximum in 'Arka Ashish', followed by 'Arka Ahuti'. 'Punjab Chhuhara' and 'Lerica' were the other two varieties which had moderate viscosity. The chemical analysis carried over after a storage period of 6 months also confirmed the above results (Table 2). It is interesting to note that the variations observed in the TSS and total solids between 0 and 6 months storage of puree can be due to differences in samples used for the analysis.

Sensory quality: Since variations in TSS of puree of different samples was 1° Brix or less, it was decided to carry out sensory evaluation without altering the composition of puree. The sensory evaluation studies indicated that 'Arka Ashish' was best suited for processing, since it scored maximum for colour, consistency, flavour and overall acceptability score (Table 3). This was closely followed by 'Arka Ahuti', which performed similar to 'Arka Ashish' in all aspects except colour. The good performance of 'Arka Ashish' in sensory evaluation was attributed to its higher lycopene content, very good viscosity, apart from the flavour. The other good varieties viz., 'Lerica' and 'Pusa Gaurav', performed better than other commercial cultivars. The storage studies carried over upto 6 months also confirmed the above results (Table 3).

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Effect of Gamma Irradiation and CIPC Treatment on Processing Quality of Potatoes

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The processing quality of 5 potato cultivars, after inhibition of sprouting by irradiation or chloroisopropyl phenyl carbamate treatment, was compared during storage at 20, 15 and 10°C up to 9 months. Cultivars 'K. Jyoti', 'K Lavkar', 'Gujrat-S' and 'Talegaon', with initial reducing sugar levels of 120 to 240 mg/100 g, yielded light coloured chips and French fries. Neither of the treatments affected the processing quality of these cultivars, when stored at 15 and 20°C up to 6 months, but the storage at 10°C resulted in darker products, due to higher levels of reducing sugars. In tubers, stored for more than 6 months at 15°C, reconditioning at 27-32°C or blanching improved the colour of the products. Cultivar 'K. Badshah', with initial reducing sugar levels of 310 to 440 mg/100 g, was not suitable for processing. *In vitro* infiltration studies showed that glucose contributed to browning more than fructose or sucrose.

Keywords : Potatoes, Gamma irradiation, Chloroisopropyl phenyl carbamate, Sprouting inhibition, Storage temperature Chips, French fries, Reducing sugar, *In vitro* infiltration.

Potato chips and French fries are the important processed products of significant commercial value in the consumer market. Normally, potatoes are stored at 2-4°C to extend their market-life, control of sprouting and prevention of rotting (Smith 1977). However, accumulation of reducing sugars in cold stored tubers make them unsuitable for use in the manufacture of chips and French fries (Burton 1969; Coffin et al. 1987; Joshi et al. 1990), because of the undesirable brown colour development due to Maillard reaction (Fuller and Hughes 1984). This has been shown earlier in rice by gamma irradiation (Roy et al. 1991). On the other hand, storage of potatoes at ambient temperatures induces profuse sprouting and rotting, thereby destroying the whole produce within a short period of time (Thomas 1984).

Our earlier investigations have demonstrated that, a dose of 0.1 kGy of gamma irradiation inhibited sprouting irreversibly, regardless of storage temperature (Thomas et al. 1978). In addition, it helped in disinfestation of tuber moth and in delaying the light induced greening and solanine synthesis (Thomas 1984). Pilot scale storage studies on the efficacy of sprout inhibition of onions by gamma irradiation have also been reported (Thomas et al. 1986). Although chloroisopropyl phenyl carbamate (CIPC) is an effective sprout inhibitor, its use legally is not permitted in India and many other countries due to the growing concern about the safety of its residue (Thomas 1984; Smith 1977). Negligible information is available on the suitability of gamma irradiated Indian potato cultivars for the manufacture of chips and French fries. In the present studies, the processing quality of five potato cultivars was compared during prolonged storage at different temperatures by using gamma irradiation or CIPC for sprout inhibition.

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Materials and Methods

Tubers of potato cultivars, 'Kufri Badshah (Military)', 'Kufri Jyoti', 'Kufri Lavkar', 'Gujrat-S' and 'Talegaon' were procured from the local market in the beginning of the harvest season and held for 8 to 10 days under ambient conditions for wound healing prior to irradiation. Tubers were irradiated by using a dose of 0.1 kGy in a Cobalt-60 package irradiator at a dose rate of 33.3 Gy/min. CIPC treatment was carried out by dipping the tubers in 0.025% solution for 5 min, followed by overnight drying at ambient temperature. Irradiated and CIPC treated tubers were stored in ventilated plastic crates in 40 Kg lots at 10°, 15°, 20° and 27-32°C (room temperature). The experiments were terminated after 4 months storage at 27-32°C and after 9 months storage at lower temperatures.

Frying: Tubers, deskinned with a hand peeler, were cut longitudinally into two equal halves from bud to stem end. One half was cut longitudinally into strips of 1 x 1 cm size, while the other half was sliced into 0.5 to 1 mm thickness and round discs were cut out with a cork borer of 2.5 cm dia. The strips and discs were rinsed in water, dried using blotting paper and dried for 6 to 7 min and 3 to 4 min, respectively, in a commercial

refined groundnut oil heated to 186 to 188°C.

The quality and acceptability of French fries and chips with respect to their overall appearance, colour, taste, aroma and texture were evaluated by a taste panel of 8 persons.

Estimation of reducing sugars: Four to five randomly selected tubers from each cultivar were deskinned, cut into small pieces and mixed. Ten g pulp, in duplicate, were placed in 25 ml of 85% aqueous ethanol solution containing a pinch of calcium carbonate and heated to 80°C for 5 min, with constant stirring. After cooling, the mixture was homogenized in an Omnimixer, centrifuged and the residue re-extracted in 85% ethanol. The pooled supernatants were concentrated in vacuum by flash evaporation to a minimum volume and stored at 10°C until used. The reducing sugars were determined colorimetrically by 3.5 dinitro salicylic acid (DNS) method (Lindsay 1973), using D-glucose as standard.

In vitro *infiltration of sugars:* About 10 g discs of strips were immersed in a solution of 1%, 2% or 10% glucose, fructose, sucrose or a 1:1 mixture of 10% each of glucose and fructose in a conical flask. Vacuum was applied continuously for 30 min for effecting infiltration of the sugars. Following these, the tissues were thoroughly washed with distilled water, blotted dry and fried as described earlier.

All the chemicals used in this study were of Analar grade.

Results and Discussion

Initial frying tests of tubers, soon after their procurement, indicated that cultivars 'Kufri Jyoti', 'K. Lavkar', 'Gujrat-S' and 'Talegaon' produced light coloured chips and French fries of good quality. The reducing sugar contents in these cultivars ranged from 120 to 240 mg/100 g fresh weight (Table 1). Chips and French fries made from 'K. Badshah', having an initial reducing sugar content of 310-440 mg/100 g fresh weight (Table 1), were darker in colour and not of acceptable quality. This relationship indicates that a high level of reducing sugars in potatoes is undesirable for processing, since the consumer preference is for an attractive light brown or golden yellow product (Talburt and Smith 1975; Fuller and Hughes 1984).

Gamma irradiation at 0.1 kGy completely prevented sprouting in all the five cultivars, irrespective of the storage temperature. Though

TABLE 1. REDUCING SUGARS CONTENT (mg per 100 g FRESH TISSUE) OF 5 POTATO CULTIVARS IMMEDIATELY AFTER PROCUREMENT, GAMMA IRRADIATION AND 1 AS WELL AS 6 MONTHS STORAGE AT 15° AND 10°C.

Cultivars	Concentration of reducing sugars				
	Initial	After irradiation and storage at			
		10°C		15°C	
		Months		Months	
		1	6	1	6
'K. Badshah (Military)'	390±50	462±90	736±222	402±55	603±150
'K. Jyoti'	193±22	322±36	445±100	198±35	255±21
'K. Lavkar'	150±22	305±42	470±112	174±25	234±24
'Gujrat-S'	196±26	328±50	653±163	210±39	238±24
'Talegaon'	207±27	337±37	532±117	218±35	258±25

Each value represents the mean of 4 replicates.

CIPC controlled the sprouting, its effective concentration was dependent on the storage temperature. Liu et al (1990) reported that 300-500 µg CIPC/litre effectively inhibited sprouting in 10°C stored 'Kennebec', 'Wu-Foon' and 'Cardinal' potato tubers, without affecting their processing qualities upto 8 months. In our earlier studies, it was found that 0.05% CIPC completely prevented sprouting even in ambient temperature stored potatoes (unpublished data). Since this concentration was several times higher than the requirement, a concentration of 0.025% was used in the present studies. Complete sprout inhibition was observed in 0.025% CIPC treated potatoes stored at 10°C, but tubers stored at 15 and 20°C showed restricted growth of rosette sprouts after 3 or 4 months and these sprouts increased in size during further storage. After 6 months storage at 20°C, 65-70% of tubers in all cultivars showed sprouting, the sprout lengths varied from 3 to 5 mm in size, while in potatoes, 60% of the tubers had sprouts of 2 to 4 mm in size, when stored at 15°C. These results show that gamma irradiation is a better effective sprout inhibitor than CIPC, since irradiation inhibited sprouting regardless of storage temperature, while CIPC at 0.025% concentration was not so effective above 10°C storage. At higher concentrations of CIPC, there is a risk of higher residue levels of the chemical in the tuber.

The storage changes in the reducing sugar levels of gamma irradiated and CIPC treated tubers of all the five cultivars, as influenced by temperature, are shown in Figs. 1-5. Neither treatment of the sprout inhibition affected the quality of chips and French fries of 'K. Jyoti', 'K. Lavkar', 'Gujrat-S' and

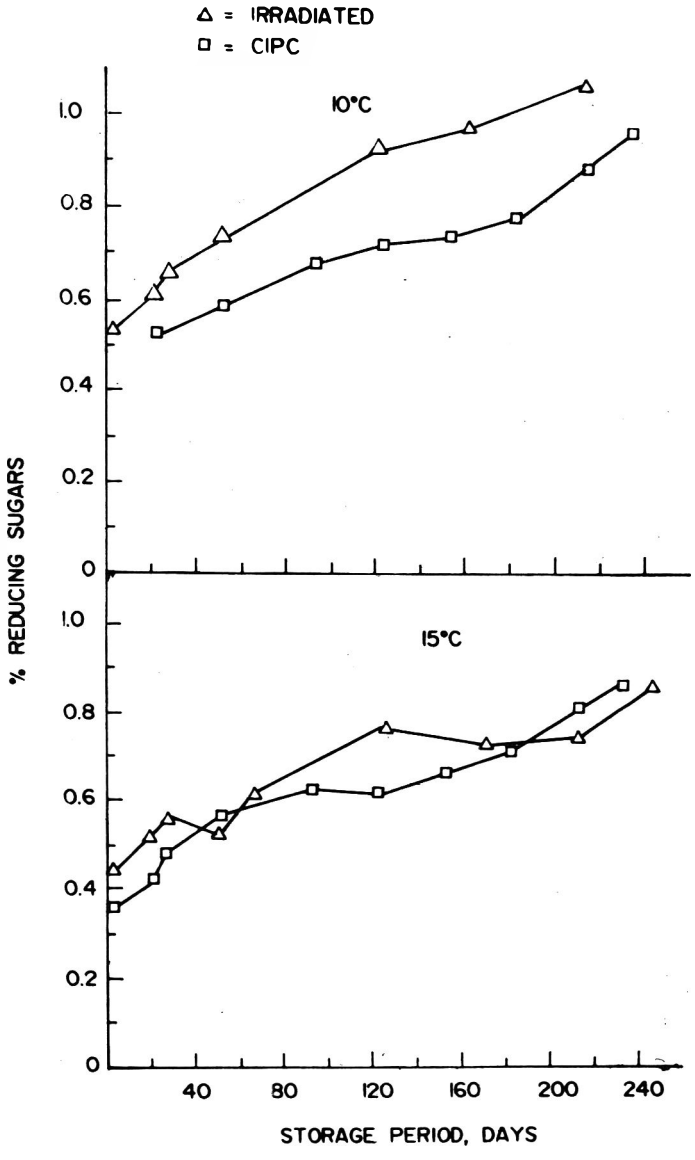

Fig. 1. Changes in the levels of reducing sugars in gamma irradiated and CIPC treated potato tubers of cultivar 'Kufri Badshah'.

'Talegaon', with the use of potatoes stored at 15°C and 20°C up to 6 months. The levels of reducing sugars ranged between 180 and 270 mg/100 g fresh weight in these samples, while no particular trend, attributable to cultivar of sprout control treatment, was discernible.

Our earlier studies have shown that sprouting in several potato cultivars could be completely prevented by irradiation at a dose of 0.1 kGy and these could be kept for 6 months at 15°C with less than 10% losses on account of microbial rottage. This procedure was, therefore, recommended as an alternative to commercial cold storage at 2-4°C (Thomas et al. 1978; Shirsat et al. 1991). The suitability of irradiated potatoes for processing was, however, not evaluated. The present study showed that the irradiation maintained the quality of the chips and French fries during 6 months storage at 15° or 20°C in those potato cultivars having reducing sugars of about less than 250 mg/100 g fresh weight at the time of treatment (Table 1). Although storage exceeding 6 months may result in increased formation of reducing sugars, reconditioning at room temperature (27°-32°C) for 1 to 2 weeks or blanching (1 to 5 min in water heated to 98°C) lowered the reducing sugar contents

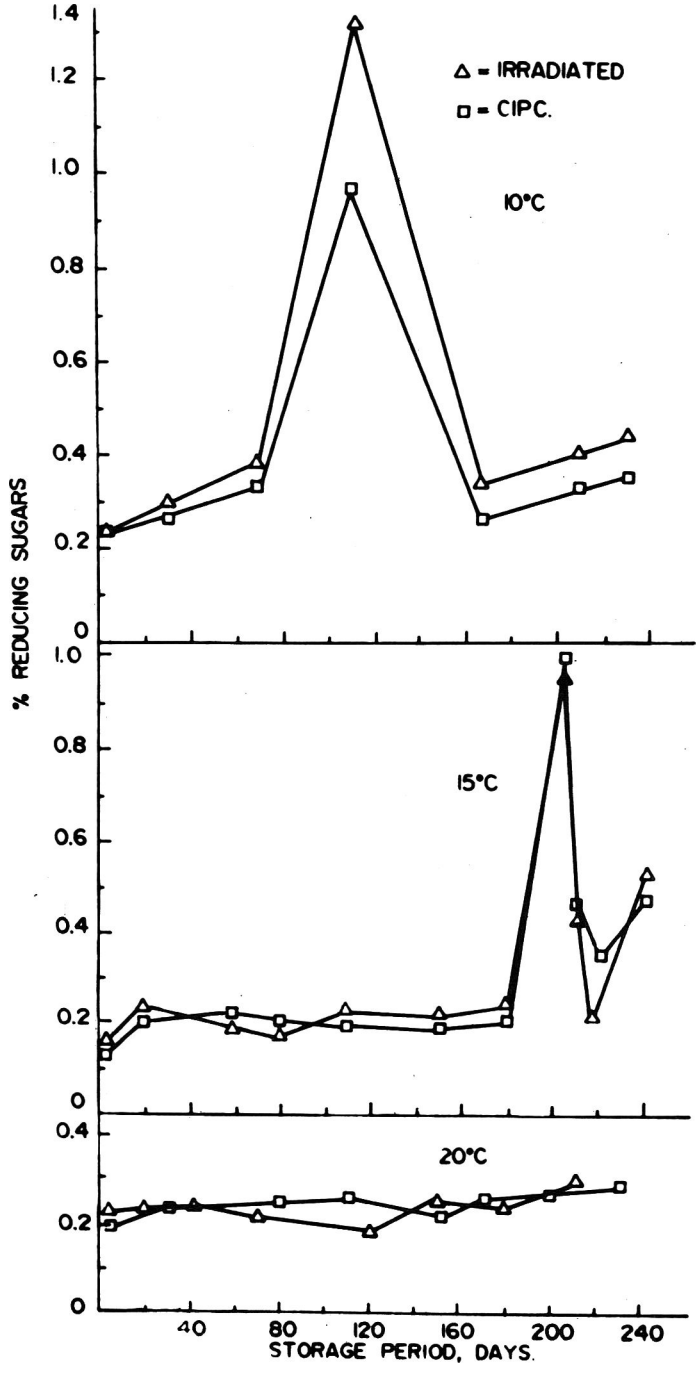

Fig. 2. Changes in the levels of reducing sugars in gamma irradiated and CIPC treated potato tubers of cultivar 'Gujrat-S'.

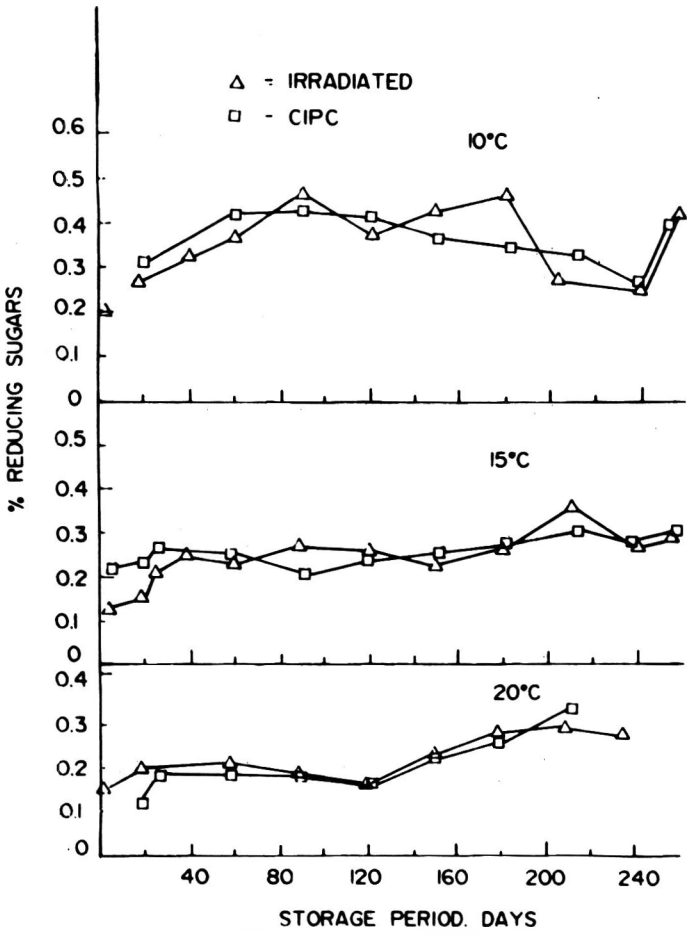

Fig. 3. Changes in the levels of reducing sugars in gamma irradiated and CIPC treated potato tubers of cultivar 'Kufri Jyoti'.

in 'K. Jyoti' 'K. Lavkar' 'Gujrat-S' and 'Talegaon' cultivars. Such tubers were found to be suitable for processing (data not shown). However, in case of 'K. Badshah', neither the reconditioning nor the blanching improved the chipping quality.

It has been reported that in cultivar 'Russet Burbank', grown in Canada (Borsa et al. 1989), and in 'Kennebec', 'Wu Foon' and 'Cardinal', grown in Taiwan (Liu et al. 1990), the irradiation followed by extended storage at 10°C did not affect their chipping quality. The unsuitability for processing of 'K. Jyoti', 'K. Lavkar', 'Gujrat-S' and 'Talegaon' cultivars, due to increased formation of reducing sugars following irradiation and storage at 10°C, may be due to the differences in cultivars.

Darkening of potato chips on account of Maillard reaction is mostly determined by the reducing sugar contents of the tubers (Borsa et al. 1989; Roe and Faulks 1991), although sucrose may also contribute to chip colour (Leszkowiat et al. 1990) as it is an intermediate product of reducing sugars from starch (Isherwood 1976). Vacuum infiltration of tuber strips with 1 to 2% sucrose did not alter the colour of the chips and French fries in cultivars 'K. Jyoti', 'K. Lavkar', 'Gujarat-S' and 'Talegaon', while infiltration with 10% sucrose caused only slight darkening. On the other hand, infiltration with 1 and 2% glucose and fructose improved the colour of the French fries to an acceptable golden yellow colour. At 10% level, these sugars caused intense darkening of the French fries, maximum effect being with glucose. Thus, the infiltration studies with sugars corroborate the predominant role of glucose in the browning of chips and French fries than that of fructose or

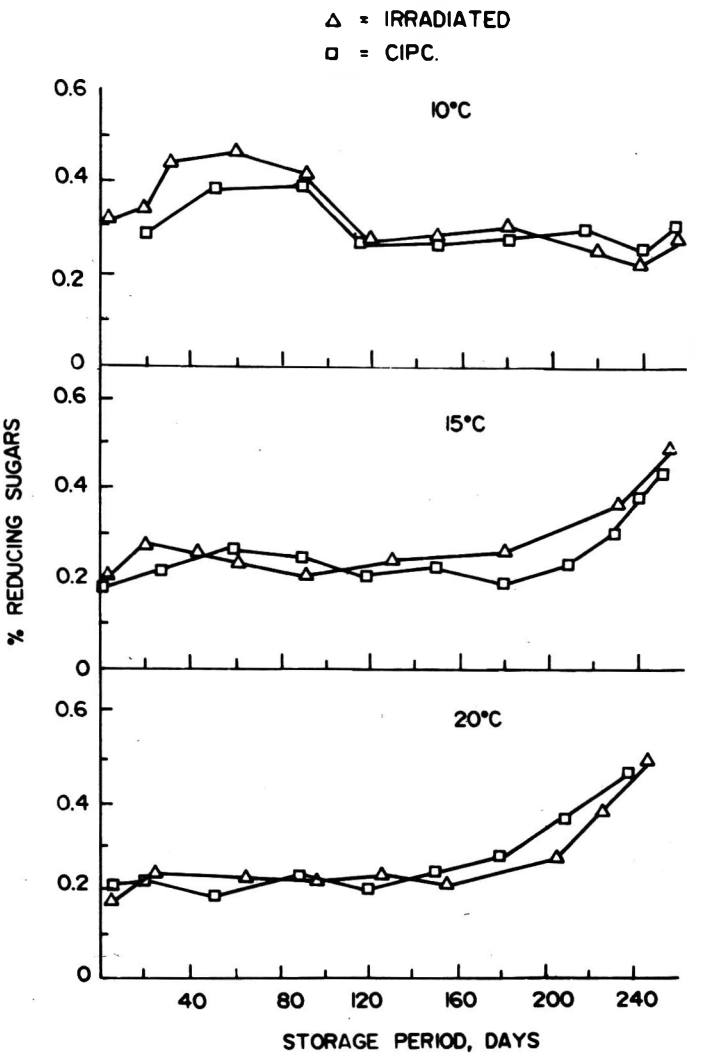

Fig. 4. Changes in the levels of reducing sugars in gamma irradiated and CIPC treated potato tubers of cultivar 'Kufri Lavkar'.

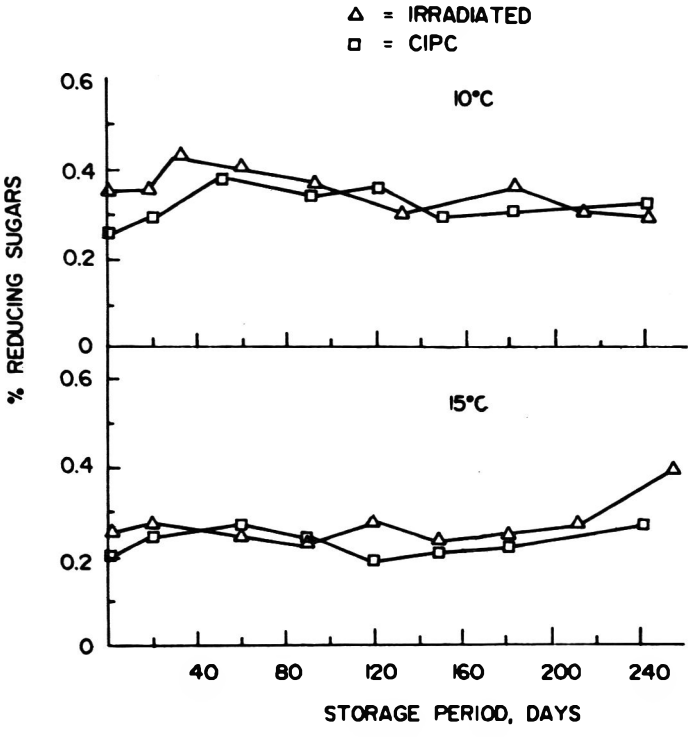

Fig. 5. Changes in the levels of reducing sugars in gamma irradiated and CIPC treated potato tubers of cultivar 'Talegaon'.

sucrose as reported by others (Roe and Faulks 1991).

In conclusion, this study demonstrates that gamma irradiation retained the processing qualities of potato cultivars during extended storage at 15°C and above.

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Effects of Different Levels of *Callosobruchus chinensis* L. Infestation on Proximate Principles, True Protein, Methionine and Uric Acid Contents of Greengram and Redgram

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The effects of insect infestation on proximate principles, calories, true protein, non-protein nitrogen, methionine and uric acid contents in greengram and redgram were studied. With the increase in level of infestation, a significant increase was observed in all parameters studied, except in case of calories, true protein and methionine contents. The values in latter cases decreased significantly. Increase in uric acid was manifold. Thus, the qualitative and quantitative damages to grains are related to the extent of infestations and such grains should be avoided for human consumption.

Keywords : Greengram, Redgram, Non-protein nitrogen, Calories, Methionine, True protein, Protein efficiency ratio, Feed efficiency ratio.

Legumes, which form a major constituent of diets of masses in developing countries, are rich sources of proteins. Greengram *(Vigna radiata* L Wilzeck) and redgram *(Cajanus cajan* L) are widely grown and consumed. These pulses are stored for about an year or until the harvest of next crop. During the period of storage, heavy loss is inflicted by insects. Among the insects, bruchides are the most important pests, affecting the stored legumes, both quantitatively and qualitatively.

Physico-chemical changes such as weight loss, decrease in true protein, methionine, calories, protein efficiency ratio (PER) and feed efficiency ratio (FER) are noticed in the bruchide infested pulses. (Daniel et al. 1977; Gupta et al. 1981, 1984; Shehnaz and Theophilus 1975). No detailed information is available on the effect of specific levels of infestation on proximate principles, energy, true protein, methionine, uric acid contents and also PER and FER of greengram and redgram. In this study, an attempt was made to see the impact of graded infestation levels on these parameters in greengram and redgram.

Materials and Methods

Greengram (*Vigna radiata* L) Wilzeck 'K-85' was procured from the Directorate of Farms of the University, while redgram *(Cajanus cajan)* was from local market.

Insect culture: The pulse beetles *(Callosobruchus chinensis* L) were obtained from the Department of Entomology of the University, in 500 g of chick pea in a container covered with muslin cloth. Then, the culture of C. *chinensis* was maintained at the temperature of 28 ± 2°C and relative humidity of 75±2%.

Sample preparation: Pulses were cleaned manually to get rid of dust and other foreign materials. Pulse samples, weighing 750 g each, were placed in triplicate, in plastic airtight containers of 2 kg capacity. In each container, 80 pulse beetles (3 to 4 days old) were released, covered with lids and kept for 28 days at room temperature (33°-40°C). C. *chinensis* usually completes its life cycle within 23 to 28 days, depending upon climatic conditions. Infestation in grains were first examined after an interval of 28 days, followed by observations after 15 days interval to attain the desired infestation level. The infestation levels of 10, 20, 30, 40, 50 and 60% were selected on the basis of examination of holes present in the grain. The grains, along with the containers, were deep-frozen for 72 h to kill the developing larvae/pupa and the adult insects. The insect excreta and frass were removed manually, and the samples were stored in the refrigerator till further analysis. The 1000 grains in triplicate were taken for each sample. The grains were examined for the presence of holes and % of damaged grains was calculated.

Embedded larvae (%) were determined by soaking 1000 grains, in triplicate, from each infestation level for overnight and then dissecting the grains with a sharp blade. About 100 g clean sample was ground in a Cyclone mill to pass through a 40 mesh sieve and stored in airtight

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polythene containers in refrigerator till further analysis.

Chemical analysis: Proximate principles were analysed according to standard methods (AOAC 1980). Energy value was determined by choromic oxide method (O'Sheer and Mayure 1962). The nitrogen content was estimated by micro-Kjeldahl method (AOAC 1980) and it was multiplied by a factor 6.25 to obtain crude protein values. The non-protein nitrogen (NPN) values were estimated according to the method of Kapoor et al (1975). The extraction of uric acid was done by the method of AOAC (1980) method for estimation by the method of O'Ser (1971). The fat-free samples were estimated for methionine by the methods of Gupta and Das (1954) and McCarthy and Palle (1959). The data were subjected to analysis of variance and correlation matrix (Snedecor and Cochran 1968).

Animal experiment: Ninety male albino 'Wistar, weanling rats, aged 21 days and weighing 32 ± 5 g were obtained from the germ-free small animal house of the University. These were divided into 9 groups. One control group was fed casein and other two groups were fed un-infested greengram and redgram, respectively. Remaining 6 groups were given each infested pulse at 20, 40 and 60% levels of infestation. Rats were caged individually in polypropylene metabolic cages and fed with weighed amounts of the feeds. Water was given *ad libitum* for 28 days. All the diets contained (g/100 g diet) sucrose 10, groundnut oil 10, mineral mixture 4, vitamin mixture 1, cellulose 5, choline chloride 0.02, in addition to specific amount of pulses and starch (Table 1). The composition of mineral and vitamin mixtures is as recommended by NAS (1972) committee. The ingredients were mixed thoroughly to ensure uniform distribution of vitamin and salt mixtures and passed through 70 mesh sieve. Feed efficiency ratio (FER) and protein efficiency ratio (PER) were determined by the method of Chapman et al (1959).

TABLE 1. PULSE AND STARCH CONTENTS OF THE DIETS (g/100 g DIET)

Ingredient	Pulse	Starch
Uninfested greengram	38.81	57.18
Infested greengram, %		
20	35.58	34.48
40	31.60	38.38
60	26.90	43.08
Uninfested redgram	51.07	18.91
Infested redgram, %		
20	41.40	28.58
40	33.80	36.18
60	30.30	39.68

Protein level in diet was adjusted at 10% control diet contained 12.8 g casein and no pulse

Results and Discussion

The weight of *C. chinensis* infested greengram and redgram increased upto the 20% level of infestation and then, there was significant decrease in the weight as the % infestation increased (Tables 2 and 3). The % larvae inside the grain increased significantly upto 30% level of infestation in both the pulses. The level started decreasing as the infestation % increased. Weight/volume ratio (density) first increased significantly

TABLE 2. PHYSICAL AND CHEMICAL CHANGES IN INSECT INFESTED GREENGRAM

Attributes	Level of infestation, %								
	0	10	20	30	40	50	60	SE (d)	CD (P∠0.05)
Weight, g	750.0	764.3	755.3	746.3	636.8	583.5	413.5	6.5	13.6
Embedded larvae, %	0.8	23.7	43.6	59.3	49.3	42.0	35.0	2.4	4.3
Density	1.3	1.4	1.4	1.3	1.3	1.2	1.1	1.01	0.03
Moisture, %	10.1	11.0	11.4	12.4	12.8	13.0	13.3	0.10	0.21
Ash, %	3.3	3.8	4.1	4.9	5.1	5.3	5.8	0.04	0.10
Crude protein, %	21.8	26.6	28.1	29.6	31.6	34.4	37.2	0.05	0.10
Crude fibre, %	3.9	4.2	5.4	6.0	6.8	7.1	7.6	0.08	0.16
Crude fat, %	1.3	1.4	1.7	1.8	2.0	2.2	2.3	0.02	0.04
Energy, Kcal	338.0	331.0	324.0	318.0	308.0	295.0	271.0	1.74	3.63
Non-protein nitrogen, %	0.02	0.2	0.9	1.9	2.4	3.1	3.9	0.001	0.002
True protein, %	21.6	24.1	22.7	17.6	16.9	14.8	12.8	0.12	0.24
Uric acid, mg %	1.6	613.7	1025.6	1333.3	1846.1	3359.0	4871.8	87.9	191.6
Methionine, mg %	305.4	289.7	269.7	249.8	232.9	217.9	196.8	4.16	9.06

The figures in table are mean by triplicate analysis.

TABLE 3. PHYSICAL AND CHEMICAL CHANGES IN INSECT INFESTED REDGRAM

Attributes	Level of infestation, %								
	0	10	20	30	40	50	60	SE (d)	CD (P∠0.05)
Weight, g	750.0	763.0	754.3	641.7	593.8	495.0	443.0	6.0	12.5
Embedded larvae, %	0.3	28.2	40.2	53.3	49.3	47.8	37.0	3.0	6.2
Density	1.4	1.6	1.8	1.6	1.4	1.3	1.2	0.03	0.05
Moisture, %	7.9	8.8	9.3	10.5	11.5	12.4	13.2	0.20	0.43
Ash, %	18.6	21.0	24.1	27.2	29.5	30.1	33.0	0.01	0.03
Crude protein, %	4.3	4.5	4.8	5.0	5.3	5.7	6.0	0.03	0.05
Crude fibre, %	4.9	5.1	5.7	6.0	6.1	6.8	7.2	0.04	0.09
Crude fat, %	1.8	2.1	2.3	2.6	2.9	3.1	3.3	0.03	0.06
Energy, Kcal	358.0	354.0	346.0	322.0	312.0	297.0	295.0	0.4	0.8
Non-protein nitrogen, %	0.01	0.4	1.0	1.7	2.4	2.6	3.3	0.001	0.002
True protein, %	19.5	18.4	20.0	17.1	14.3	13.7	12.3	0.5	1.1
Uric acid, mg %	0.9	352.0	782.0	1141.0	1525.6	2910.2	3653.8	20.7	45.2
Methionine, mg %	281.3	261.9	233.9	214.1	177.6	148.2	119.2	4.2	9.1

The figures in table are mean by triplicate analysis.

and then started decreasing (Tables 2 and 3). The changes in the embedded larvae, density and initial increase in weight might be due to presence of insect larvae and pupal stages within grains. As the number of holes increased, there was loss in the above attributes probably due to consumption of endosperm portion of the grain by insects. Similar results have been reported by other workers (Vir and Jindal 1981; Gupta et al. 1984b; Singh and Sharma 1981; Srivastava et al. 1988; Vimala and Pushpamma 1983). Damage was more in greengram than in redgram, probably due to small size of the former.

Data presented in Tables 2 and 3 show significant changes in proximate composition with the increase in the level of infestation. Maximum changes in these constituents were observed in 60% infested grains. Increase in moisture was observed and can be attributed to increase in insect population, insect excreta and metabolic activity of the insects. Similar results have been reported earlier by other workers (Daniel et al. 1977; Gupta et al. 1981; Shehnaz and Theophillus 1975; Swaminathan 1977). Increases in ash, crude protein, fat and crude fibre contents might be due to the fact that insect consumed mostly endosperm portion of legumes, thereby leaving behind the husk which is rich in these constituents (Gupta et al. 1984b; Khurb 1981; Shehnaz and Theophillus 1975). Increase in crude protein (Tables 2 and 3) is probably due to increase in uric acid, NPN and also due to presence of insects, insect body fragments and body parts inside the grains. As is evident from the data in Tables 2 and 3, significant decrease in calorific value of pulses occurred as the level of infestation increased. Maximum loss was observed in 60% infested grains. The decrease can be attributed to consumption of endosperm by insects, which contributes major portion of the pulses.

NPN and uric acid contents increased, while true protein and methionine contents decreased significantly in both the pulses with the increase in the level of infestation (Tables 2 and 3). The significant increase in the non-protein nitrogen content might be due to the presence of insect excreta and the insect body fragments within the grain. The increase in NPN was more in greengram than redgram. Similar reports by other workers are also available (Shehnaz and Theophillus 1975; Pingale et al. 1954; Khurb 1981; Srivastava et al. 1988). The increase in crude protein was found to be significant (Tables 2 and 3). This may be due to increase in non-protein nitrogen contributed by the insect excreta and body fragments. Bruchides have a tendency of consuming carbohydrate-rich endosperm and as a result, the protein-rich bran is left behind (Shehnaz and Theophillus 1975; Gupta et al. 1984b). This also is responsible for crude protein increase. True protein decreased with increase in the level of infestation. The decrease may be due to higher NPN substances produced by insects and contribution of these in the estimated values of crude protein and NPN. The negligible quantities of NPN and uric acid, found in control samples of legumes, increased significantly ($P<0.05$) as the infestation level increased (Tables 2 and 3). Manifold increase was found in uric acid in 60% infested grains. It is worth noting that the uric acid is one of the end products of protein metabolism in insects (Pixton 1965; Swaminathan 1977).

TABLE 4. EFFECT OF INSECT INFESTATION ON PER AND FER OF GREENGRAM AND REDGRAM

Attributes	Diets						
	Casein	Uninfested pulse	Infested (%)			SE (d)	CD (P∠0.05)
			20	40	60		
Greengram							
Feed consumed, g	148.4±3.5	136.0±3.9	134.5±2.7	123.3±5.1	149.8±3.8	6.2	12.4
Protein consumed, g	14.8±0.3	13.6±0.4	13.5±0.3	12.3±0.5	15.0±0.4	0.6	1.2
Weight gain, g	52.1±6.0	37.9±4.9	3.6±0.8	3.5±0.3	1.3±0.2	0.5	1.1
PER	3.5±0.4	2.8±0.5	0.4±0.2	0.3±0.1	0.2±0.1	0.2	0.3
FER	0.4±0.04	0.3±0.05	0.04±0.01	0.03±0.01	0.02±0.01	0.02	0.04
Redgram							
Feed consumed, g	148.4±3.5	176.4±4.5	154.6±5.6	155.8±4.8	158.2±3.4	3.4	7.6
Protein consumed, g	14.8±0.1	17.6±0.5	15.5±0.6	15.6±0.5	15.8±0.3	0.4	0.8
Weight gain, g	52.1±6.0	35.0±4.3	30.3±3.8	20.1±4.8	17.1±5.1	1.4	2.8
PER	3.5±0.4	3.0±0.4	2.0±0.2	1.3±0.2	1.1±0.1	0.1	0.2
FER	0.4±0.04	0.3±0.05	0.02±0.02	0.01±0.02	0.01±0.01	0.01	0.02

Values in table are mean ± SD of observation of 10 rats

The insect population was higher at higher levels of infestation, which increased the uric acid and non-protein nitrogen contents of pulses. The increased uric acid and total nitrogen contents of pulses, stored for varied periods, have also been reported by Rajan et al (1975), Gupta et al (1984a, b), Shehnaz and Theophillus (1975), Pingale et al (1954) and Daniel et al (1977). Uric acid content was also considered as an index of the insect population density of the legumes by Gupta et al (1984b). Uric acid content of pulses is also well related to kernel damage (Swaminathan 1977). The significant increments in the uric acid levels from 10 to 60% infestation levels are of significant importance from the hygienic, nutrition and acceptability points of view and also the gout causing potential of the infested legumes (Swaminathan 1977; Passmore and Eastwood 1987).

Decrease in methionine content at all levels of infestation (Tables 2 and 3) was significant. In greengram, the methionine loss was upto 35.7% as against 57.6% in redgram. Methionine is one of the ten amino acids required by insects (Gilmour 1961). The decrease in methionine may be related to its consumption by insects for their growth and population build up. For the vegetarians who obtain all their proteins from cereals and legumes, the implication of methionine reduction in the methionine deficient nature of the legumes is of significance and may result in amino acid imbalance.

With increase in level of infestation, FER and PER decreased significantly (Table 4). Decrease was higher in greengram as compared to redgram at 60% level of infestation. Weight gain also decreased with increase in level of infestation, the increase being only 1.33 g in greengram and 17.05 g in redgram after 28 days. The poor growth rate might be due to poor quality of infested legumes. Similar results have been reported by Shehnaz and Theophillus (1975) in *Bengal* gram and field bean infested diets fed to rats. The decreases in FER and PER might be due to poor quality of proteins in insect infested grains, as the NPN and uric acid contents were higher in insect infested grains. Similar results have also been reported by Khurb (1981).

There was significant negative correlation between level of infestation and weight, density, true protein, energy and methionine (Table 5). All other parameters, like moisture, ash, crude protein,

TABLE 5. CORRELATION MATRIX BETWEEN LEVEL OF INFESTATION AND DIFFERENT PARAMETERS

Attributes	Correlation	
	Greengram	Redgram
Weight	- 0.879	- 0.883
% Embedded larvae	0.674	0.582
Density	- 0.817	- 0.821
Moisture	0.965	0.990
Ash	0.986	0.990
Crude protein	- 0.980	0.990
Crude fibre	0.987	0.990
Energy	- 0.926	- 0.995
Crude fat	0.989	0.997
Non-protein nitrogen	0.995	0.994
True protein	- 0.987	- 0.977
Methionine	- 0.999	- 0.995
Uric acid	0.965	0.937

crude fat, NPN and uric acid showed highly significant positive correlation (Table 5).

Different levels of infestation of stored greengram and redgram resulted in both quantitative and qualitative losses. The stored pulses also became unhygienic due to the presence of insect excreta. The nutritive value is reduced and the presence of uric acid in higher amounts might induce gout in the consumers. Therefore, consumption of grains infested at higher levels must be discouraged.

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Distribution Pattern of Bittering Principles in Kinnow Fruit

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Analysis of different parts of kinnow fruit indicated that the naringin content was the highest (0.422 mg/g) in peel, followed by juice (0.230 mg/ml) and seed (0.134 mg/g). In contrast, the highest limonin content was found in seed (9.52 mg/g), followed by peel (4.69 mg/g) and juice (0.218 mg/ml). The juice stored at 25°C developed full bitterness at 4 h as against the time of 7 h for juice stored at 12.5°C.

Keywords : Kinnow, Limonin, Naringin, Distribution pattern, Bitterness.

Kinnow, a hybrid of king and willow leaf mandarins (*C. nobilis* x *C. deliciosa*), is known for its superior characters such as heavy bearing, wide adaptability and fruit quality (Jawanda 1978), though it has a shorter shelf-life (Jawanda and Singh 1973). A considerable research work to enhance the storage life of the fruit has been reported (Singh 1981; Nagar 1993). However, the information on processing of kinnow mandarins is scanty (Renote and Bains 1982; Sandhu et al. 1990). Some studies have been done on the physico-chemical changes during storage (Sandhu et al. 1985) and also on thermal processing (Nath and Ranganna 1977) and its utilisation (Pruthi et al. 1984). Cultivation of kinnow has assumed a considerable significance during recent years in Northern India (Sandhu et al. 1990), thereby demanding necessary steps to develop technology for its processing. The major problem with the acceptability of the kinnow juice is the rapid development of bitterness in the juice. Limonin and naringin (naringenin 7-neohesperidose) have been identified as the principles causing bitterness in kinnow (Barwal 1984). However, the information on their total contents and distribution in different parts of the fruit is not available. Therefore, determination of the limonin and naringin contents in different parts of kinnow fruit (peel, seed and juice) and time required to develop full bitterness in the extracted juice are essential pre-requisites to citrus processors. Investigations carried out on these aspects are discussed in this communication.

Kinnow fruits were harvested during mid-January, 1990 from a local orchard in Kangra district (Himachal Pradesh). The juice was extracted manually by squeezing. The seeds were separated and washed to remove any juice adhering to them. Samples of fresh peel, along with attached membranes, were macerated in a Waring blender for 5 min. Seeds were dried for 5-6 h in a dehydrator at a temperature of 60 ± 1°C and finely crushed using a mortar and pestle. The limonin extract was obtained from the samples by overnight extraction with chloroform and the samples were analysed by the colorimetric method of Vaks and Lifshitz (1981), whereas, naringin content was estimated by the method of Davis (1947).

A freshly extracted juice was stored at room temperature (12±1°C) and controlled temperature (25±1°C) for 10 h, The samples were drawn for the estimation of limonin and naringin at an interval of 60 min.

The results showed a wide variation in distribution of limonin and naringin contents in various parts of the fruit. The seeds contained the highest limonin (9.50 mg/g), followed by peel (4.69 mg/g) and juice (0.218 mg/ml). Gaonkar and Bamzi (1989) have also reported similar findings in case of limonin contents in grapefruit, oranges, lime and lemon. The highest naringin content was in peel (0.420 mg/g), followed by juice (0.230 mg/ml) and seeds (0.134 mg/g). Higher naringin content in the juice might be due to its water solubility and consequent extraction in juice. Similar trend has been reported earlier for other citrus fruits (Yusof et al. 1990). However, the naringin content in skin of rough lime was more than that in the juice and seed (Yusof et al. 1990). The respective values of limonin in different parts of kinnow were quite comparable with those in grape fruit seeds, whereas naringin content was considerably lower in kinnow, than in other citrus species (Yusof et al. 1990). A comparison of limonin content of kinnow with its threshold values (Guadagni et al. 1976) indicated that the

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kinnow fruit had considerably higher values for limonin. The bitterness of juice is, therefore, intolerably high and juice needs to be debittered to make it acceptable.

The data (Fig. 1) show that the development

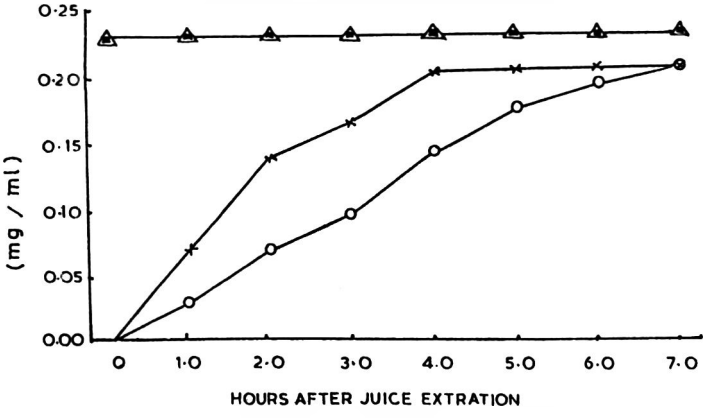

Fig. 1. Changes in bittering principles of kinnow juice held at 12.5 and 25.0°C. O——O Limonin at room temp. (12.5±1°C), X——X Limonin at controlled temp. (25±1°C), ■——■ Naringin at room temp. (12.5±1°C), Δ——Δ Naringin at controlled Temp. (25±1°C).

of bitterness, i.e. the conversion of limonoate-A-ring lactone (non-bitter precursor of limonin) to limonin get completed within 7 h of juice extraction at room temperature (12 ± 1°C), whereas it took 4 h after juice extraction at 25 ± 1°C. However, no change in naringin content could be observed with time and temperature. Similar result has also been reported earlier (Yusof et al. 1990). Therefore, it could be concluded that the development of bitterness in kinnow juice was influenced by the temperature at which juice is held, as has also been emphasized in earlier studies (Emerson 1948; Chandler and Kefford 1966). Since the limonin content of the juice is influenced by temperature, the development of bitterness cannot be prevented, but could be delayed to some extent by appropriately selecting the temperature of storage. The processors could definitely make use of the time to employ suitable debittering technique before pasteurizing the kinnow juice. In an earlier study, heat processing of the juice was reported to result in the development of bitterness in kinnow (Renote and Bains 1982). Our results are, however, contrary to these findings, as bitterness was found to develop even without heat processing. Thus, it could be stated that bitterness develops whether the juice was heat processed or not and suitable debittering techniques have to be applied to make the juice acceptable from sensory quality point of view.

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Biochemical and Enzymatic Changes in Apple During Cold Storage

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Biochemical and enzymatic changes in apple during cold storage were studied. Total soluble solids, total sugars and soluble protein contents increased upto 150 days of storage and thereafter declined. In contrast, titratable acidity, total phenols and pectin contents followed a linear declining trend throughout the storage period of 7 months. The activity of polygalacturonase and cellulase increased upto 150 days of storage, thereby leading to softening of apple. The activities of these enzymes declined thereafter.

Keywords : Apple, Polygalacturonase, Cellulase, Cold storage, Biochemical changes, Enzymatic changes, Storage behaviour.

It is generally agreed that fruit ripening is a biologically active process, often involving high metabolic activity, increased activity of some enzyme systems and ultimate deterioration of fleshy organs (Looney 1970). As soon as fruit is harvested, it is left to survive on its own as the metabolites, being utilized to maintain its physiological processes, are not replenished any more. Besides, once they are infected with *Aspergillus niger*, these induce a variety of biochemical changes (Massand and Chhatpar, 1980). Earlier studies on storage behaviour showed significant relationship between fruit maturity and sensory quality (Krishna Prakash et al. 1985). In the present investigation, an attempt has been made to determine the pattern of biochemical and enzymatic changes in apple fruit during cold storage, with a view to study its storage behaviour.

'Red delicious' apple fruits were harvested at proper maturity (130 days after full bloom), packed in corrugated fibre board cartons and stored in walk-in-cold chamber, maintained at 0 ± 1°C and 90-95% relative humidity. The experiment was laid out in completely randomized design with three replications and each replication comprised of 10 kg fruits. Ten fruits from each replication were drawn at 90, 150, 180 and 210 days of storage and analysed for various biochemical constituents and enzymatic activities. Waxol dipping of apple is known to extend the storage without much loss in sensory quality (Habibunnisa et al. 1988). These studies were carried out continuously for two years (1989-90 and 1990-91). As the trend of results was consistent during both the years, the data of only a single year are presented.

The total soluble solids (TSS) of fruit juice were recorded on the direct reading digital refractometer with auto-temperature correction (Atago-DBX-50, SOMSO Company Ltd., Tokyo, Japan). Total sugars and titratable acidity (as malic acid) were determined by standard procedure (AOAC 1980). Soluble protein, total phenols and pectin contents were determined as per the methods adopted by other workers (Lowry et al. 1951; Bray and Thorpe 1954; Ruck 1963). The activities of polygalacturonase (PG) and cellulase were studied as per the recommended methods (Mahadevan and Sridhar 1982; Abeles and Takeda 1990).

The data on the various biochemical constituents and enzymatic activities are presented in Table 1. It is seen that the TSS and total sugars, which were fairly low at harvest (13.48 °Brix, 8.90%), increased as the storage period advanced. These reached a peak value at 150 days of storage (16.20 °Brix and 12.58%), and declined thereafter. The increase in TSS and total sugars upto 150 days of storage may be due to hydrolysis of starch into sugars. On completion of hydrolysis of starch, the further increase in TSS/sugars did not occur. Hence, decline in these attributes is predictable (Smith et al. 1979).

A declining trend in the titratable acidity is rather sharp in the beginning with the advance of storage period. It decreased gradually thereafter. The titratable acidity varied from 0.25 to 0.05% during storage. A decrease in titratable acidity during ripening and storage could be attributed to an increase in malic enzyme and pyruvate decarboxylation reactions during the climacteric period (Wills et al. 1980). Apple fruit cells have been reported to use organic acids, principally malic

TABLE 1. BIOCHEMICAL AND ENZYMATIC CHANGES IN APPLE DURING STORAGE

Description	At harvest	Storage				
		90	150	180	210	CD at 5%
TSS, °B	13.48	15.60	16.20	12.33	10.94	0.13
Total sugars, %	8.90	11.5	12.58	9.63	8.70	0.12
Titratable acidity, %	0.25	0.16	0.10	0.07	0.05	0.02
Soluble protein, mg/g	2.25	2.61	2.95	1.20	1.05	0.15
Total phenols, µg/g	89.35	65.90	38.00	31.00	27.50	3.15
Pectin, as % Ca-pectate	0.71	0.50	0.40	0.31	0.21	0.03
Polygalacturonase*	16.41	24.82	31.08	21.90	17.30	0.60
Cellulase*	11.40	12.40	13.06	9.15	8.00	0.51

Mean values of three replicates. * Unit definition as per Mahadevan and Sridhar (1982) and Abeles and Takeda (1990).

acid, as respiratory substrate during ripening and storage (Rhodes et al.1968).

The soluble protein contents were lower at the time of harvest (2.25 mg/g) and increased gradually (2.95 mg/g) with the increase in storage period upto 150 days. Thereafter, a sharp decline ensued, recording 1.05 mg/g soluble protein at the end of 7 months storage. The synthesis of protein during ripening might be due to an increase in malic enzyme activity in apple fruits (Ulrich 1974). More direct evidence for the involvement of protein synthesis in the ripening of fruits is provided by the preferential incorporation of radioactive amino acids into certain proteins during early climacteric in apple (Dilley 1962).

A continuous decline in the total phenolics, with the advance in storage period was observed. The maximum level of total phenols (89.35 µg/g) was recorded after harvest, which reduced to 27.50 µg/g at the end of storage period studied. It has been reported that the concentration of phenolics decreased during storage of fruits (Hulme et al. 1971), thereby making the fruit susceptible to pathological breakdown (Nalubizon 1976).

There was a gradual linear decline in pectin content with the advance in storage period. The pectin contents ranged between 0.71 and 0.21% from harvest upto 210 days of storage. The decline in pectin content during storage is due to conversion of insoluble protopectin into soluble pectin (Hulme 1958). Polygalacturonase and cellulase activities of apple fruits were quite low (16.41 and 11.40% loss in viscosity) at the commencement of sampling (Table 1). These increased with the storage period upto 150 days (31.08 and 13.06%) and then declined at the end of storage period studied. The activities of these enzymes have also been reported to be low or absent in unripe fruits, but these increased during ripening (Bartley 1978). The cell wall bound polygalacturonase is known to be involved in the solublization of pectins (Rhodes 1980). Similarly, cellulases are considered to be responsible for the hydrolysis of cellulose fibrils of the cell wall (Babbitt et al. 1973). These ultimately lead to softening of the fruits.

It may be concluded that the apple fruits remained in good condition upto 150 days of storage at 0 ± 1°C and 90-95% relative humidity. Thereafter, the fruits started becoming soft and mealy due to metabolic degradation processes, such as abrupt fall in the level of various biochemical constituents and decline in enzymatic activities.

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Physical Characteristics and Composition of Certain New Varieties of Soybeans

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Six new varieties of soybean showed 7.3-8.6% hull, 0.69-0.74 g/cc bulk density, 1.05-1.18 g/cc true density, 94.3-145.6 g 1000 grain weight and 5.6-7.6 kg hardness in vertical position and 13.0-19.8 kg in horizontal position. Colour of varieties ranged from light yellow to golden yellow. The varieties contained (%) 34.3-40.7 protein, 16.5-18.3 fat, 4.1-4.6 crude fibre, 4.5-5.3 total ash and 20.0-25.4 carbohydrates (by difference). The other components include (mg/100 g seeds) 247.4-307.7 calcium, 496.1-540.9 phosphorus, 10.3-13.4 iron and 575-720 total phenols, in addition to trypsin inhibitor activity of 21.1-25.2 TUI/mg sample.

Keywords : Soybeans, Physical characteristics, Chemical composition, Minerals, Phenols.

Soybean varieties differ in their physico-chemical characteristics and these variations influence the quality characteristics of products prepared from the seeds (Snyder and Kwon 1987). The suitability of soybean varieties for preparation of different soy foods in countries like USA, Japan and China has been investigated extensively (Smith et al. 1960; Wang et al. 1983). Though soybeans were introduced in India during mid sixties, information on quality characteristics of Indian varieties is limited. A number of soybean varieties have been developed by the plant breeders in the country during the past few years and a few varieties have been studied for their chemical composition and cooking characteristics (Smita and Vaishali 1989; Om Kumar et al. 1992). These varieties differ in their agronomic requirements and resistance against yellow mosaic virus. The present communication reports physico-chemical characteristics of the grains of six new varieties of soybeans. Such information would be useful in selecting soybean varieties for desired end uses, such as oil extraction, manufacture of soy milk and tofu.

Dry mature seeds of soybean varieties 'PK-262', 'PK-416', 'PK-471', 'PK-472', 'PK-515' and 'PK-564', grown during the *khariff* season, 1990-91, were procured from Crop Research Centre of the university. The beans were cleaned and stored in air tight containers at ambient temperature (20-25°C) until use. Hull content of each variety was determined by dehulling one kg grains in a dehuller, designed and fabricated by Post-harvest Process and Food Engineering Department of the university (Agrawal 1974). Hardness was measured in vertical and horizontal positions of soybean grain, using a Hardness Tester (Kiya Seisakusho, Japan). Bulk and true densities were determined by the method of Bhattacharya et al(1972), while colour was observed visually. Thousand grain weight was found out by weighing 1000 sound grains of each variety.

Moisture, fat, ash, crude fibre and calcium contents of whole soybeans were estimated by standard procedures (AACC 1976). Protein content was determined by Kjeldahl method (AOAC 1984), using a protein conversion factor of 6.25. Phosphorus, iron and phenol were determined by colorimetric methods as described by Ranganna (1986), while trypsin inhibitor activity was measured by the method of Kakade et al (1974). Statistical analysis of data was done as per Snedecor and Cochran (1967).

The hull contents of varieties ranged from 7.3 to 8.6% (Table 1). Varieties 'PK-515' and 'PK-472' exhibited the maximum and minimum hull contents, respectively. These varieties would, therefore, yield minimum and maximum amounts of soy dhal, respectively upon dehulling of soybeans. The force required to crush the grains in horizontal position (line of cleavage) was more than double as compared to that required in vertical position (perpendicular to the line of cleavage). Variety 'PK-262 'required maximum force in horizontal as well as vertical position, whereas variety 'PK-472' required minimum force to crush the grains in vertical position. Hardness of soybeans was found to be significantly affected by the protein content of the grains. A

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positive correlation of 0.8199 was observed between protein content of whole soybeans and grain hardness (vertical position), whereas correlation between hardness in horizontal position and protein content was 0.9276. The bulk and true densities of different varieties did not differ significantly. Colour of seed coat of different varieties was yellow to golden yellow. Thousand grain weight of varieties ranged from 94.3 to 145.6 g. Varieties 'PK-472' and 'PK-515' exhibited maximum and minimum grain weights, respectively. The values of the grain hardness, densities, colour and 1000 grain weight were similar to those observed by Jain (1985) for different Indian varieties.

Moisture content of different varieties (Table 1) ranged from 9.8 to 12.7%, which was greater than the value for 'Bragg' variety reported earlier (Gupta et al. 1976) Protein and fat contents in varieties were 34.3-40.7% and 16.5-18.3%, respectively. Varieties 'PK-262','PK-515' and 'PK-564' contained equal and maximum protein contents. Varieties 'PK-416' and 'PK-472' exhibited maximum fat contents and were, therefore, more suitable for oil extraction. Crude fibre contents of varieties 'PK-416' and 'PK-515' were significantly lesser than other varieties. Total ash contents in varieties were 4.5-5.3%, while varieties 'PK-471', 'PK-515' and 'PK-564' showed almost equal quantities. The carbohydrates in different varieties varied between 20.0 and 25.4%, while varieties 'PK-472' and 'PK-564' exhibited maximum and minimum contents, respectively. The variations in carbohydrate contents among different varieties are attributed to differences in the contents of other constituents. All the varieties, except 'PK-416', contained greater amounts of protein than 'Bragg' variety (Kapoor et al. 1975). These varieties contained lesser amounts of crude fat, ash and carbohydrates, but greater amounts of crude fibre than 'Bragg' variety. The values of proximate constituents observed in the present study are in conformity with data of other Indian varieties (Deodhar et al. 1973; Sood et al. 1977; Jain 1985).

Soybean varieties exhibited wide variations in the mineral contents (Table 1). Varieties 'PK-262' and 'PK-564' contained significantly greater amounts of calcium than other varieties. Variety 'PK-515' contained the highest amounts of phosphorus. Gupta et al (1976) reported 0.39 and 0.57% calcium and phosphorus, respectively in 'Bragg' variety of soybean. The values of calcium and phosphorus contents for different varieties observed in the present study are lower than those for 'Bragg' variety. The iron contents in different varieties ranged from 10.3 to 13.4 mg/100 g. Varieties 'PK-472' and 'PK-262 'contained

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF DIFFERENT VARIETIES OF SOYBEANS

Characteristics	Varieties					
	'PK-262'	'PK-416'	'PK-471'	'PK-472'	'PK-515'	'PK-564
Hull content, %	8.1	8.0	8.4	7.3	8.6	8.2
Grain hardness, kg						
Vertical position	7.6	5.8	6.2	5.6	7.5	7.2
Horizontal position	19.8	13.0	17.8	14.5	18.0	17.0
Bulk density, g/cc	0.71	0.70	0.69	0.74	0.71	0.70
True density, g/cc	1.11	1.05	1.05	1.11	1.18	1.18
1000 grain weight, g	118.4	133.8	117.2	145.6	94.3	110.2
Colour	Golden yellow	Light yellow	Light yellow	Light yellow	Light golden yellow	Light golden yellow
Moisture, %	10.1^{e}	12.7^{a}	9.8^{c}	11.4^{b}	10.4^{c}	11.8^{b}
Protein, %	40.7^{a}	34.3^{d}	39.4^{b}	36.1^{c}	40.7^{a}	40.7^{a}
Crude fat, %	17.6^{c}	18.3^{a}	17.5^{c}	18.2^{ab}	16.5^{d}	17.7^{bc}
Crude fibre, %	4.6^{a}	4.3^{bc}	4.4^{ab}	4.4^{ab}	4.1^{c}	4.6^{a}
Ash, %	4.5^{c}	5.0^{b}	5.3^{a}	4.6^{c}	5.2^{ab}	5.2^{ab}
Carbohydrates (by difference), %	22.5	25.4	23.6	25.3	23.1	20.0
Calcium, mg/100g	307.7^{a}	247.4^{c}	265.5^{b}	253.4^{bc}	259.4^{bc}	295.6^{a}
Phosphorus, mg/100 g	511.3^{c}	516.2^{c}	532.8^{b}	496.1^{c}	540.9^{a}	504.0^{d}
Iron, mg/100 g	13.4^{a}	10.5^{c}	10.5^{c}	10.3^{c}	12.7^{b}	13.2^{a}
Phenols, mg/100 g	575^{f}	687^{b}	655^{c}	720^{a}	636^{d}	602^{c}
Typsin inhibitor activity, TUI/mg	21.1^{d}	24.2^{b}	23.9^{bc}	23.6^{c}	25.2^{a}	24.8^{a}

* Means in each row followed by different letters differ significantly at 5% level.

the minimum and maximum amounts of iron, respectively. These values were higher than those reported for 'Bragg' variety (Gupta et al. 1976). There were significant differences in the contents of total phenols among different varieties. Ramakrishna (1986) observed a broader range of values (0.4-0.7%) than those observed in the present study. Trypsin inhibitor activity values of different varieties were 21.1 to 25.2 TUI/mg of soybeans. Varieties 'PK-515' and 'PK-262' exhibited maximum and minimum trypsin inhibitor activity, respectively. Jain (1985) has also reported similar values for different Indian varieties.

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Fungal Population Associated with Raw Materials and Intermediate Products of Lager Beer Produced from Nigerian Sorghum Grains

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Fungi were enumerated on potato dextrose agar, malt extract agar, Czapek Dox agar and wort agar during the processing of sorghum for the production of lager beer. Isolates were purified and characterized. *Aspergillus* sp., *Rhizopus* sp., *Mucor* sp. and *Alternaria* sp dominated the populations on malt extract agar, while *Penicillium* sp, *Curvularia* sp. *Dreschlera* sp. and *Neurospora* sp formed the rest of the populations.

Keywords : Fungal population, Lager beer from Nigeria, Sorghum grain, Hop pellet, Yeast inoculum, Intermediate and final products.

Manufacture of beer is one of the important industries in Nigeria. With increase in population, slow economic growth and development, there is a need to harness locally produced food materials for feeding the expanding brewing industries. This will reduce import of barley, thereby saving the much needed foreign exchange earnings. Sorghum has been accepted as a cereal, suitable for producing African beer (Okafor and Anichie 1987) and is widely used as an adjunct with other cereals for the production of sparkling and clear lager beer (Anichie 1982; Dhamija and Singh 1978).

It is well known that, during food processing, a wide variety of microorganisms are found as contaminants on raw materials, intermediates and final food products and is often controlled by fumigation (Raghunathan et al. 1974). Some of these microbes have been reported to cause spoilage of foods or are pathogenic to consumers. In Nigeria, the sorghum cultivation is confined to Nothern parts and consequently, most brewing industries buy sorghum from different sources and markets. This, in turn, increases the probability of the grains carrying different microbes and it becomes necessary to conduct quality tests on the raw materials. The research investigations to compliment the routine tests can throw more light on the source and identity of the microbes not only in the raw materials but also in intermediates and final products. Knowledge on the microbes may lead to understanding the origin and ecology of the population and possible effective ways of controlling them. This investigation, therefore, seeks to find the identity of fungal population during the processing of sorghum for lager beer production and discusses their role in causing health hazards.

Raw materials : Sorghum (*Sorghum bicolor* L. Moench) a 'local red Nigerian' variety, was obtained from Oba market, Akure, Nigeria. Hop pellets and yeast were supplied by Bendel Breweries, Benincity, Nigeria and Nigerian Breweries Limited Lagos, Nigeria, respectively.

Malting : The modified method of Skinner (1976) was used for beer making. Sorghum grains (400 g) were steeped in tap water for 16 h at 28°C and spread on a previously washed concrete floor. The grains were turned and sprinkled with water three times daily at 8 h interval for 5 days at a temperature between 25-35°C. Malting was stopped, when the plumule and radicle length measured about 3.8 cm. Malting was checked by chewing few grains on each successive day, for a sweet taste development (usually the fifth day). The germinated grains were separated and oven dried at 50°C. Roots and shoots were removed by gentle brushing, weighed to determine the malting loss (Kumar et al. 1991; Chandrashekar and Desikachar 1983). Malted sorghum was ground fairly coarse in a blender mill to obtain a grist and later the grains were sieved.

Mashing : An intensive mashing with three stage decoction system was used. The ground, malted sorghum (240 g) was stirred with water (860 ml). The mash was maintained at 35°C for 30 min in a water bath with constant stirring and then allowed to rest for 5 min. The temperature was

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increased gently to 52°C and then allowed to rest for another 20 min at this temperature. One-third of the mash was taken and heated to 72°C and allowed to rest for 20 min, before it was returned to the main mash. This diversion process was repeated three times.

Saccharification test : Conversion of starch to sugar was tested by addition of few drops of 0.2 N iodine solution to the mash sample. The mash was then boiled for 10 min to inactivate the enzyme. After 20 min, it was filtered to obtain the sweet wort. The resulting sweet wort was heated to boiling for 1.5 h, before adding hop pellets (1.5 g) and HCl (0.2 ml). Then, it was cooled for 30 min and filtered.

Pitching of the wort : With the addition of *Saccharomyces carlsbergensis*, the filtered wort was incubated at 5°C, during which it was agitated at 30 min interval for a total of 4 h. Then, it was incubated at 10°C.

Primary fermentation : The yeast in the decanted mixture contained alcohol and sugar. After two weeks, the matured beer was filtered through Kiselghur and Whatman No. 1 filter paper. Sugar and dried yeast were added to the beer and allowed to stand for 1h.

Bottling and pasteurization : The matured filtered beer was poured into sterile clean green bottles, corked and pasteurized at 68°C.

Isolation and identification of fungi : Sorghum seeds used for the production were surface sterilized in 1% sodium hypochlorite solution and then washed in 5 changes of sterile distilled water, before plating out on potato dextrose agar (PDA), malt extract agar (MEA), Czapek Dox agar (CDA) and wort agar (WA).

Samples from the mash, wort, unpasteurized and pasteurized beer were collected at various stages. Five g. sample was suspended in 45 ml of bacteriological peptone in 100 ml capacity flasks, agitated for 5 min at 500 rpm on a rotatory shaker (Labline Instruments, Illinois, USA, Model 3521) and allowed to settle. The supernatant was filtered into test tube using No.1 Whatman filter paper. Serial dilutions were made and 0.1 ml was pipetted on to sterile media, as named above. All the media were supplemented with 0.3% streptomycin sulphate to prevent the growth of bacteria. Incubation was at 28°C upto 7 days. During this period, fungal growth was observed daily under the microscope for morphology and structural characteristics. Fungal colonies were also counted and their characteristics were compared with literature description and illustrations (Barnett and Hunter 1972; Konen and Roberts 1985).

Variations in the population of fungi were observed on different media (Table 1). Fungi populations from water had the least counts, while hop pellets contained the highest population. *Alternaria* sp. were isolated from yeast, while *Rhizopus*, *Aspergillus*, *Dreschlera*, *Fusarium*, *Curvularia* and *Penicillium* species were found on malted sorghum.

TABLE 1. FUNGI POPULATIONS ISOLATED FROM VARIOUS MEDIA USED

Sample	Colony-forming units (cfu) per g or ml on Potato dextrose agar	Malt extract agar	Czapek Dox agar	Wort agar	Mean value
Sorghum grains	1424	2016	1368	632	13.6×10^2
Water	2	3	2	1	2
Malted sorghum	2320	3064	1816	800	2×10^3
Hop pellets	15980	8800	10000	5220	1×10^4
Yeast culture	120	72	48	160	1×10^2

The samples of mash (before and after saccharification) wort and beer (unpasteurized and pasteurized) were negative for fungi in all media.

Curvularia, *Mucor* and *Neurospora* species formed the fungal population on hop pellets (Table 2). Most of these species are spore-formers and are well known storage fungi, which are common on cereal seeds. The Zygomycetes, comprising of *Rhizopus* sp. and *Mucor* sp, and the Ascomycetes (*Neurospora* sp.) constitute 25 and 10% of the total population, respectively. The Deuteromycetes formed the largest group.

It is naturally expected that microorganism will be found on the surface of seeds like sorghum and

TABLE 2. DISTRIBUTION OF FUNGAL SPECIES ON VARIOUS COMMODITIES

Fungal species	Commodities: Sorghum grains	Water	Malted sorghum	Hop pellets	Yeast culture
	Colony forming units (cfu) per g or ml				
Alternaria sp.	672	0	800	0	400
Aspergillus sp.	691	0	1505	0	0
Curvularia sp.	545	0	862	2×10^4	0
Dreschlera sp.	550	0	890	0	0
Fusarium sp.	522	0	802	0	0
Mucor sp.	670	4	0	1×10^4	0
Neurospora sp.	544	1	800	3×10^3	0
Penicillium sp.	556	0	114	0	0
Rhizopus sp.	690	3	2×10^3	0	0
Total	5×10^3	8	8×10^3	4×10^4	4×10^2

other raw materials, since there had been earlier reports of their occurrence (Kommedahb and Chang 1970). What is relevant in this studies is the beer production. It is also possible that the microbes were contaminants from the processing procedure or the environment itself. *Aspergillus* sp. and *Penicillium* sp. are soil saprophytes. They produce numerous sporangiospores and resistant conidia, which are the agents for survival and dispersal (Eve 1980). These fungi attack grains and have undesirable effects, such as production of toxins, which may cause severe illness such as haemorrhage, liver damage and death. *Aspergillus* sp. produce aflatoxin, which may cause pathological changes in the liver. *Fusarium* sp. also produce toxins, which could have pathological changes in the blood and symptoms usually include fever, headache, diarrhoea, nausea and vomitting during the first few days (Fraziere and Westhoff 1978).

The various fungi found in this investigation have some similarities with those reported by earlier workers on barley (Harrison et al. 1974), *burukutu* and *pito* (Ogbonna et al. 1983). None of these toxigenic fungi was found in the mash down to the final product. Therefore, there is less fear about the effect of their toxins through beer consumption on human beings.

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Bacteriological Quality of Ice Cream Marketed in Tirupati, A Pilgrimage Town of India

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Counts of mesophilic aerobes, coliforms, aerobic spore formers and staphylococci in market samples of icecream varied in relation to the sources and packages. Higher counts of coliforms were prevalent in samples collected from local vendors. Icecream samples from parlours and hotels showed higher staphylococcal counts. With reference to the packages, icecream sold in cups had higher counts of coliforms and staphylococci, while aerobic spore formers were on a higher side in icecream bars.

Keywords : Icecream, Bacteriological quality, Standard plate count, Coliforms, Staphylococci, Aerobic spore formers.

Ice cream is a popular delicious frozen dairy product. In spite of the standards laid down for the microbiological quality of ice cream (ISI 1964), the quality of ice cream sold in market falls far below the stipulated microbiological standards (Ajab Singh et al. 1977; Patel and Vyas 1971). The practices of hygiene and sanitation, prevailing in ice cream industry, give ample scope for the entry of bacterial contaminants, which pose serious health hazards. The present study was undertaken to evaluate the microbiological quality of ice cream sold in the town of Tirupati, which is the biggest pilgrimage centre of India and consequently, a vast population of people have access to this frozen dairy product.

Samples of different commercial varieties of ice cream, sold in cups and in the form of bars, were collected from hotels, parlours and street hawkers. Ice cream samples, in their original packings, were brought to the laboratory in an ice box. A total of 47 samples of ice cream were analysed in the present study. Prior to analysis, samples of ice cream were tempered to 25-35°C. Counts for mesophilic aerobes (SPC), and coliforms in ice cream samples were determined as per the procedures laid in Indian Standards Specification (ISI 1964). Staphylococci were enumerated, following the procedure of Harrigan and McCance (1976). For enumeration of aerobic spore formers, the samples were heated at 80°C for 10 min to destroy the vegetative forms and cooled to room temperature, before plating on agar used to enumerate mesophilic aerobes. The plates were incubated at 37°C for 24 h (Buchanan and Gibbons 1974). The data obtained in the present study were analysed by the standard statistical analytical methods (Snedecor and Cochran 1967), using the $\log_{10}$ values of observed microbiological counts.

The counts of mesophilic aerobes (SPC), coliforms, staphylococci and aerobic spore formers in market samples of ice cream in relation to sources and packages are presented in Table 1.

TABLE 1. INCIDENCE OF MESOPHILIC AEROBES, COLIFORMS, STAPHYLOCOCCI AND AEROBIC SPORE FORMERS IN MARKET ICE CREAM

	Sample analysed	Bacterial counts (range), x 10^2 Cfu/ml: Mesophilic aerobes	Coli-forms	Staphy-lococci	Aerobic spore-formers
Source					
Hotels	11	100-1800 (713.6)	0-5 (1.7)	0-190. (3.9)	2-2.95 (1.6)
Parlours	16	140-3650 (1498.8)	0-11.5 (2.7)	0-19.5 (4.7)	1.9-11.3 (4.4)
Local vendors	20	250-6500 (2062.5)	0-4.85 (1.2)	0-5.5 (1.4)	0.3-25 (4.8)
Packages					
Cups	32	100-6500 (1419.1)	0-11.5 (1.8)	0-19.5 (3.7)	0.2-11.3 (3.0)
Bars	15	400-4600 (1844.7)	0-5.5 (1.1)	0-18 (1.9)	0.3-25 (6.0)

Figures in parentheses indicate mean values

About 12.5 and 40% of samples collected from parlours and vendors had SPC exceeding the laid down standard of 250 x 10^3 Cfu/ml. Among the packages, 18.7 and 26.6% of cups and bars had SPC more than 250 x 10^3 Cfu/ml. On the same lines, 54.5,81.3 and 65% of ice cream samples collected from hotels, parlours and local vendors had coliform counts exceeding 90/ml, which was more predominant among cups (78%). Among the

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TABLE 2. ANALYSIS OF VARIANCE OF VARIOUS BACTERIAL PARAMETERS (IN LOG UNITS) OF ICE-CREAM COLLECTED FROM DIFFERENT SOURCES (HOTELS, PARLOURS AND LOCAL VENDORS) AND DIFFERENT PACKAGES (CUPS AND BARS)

Bacterial counts	Different sources			Different packages		
	Sum of squares	Mean sum of squares	'F' value	Sum of squares	Mean sum of squares	'F' value
Mesophilic aerobes	1.2448 6.2851	0.6224 0.1428	4.3585*	0.5420 6.9881	0.5420 0.1553	3.4900^{NS}
Coliforms	5.5526 54,1821	2.7763 1.2314	2.2546^{NS}	1.9290 54.2157	1.9290 1.2048	1.6011^{NS}
Staphylococci	1.2954 47.5995	0.6477 1.0818	0.5987^{NS}	4.3012 44.5940	4.3012 0.9910	4.3403*
Aerobic spore-formers	1.7656 0.4656	0.8828 0.0106	83.2800**	0.4013 8.6770	0.4013 0.1928	2.0800^{NS}

*P≤0.05, ** P≤0.01, NS : Not significant, DF for sources is 2 and for error is 44 for all the common traits, DF for packages is 1 and for error is 45 for all the common traits.

three types of sources, samples of ice cream from hotels and parlours had higher counts of staphylococci and aerobic spore formers, with not much difference among packages.

In earlier studies (Suryanarayana Rao and Dudani 1962; Thatti et al. 1969), about 50% market ice cream samples had SPC of more than 250 x 10^3 Cfu/ml. Similarly, 57% of market ice cream samples collected in Bombay had coliform counts exceeding 90 Cfu/ml (Thatti et al. 1969). With reference to the count of coliforms in ice cream sold in different packages, a similar observation to the present study was made by Sarada and Mushtari Begum (1991), wherein ice cream bars had lower coliform counts. The counts of staphylococci and aerobic spore formers recorded in the present study also finds agreement with the counts recorded in earlier studies (Rajalakshmi 1983; Pillai et al. 1990).

Analysis of variance between the 3 sources of samples and between 2 forms of packages are presented in Table 2. With reference to counts of mesophilic aerobes and aerobic spore formers, there were significant differences between samples from the 3 sources. However, no significant difference was observed between the 2 packages. On the other hand, in case of counts of coliforms and staphylococci, no significant differences were observed between the 3 sources. In relation to packages, significant difference was observed in the staphylococcal counts, while it was not so in case of coliform counts.

The results of the present study indicated that a considerable number of ice cream samples, marketed in the pilgrim town of Tirupati, harbour a good number of coliforms, staphylococci and aerobic spore formers, which may pose serious public health hazards.

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Removal of Glucose from Hen Egg Using Glucose Oxidase and Catalase Co-immobilized in Hen Egg White Foam Matrix

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A novel technique has been developed for obtaining a porous hen egg white foam matrix containing co-immobilized glucose oxidase and catalase. The technique involved cross-linking of the foamed egg white along with the enzymes using 0.75% glutaraldehyde for 1 h. The foamed matrix was mechanically very stable and could be reused in 10 batches for the removal of glucose from eggs, without loss in activity.

Keywords : Glucose removal, Eggs, Glucose oxidase, Catalase, Immobilized enzymes, Hen egg white foam matrix.

One of the major problems in the manufacture of dehydrated egg products, is the occurrence of browning because of the presence of glucose (0.4%) in egg melange (Scott 1953). This not only affects the quality of the product but also decreases its solubility and nutritional value. Removal of glucose from egg prior to dehydration is normally carried out by fermentation with yeast cells (Satyanarayana Rao and Murali 1985), mainly because of its economic feasibility. This process, however, occasionally leads to mustiness and off-flavour development during storage (Kline et al. 1954). Contamination of the final product by yeast also limits its application in various food mixes. Some of these problems have recently been obviated by using immobilized yeast cells (D'Souza and Godbole 1989). Enzymatic removal of glucose from egg, using glucose oxidase and catalase, is the most preferred method (Pitcher 1980). Commercial application, however, has been hampered mainly due to the high cost of enzymes. In order to obviate these problems, efforts are being made recently to immobilize these enzymes for their reuse (Sankaran et al. 1989). The present communication delineates a novel method for co-immobilization of glucose oxidase and catalase in hen egg white foam matrix and its use in the removal of glucose from egg melange.

Glucose oxidase (EC 1.1.3.4) (430 U/mg, from *Aspergillus niger*), catalase (1,000 U/mg, from beef liver) were obtained from Sigma Chemicals Co., St. Louis, USA while glutaraldehyde was obtained from Polyscience Inc. Warington. Leghorn variety of eggs were procured from the local market.

Immobilization of glucose oxidase and catalase in hen egg white foam matrix : Fresh egg white (100 ml) was mixed with 250 mg of glucose oxidase and 250 mg of catalase, followed by glutaraldehyde to a final concentration of 0.75%. The mixture was immediately foamed by vigorous stirring, using an overhead stirrer. The foam was kept undisturbed for 1 h at 26°C, to allow for cross-linking, after which it was cut into blocks of $3 \times 3 \times 3$ cm^3 each, and washed with distilled water. Excess aldehyde groups were blocked by incubation in 0.5 M ethanolamine (pH 7.0) for 1 h. The blocks of foam were washed again with water and used as a source of immobilized glucose oxidase and catalase. The unfoamed blocks were prepared by mixing egg white with glutaraldehyde without foaming, followed by incubation under stationary conditions for 1 h at 26°C. The gel blocks were washed as described earlier.

Removal of glucose from eggs : Fresh eggs were washed, soaked in 2% bleaching powder solution for 30 min, followed by water and surface dried. The eggs were broken and the egg white and yolk were blended using an emulsifier (type L56-3. Remi Motors, Bombay). The egg was emulsified at 1000 rpm for 2 min. Egg melange (135 ml), equivalent to 3 eggs, was mixed with two (3 cm)^3 blocks of immobilized enzyme and stirred gently on a rotary shaker at 26°C for the required time interval.

Analytical methods : Glucose oxidase and catalase were assayed as described earlier (Kaul et al. 1986; D'Souza and Nadkarni 1980a). Glucose was estimated using glucose oxidase and peroxidase (Bergmeyer and Bernt 1965). It involved dilution of egg melange (1 ml) with 8 ml distilled water, and precipitation of proteins by 0.5 ml of 10%

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sodium tungstate and 0.5 ml of sulphuric acid.

The conditions for preparation of hen egg white foam matrix containing glucose oxidase and catalase were optimized. Foaming of egg white, along with glutaraldehyde was found to be essential for stabilization of the foam matrix. Addition of 0.75% glutaraldehyde (final concentration), followed by incubation for 1 h under stationary conditions, resulted in a mechanically stable foam. The effective volume occupied by the foam matrix obtained from 100 ml of egg white was about 300 cm^3. The matrix was spongy, porous and mechanically stable to shaking and stirring conditions.

Efficiency of glucose removal from egg melange, using unfoamed and foamed hen egg white matrix containing bound enzymes, was studied in a batch process. The kinetics of glucose removal is shown in Fig. 1. Matrices obtained from the same initial volume of egg white have been used for comparison. Freshly prepared foamed matrix was able to remove over 95% of glucose in 5 h, as compared to the unfoamed matrix, which could remove only 40% glucose under similar conditions. Controls containing hen egg white foam blocks, without the enzyme, did not show any desugaring ability.

In order to examine the stability towards reuse, the enzyme bound foam matrix was retrieved from the egg melange and washed thoroughly with water and reused in a fresh batch of melange. The

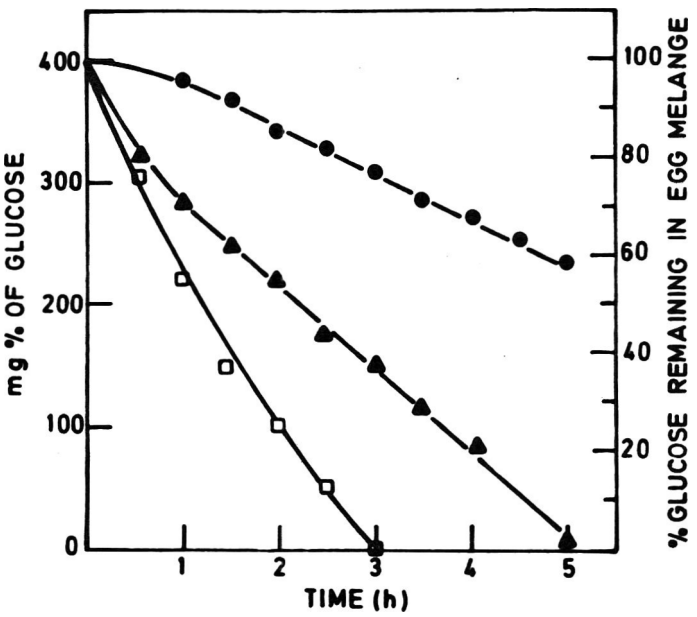

Fig. 1. Kinetics of glucose removal by hen egg white matrix from egg. □ Reuse 1-9, ▲ foamed matrix (1st use), ● unfoamed matrix

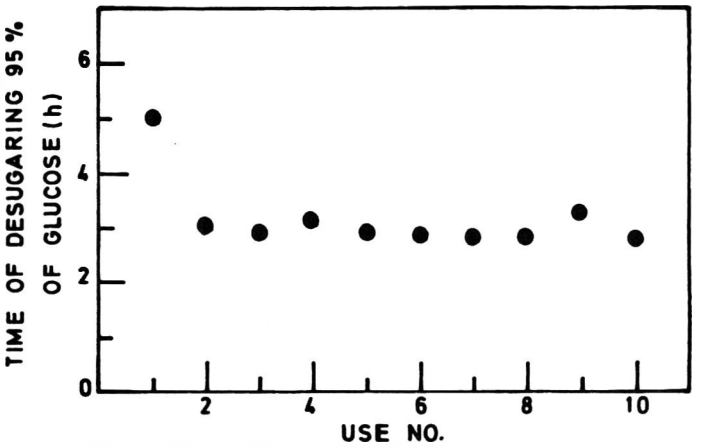

Fig. 2. Reuse of immobilized enzyme for desugaring.

efficiency of the foamed block, to remove glucose from eggs, was found to increase after initial use. Thus, from the second use onwards, the time required for desugaring was reduced to 3 h (Figs. 1 and 2). Similar enhancement in immobilized enzyme activity has been reported with other systems (Messing 1985). This has been attributed to the enzyme active sites, initially being bound to the carrier or to adjacent enzyme molecules. Since the affinity of the active site is greater for the substrate, than that for either the carrier or the adjacent enzyme molecules, these sites become available, as the substrate (glucose) diffuses into the vicinity of the bound sites in the depth of the pore (Messing 1985). The immobilized enzymes when not in use, were stored at 4°C in 50 mM phosphate buffer (pH 7.0) without loss of activity, for a study period of 15 days.

Even though glucose oxidase has been immobilized on various particulate supports for the preparation of gluconic acid (D'Souza and Nadkarni 1980b), removal of oxygen from beer (Hartmeier and Willox 1981) and initiation of lactoperoxidase systems in milk (Kaul et al. 1986), not many reports have appeared on the use of this technology for removal of glucose from eggs (Sankaran et al. 1989). This is mainly due to limitations of particulate supports in view of high pressure drops, hindered flow characteristics and also difficulties in the complete and easy retrieval of immobilized enzyme from stirred tank reactors, while treating viscous subtrates like egg melange (Sankaran et al. 1989). Some of these problems can be obviated by using immobilized glucose oxidase on porous supports with large dimensions as in the present study. Porous supports are currently gaining considerable importance in biotechnology, in view of their low diffusional problems (Shankar et al. 1985). As compared to the complex techniques currently used for the

preparation of such porous supports, the unique property of egg white to form a stable foam has been utilized in the present study to develop a novel technique for obtaining a highly porous hen egg white foam matrix. It has simplicity in its preparation. In addition to immobilizing glucose oxidase, it can find future potential for the immobilization of other enzymes and cells. Ease of availability in a ready-to-use form, low cost, and a palatable protein nature make it a useful support for immobilization (D'Souza and Nadkarni 1981; D'Souza et al. 1985; Kaul et al. 1984).

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Effect of Soy Milk Supplementation on the Coagulation Time, Green Cheese Composition and Losses of Milk Components in Whey

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Except for proteins, the cow milk contained higher total solids, fat, acidity and ash as compared to soy milk. The coagulation time of cow milk with calf or microbial rennet was similar. The addition of soy milk to cow milk increased the coagulation time of the blend. Yield and titratable acidity of whey decreased with the increase in proportion of soy solids in blend, whereas reverse was true for the total solids, protein and ash. The yield of cheese increased with increase in the proportion of soy solids in the blend. The moisture content of cheese made from cow milk using both the enzymes was similar, but it increased with the increase in the soy solids in the blends. A slight decrease in protein and fat of cheese made from various cow:soy milk blends with the microbial rennet was observed. A slight increase in salt content was found in cheese made with higher soy solids in the blend. Increases in titratable acidity, soluble protein and free fatty acid values of cheese were observed with increases in proportions of soy solids in the cow:soy milk blends.

Keywords : Soy cheese, Soy milk, Cow milk, Calf and microbial rennets, Coagulation time, Cheese composition, Whey losses.

Cheese is considered as the best way of conserving surplus milk (Davis 1965). Among the cheese varieties, cheddar, a medium hard cheese, is popular all over the world and this is the variety that is usually made in India due to its better keeping quality, consumer acceptability and mild flavour (Scott 1979). In spite of significant increase in milk production in the country, fluid milk is not available to a common man due to enormous increase in population. Soy proteins are unique among plant proteins by virtue of their relatively high biological value and essential amino acid content and are widely used in meat preparations (Padda et al. 1985). Therefore, the fortification of various milk products with soybean could solve the problem of milk non-availability (Kellor 1971; Schroder et al. 1973; Kumar and Angelo 1983). Since the use of calf rennet is banned in the country due to religious sentiments, microbial rennet is being used for cheese manufacture. Hence, a microbial rennet has been used in the present studies for making cheese from admixtures of cow milk and soy milk. Cheese, thus obtained, was compared with that obtained by using calf rennet.

Source of materials : Cow milk was obtained from Livestock Research Centre, Nagla of the University. Mature soybeans of variety 'PK-262' were obtained from the University Farm. Modilase, double strength microbial rennet, produced from *Mucor miehei*, and calf rennet were obtained from Chr. Hansens Laboratory Inc. Denmark and Australia, respectively. *Streptococcus lactis* was obtained from National Dairy Research Institute, Karnal. Bulk culture was prepared by using cow skim milk.

Preparation of soy milk and cheese : Soy milk was prepared as per method described by Nelson et al (1976). Standardized cow milk (casein/fat=0.7) was pasteurized to 145°F for 30 min and soy milk, heated to 100°C for 5 min, was added to it in different proportions (95:5, 90:10, 85:15, 80:20 and 75:25). Required amount of bulk starter culture and coagulant were added to different blends of cow milk and soy milk at 30°C. The resultant curd was cut with cheese knives and scalded upto 37°C. After cooking, the whey was measured and analysed for milk component losses. Cheese samples were also analysed for their composition.

Analytical methods : Moisture in cheese was determined gravimetrically (MIF 1959), while protein, pH and total solids were determined by standard methods (ISI 1961). Fat, ash and titratable acidity of cheese samples were determined by the methods recommended by AOAC (1975). The soluble protein was determined by the method described by Kosikowski (1966). The free fatty acids (μ moles/

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TABLE 1. COMPOSITION, TITRATABLE ACIDITY, pH, COAGULATION TIME OF COW MILK, SOY MILK AND THEIR BLENDS[1]

Blend Cow milk : Soy milk	Moisture, %	Total solids, %	Fat, %	Protein, %	Ash, %	Titratable acidity, % lactic acid	pH	Clotting* time, sec
100 : 00	87.2	12.8	4.50	3.28	0.65	0.14	6.6	176+
100 : 00								175++
00 : 100	92.6	7.4	1.30	3.34	0.43	0.03	7.9	ND
95 : 5	87.6	12.4	4.00	3.45	0.61	0.09	7.0	274
90 : 10	87.8	12.2	4.07	3.29	0.58	0.10	6.9	381
85 : 15	88.1	11.9	4.10	3.28	0.56	0.11	6.8	559
80 : 20	88.4	11.6	4.15	3.31	0.54	0.12	6.7	738
75 : 25	88.6	11.4	4.19	3.29	0.31	0.13	6.7	906

[1] Average of triplicate experiments *Calf and microbial rennets were used for clotting cow milk, while microbial rennet alone was used for clotting cow milk:soy milk blends,+ : Clotting time with calf rennet, ++:Clotting time with microbial rennet. ND:Not determined.

g fat) were estimated as % oleic acid as per method described by Rama Murthy and Narayanan (1974). The requirement of clotting enzyme was determined as suggested by Davis (1965).

The results indicated that the cow milk was richer in total solids, fat and ash contents, except for proteins, as compared to soy milk or the blends made therefrom (Table 1). Soy milk composition did not confirm the findings of Chopra et al (1984). This could be due to alteration in soy milk process in which some of the soy solids might have been lost during filtration process. The titratable acidity of soy milk was 0.03%. The present results are not in agreement with the findings of Metwalli et al (1982a). This could be due to the use of sodium bicarbonate during blanching in the present studies, which might have resulted in lower titratable acidity as compared to other reports (Metwalli et al. 1982a; Chopra et al. 1984).

Addition of soy milk to cow milk resulted in an increase in the clotting time (Table 1). The clotting time of different blends (95:5, 90:10, 85:15, 80:20 and 75:25) of cow milk and soy milk were 274, 381, 559, 738 and 906 sec, respectively. These values were higher than those for the control sample (175 sec). Increase in clotting time could be attributed to inhibitory effect of soy milk solids on enzyme. An alternate explanation is that the majority of soybean proteins are of globulin type, which contains free-SH groups. Therefore, there is a possibility of occurrence of interaction between soy protein and milk casein, similar to that reported

TABLE 2. COMPOSITION, TITRATABLE ACIDITY (TA), pH, AND YIELD OF GREEN CHEESE[1] AND LOSSES OF MILK COMPONENTS IN WHEY[1] OBTAINED FROM CHEESE MADE FROM DIFFERENT COW:SOY MILK BLENDS USING MODILASE

Blend* Cow milk : Soy milk	Moisture, %	Total solids, %	Fat, %	Protein, %	Ash, %	Yield, %	TA, %	pH	Salt, %	Lactose, %	Soluble protein, %	Free fatty acids, μ mole/g fat
Green cheese												
100 : 00	38.3	61.7	31.5	24.0	2.20	12.38	0.90	5.34	1.4	ND	1.11	10.5
100 : 00	37.9	62.1	31.1	25.0	2.19	12.86	0.92	5.30	1.4	ND	1.16	10.0
95 : 5	40.6	59.4	29.6	23.6	2.15	13.19	1.00	5.20	1.5	ND	1.08	11.0
90 : 10	40.8	59.2	29.1	23.0	2.11	13.45	1.11	5.10	1.5	ND	1.28	11.9
85 : 15	41.6	58.5	29.6	21.0	2.04	13.47	1.14	4.90	1.6	ND	1.29	13.3
80 : 20	42.4	57.6	28.3	21.0	2.02	13.47	1.15	4.85	1.7	ND	1.43	14.8
75 : 25	49.1	50.9	28.3	20.0	1.94	14.00	1.20	4.70	1.8	ND	1.32	16.3
Whey												
100 : 00	93.0	7.0	0.7	0.80	0.58	70.95	0.18	5.00	ND	4.96	ND	ND
100 : 00	92.9	7.1	0.6	0.89	0.59	69.05	0.18	5.01	ND	5.00	ND	ND
95 : 5	92.9	7.1	0.6	0.93	0.58	66.82	0.17	5.04	ND	4.98	ND	ND
90 : 10	92.8	7.2	0.7	1.04	0.57	65.24	0.16	6.42	ND	4.90	ND	ND
85 : 15	92.8	7.2	0.7	1.08	0.58	65.00	0.14	6.51	ND	4.89	ND	ND
80 : 20	92.7	7.3	0.8	1.10	0.55	62.73	0.13	6.52	ND	4.83	ND	ND
75 : 25	92.6	7.4	0.9	1.18	0.54	60.43	0.13	6.80	ND	4.80	ND	ND

1 Average of triplicate experiments *Calf and microbial rennets were used for clotting cow milk, while microbial rennet alone was used for clotting cow milk:soy milk blends. ND : Not determined.

between casein and whey proteins, and it inhibits rennet action (Shalabi and Wheeloch 1976).

Composition of green soy cheese : It is apparent from Table 2 that the total solids in green soy cheese ranged from 50.95 to 59.40%. The total solids decreased as the proportion of soy milk increased in the blends. Increase in % moisture could be attributed to hydrophilic nature of soy proteins (Noyes 1969; Aworh et al. 1987). Fat and protein in green soy cheese ranged from 44.30 to 50.10% and 31.20 to 39.70% on dry weight basis, these values being highest and lowest in green soy cheese made from cow milk to soy milk ratio of 95:5 and 75:25, respectively. The pH values of green soy cheese samples ranged from 4.70 to 5.20, these values being lowest and highest for the cheese made from milk and soy milk blends at the ratio of 75:25 and 95:5, respectively. The soluble proteins and free fatty acids ranged from 1.06 to 1.32% and 11.00 to 16.30 μ moles/g fat, respectively. These values were at par with control green cheese samples (Metwalli et al. 1982b; El-Safty et al. 1979).

Losses of milk components in whey : It is apparent from Table 2 that moisture, lactose, ash, yield and titratable acidity showed lower values with the increase in proportion of soy solids in the blend, whereas reverse was true for total solids, fat, protein and pH. The reason for the increased amounts of whey solids could be the inhibition of rennet by the interaction products of soy proteins and whey proteins (Shalabi and Wheelock 1976). High fat losses in whey may be because of the entrapment of fat globules by the coagulated proteins, which would be proportionately reduced in the blends (Davis 1965).

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Characterization of Pectic Substances from Selected Tropical Fruits

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Pectin content of orange, lime, banana, mango, native mango, garden egg, avocado pear, pawpaw, cashew apple, star apple, tomato and guava fruits was studied. Mango had the highest pectin content of 1.8%, while tomato contained the lowest pectin (0.53%). The anhydrogalacturonic acid was the highest in mango, while it was the lowest in star apple. Solubility and viscosity of the pectin were found to increase with increasing methoxyl ester content. Jams of good gelation characteristics and acceptability were produced from some of these fruits.

Keywords : Pectins, Tropical fruits, Gelation, Jam, Pectin characteristics.

Fruits play an important role in human diet as the source of protein, calories, essential vitamins and minerals (Ihekoronye and Ngoddy 1985). In Nigeria, as well as other developing countries of the world, tropical fruits are seasonal. Abundant fruits are harvested during the peak periods, but these deteriorate quickly due to their perishable nature. Indigenous preservation techniques to curtail the post-harvest losses are not only ineffective, but also inadequate to meet the volume of production. Modern scientific methods of preservation, like refrigeration and controlled atmosphere storage, have been found to be technically and economically non-feasible in developing countries. The search of alternative uses of these tropical fruits to maximize their utilization and reduce losses is, therefore, vitally important. This study was aimed at producing jams from a number of tropical fruits. Parameters, such as levels of pectin, methoxyl ester and anhydrogalacturonic acid and gelation characteristics, were also studied.

Firmly ripe fresh fruits were purchased at Eke Okigwe market in Imo State, Nigeria and used immediately. The fruits studied included: star apple (*Crysophyllum albidium*), banana (*Musa sapientum*), pawpaw (*Carica papaya*), orange (*Citrus sinensis*), cashew apple (*Anacardium occidentale*), mango (*Mangifera indica*), avocado pear (*Avocado avocado*), garden egg (*Solanum nigrum* Linn), Lime (*Citrus aurantifolia*), tomato (*Lycoperisican esculentum*), guava (*Psidium guajava*) and native mango (*Irvingia gabonensis*). Several workers have studied the nature of pectins from the above fruits (Surinder Kumar et al. 1985; Alexander and Sulebde 1980; Dhingra and Gupta 1984). In our studies, the total pectic substance (protopectin, pectic acid, pectates and pectinates) from each fruit were extracted (Inoh et al. 1977). The methoxylester and anhydrogalacturonic acid content of pectin were determined using the method of Schultz (1965). The tests were performed, in triplicates, and the average values are reported. The physical properties (viscosity, solubility, reaction on heating and nature of precipitate) of the pectin samples were determined using the method of Towle and Christensen (1969). Jams were individually made from fresh fruits with 1% pectin content and high degree of methylation using the method of Creuss (1967). Jams were also made using a combination of fruits with pectin content more or less than 1%. The pH and sugar content of the gel were monitored during jam preparation (AOAC, 1970). Sensory evaluation was conducted by a panel consisting of trained persons using a 9 point Hedonic scale to determine the overall acceptability and preference for the banana, orange, lime, orange/banana, and orange/garden egg jams, using quality attributes such as colour, aroma, taste and texture or consistency (IFT Sensory Evaluation Division 1981).

Pectin content of fruits : Mango showed the highest pectin level and tomato the lowest (Table 1). Pectin content in these fruits decreased in the following order : banana, pawpaw, orange, cashew apple, guava, lime, garden egg, avocado pear, native mango, star apple and tomato. Thus, mango, banana, pawpaw, orange, cashew apple, guava, and lime, with pectin content of 1% and above, are expected to produce good gels (Ihekoronye and Ngoddy 1985). Mango pectin has the highest methoxyl ester content, while it was the lowest in star apple. There are two types of pectins - the

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TABLE 1. RESULTS OF METHOXYL ESTER CONTENT, ANHYDROGALACTURONIC ACID, PECTIN YIELD AND MOISTURE CONTENTS OF FRUITS

Sample	MEO, % of pectin	AGA, % of pectin	Pectin yield, % of the fruit pulp	Moisture content, % of the fruit pulp
Star apple	8.68	9.38	0.60	75.00
Banana	46.68	51.98	1.10	88.40
Pawpaw	69.44	79.74	1.60	87.24
Orange	36.37	44.37	1.35	90.45
Cashew apple	25.93	36.02	1.28	92.34
Mango	76.14	81.64	1.80	87.20
Avocado pear	46.60	51.60	0.73	83.10
Garden egg	60.15	65.75	0.90	80.00
Lime	56.28	69.65	1.00	87.50
Tomato	43.40	45.30	0.53	78.40
Guava	40.26	47.36	1.26	84.12
Native Mango	22.86	36.86	0.72	81.00

MEO - Methoxyl ester content, AGA - Anhydrogalacturonic acid content.

high methoxyl pectin (60-75% esterification) which is used for sugar - pectic acid gels, and the low methoxyl pectin (20-45% esterification) which is used for Ca^{2+} pectinate gels. Based on this classification, pectins from garden egg, pawpaw, and mango have high methoxyl pectin, while pectins from native mango, cashew apple, orange, guava, tomato, banana, avocado pear and lime have low methoxyl pectin (Table 1). The purity of the pectin samples can be judged by anhydrogalacturonic acid content. The results showed that mango pectin, with anhydrogalacturonic acid content of 81.6% is purest, while star apple pectin with anhydrogalacturonic acid content of 9.38% (Table 1), was the most impure.

Physical properties of the extracted pectins : The solubility of the pectin samples, extracted from different fruits, decreased in the following manner: mango, banana, pawpaw, orange, cashew apple, guava, lime, garden egg, avocado pear, native mango, star apple and tomato. This is in accordance with the order of decreasing methoxyl ester content. The results also agreed with those of Towle and Christensen (1969). Present results indicated that viscosity was directly related to temperature, thus displacing a non-Newtonian flow. This agreed with the study on the physical characteristics of pectin as reported by Stoddart et al (1969). However, a colloidial precipitate was obtained upon addition of 95% ethanol to the pectin solution. When centrifuged and washed with acetone, it gave a stringy white precipitate. Towle and Christensen (1969) had earlier observed similar precipitate of pectin. Ewart and Chapman (1952) had suggested that dissolving characteristics, appearance of solution and precipitate obtained following addition of alcohol are useful preliminary tests for identification of pectin.

Nature of gel formed : The pectins from mango, banana, lime, and orange met the requirements for pectin content and/or degree of methoxylation. They also gave thick gels, when heated with 70% sugar at pH between 3.2 and 3.5. Mango, however, was found to have quick setting potentials, because of their lower methoxyl ester content (Desroisier 1970). Pawpaw, in spite of its high pectin and methoxyl ester contents, did not form strong gel and this could be attributed to other factors that influenced gelation (Ihekoronye and Ngoddy 1985). The gelation of banana, lime and orange is attributed to their pectin contents. Gels prepared from cashew apple and guava at pH 3.2 with 70% sugar had flowing characteristics, because of their low methoxyl ester content. Those prepared with avocado pear, native mango, star apple and tomato could not gel, partly because the pectin contents were less than 1% and had low methoxyl ester content. The gels formed with the extracted pectin showed gel-graining characteristics, indicating the presence of excess sugar. They, therefore, have a lower requirement for dehydrating agent, a characteristic, which is common with low methoxyl ester pectin. Hence, this will be more useful in making of low sugar or calcium pectate gels (Heiman 1970). In spite of the high degree of methylation of firm and mature garden egg, the gels formed were almost flowing. This was because of the insufficient amount of pectin. As a result, when it was mixed with fruit of high pectin content, such as orange, it formed a strong gel.

Sensory evaluation : In terms of colour, orange jam was preferred to other jams and was significantly different at 5% level from orange/banana, lime, and banana jams (Table 2). However, it was not significantly different from orange/garden egg jam. Also in terms of taste, orange jam was preferred, but was not significantly different with orange/garden egg, orange/banana and lime jams. While there was no significant difference between the lime and banana jams in terms of taste, there was significant difference between orange, orange/garden egg and orange/banana jams on one hand, and banana jam on the other. Again, orange jam was found to have the best texture among all other

TABLE 2. SENSORY EVALUATION OF JAMS PREPARED FROM BANANA, ORANGE, LIME, ORANGE/BANANA AND ORANGE/GARDEN EGG.

Jam sample	Colour	Taste	Aroma	Texture
Orange	8.1^{a}	8.0^{a}	7.5^{a}	7.8^{a}
Orange/Garden Egg	6.7^{ab}	7.1^{a}	6.3^{c}	6.6^{ab}
Orange/Banana	6.2^{a}	7.1^{a}	6.6^{bc}	6.6^{ab}
Lime	5.6^{b}	6.6^{ab}	5.4^{d}	5.8^{b}
Banana	4.8^{b}	5.2^{b}	6.8^{b}	5.8^{b}

Any mean scores within each column followed by the same letter on superscript are not significantly different at 5% level by Tukey's test (≤0.05). Higher values indicate greater preference.

samples, but was not significantly different from lime and banana jams. Grading the samples in terms of decreasing order of overall acceptability is as per the order : orange jam, orange/banana jam, orange/garden egg jam, banana jam and lime jam.

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Evaluation of Nutritional Quality of Parboiled Rice

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Feeding trials were conducted on albino rats for 28 days using reference casein diet, raw rice diet and the diets based on pressure parboiled rice as well as traditionally parboiled rice. Protein efficiency ratio (PER) did not vary significantly for different groups. However, PER value was the highest for rats fed on casein diet,followed by raw rice diet, pressure parboiled rice diet and traditionally parboiled rice diet. respectively.

Keywords : Raw rice, Pressure parboiled rice, Traditionally parboiled rice, Casein, Rats, Protein efficiency ratio.

Parboiling of paddy, carried out to improve the quality of rice, is an age old practice. It involves a hydrothermal treatment, in which paddy is soaked, steamed and dried, with a view to mainly improve the milling quality. Several methods of parboiling are in vogue in different parts of world and the method of soaking paddy in hot water followed by open steaming (CFTRI 1969) has been widely practised in India, since long time. Lately, pressure parboiling of paddy by applying steam under pressure has been recommended (Iyengar et al. 1980, Ali and Bhattacharya 1982). Some studies have been done by various workers on the effect of milling on the quality of parboiled rice and control of spoilage (Raghavendra Rao et al. 1967; Vasan et al. 1981; Kulkarni and Bal 1986). Studies have been conducted on evaluation of protein quality of milled raw rice (Bresseni et al. 1971; Murata et al. 1978) in rats, using different protein levels. But studies utilising parboiled milled rice are lacking. The present study was, therefore, undertaken to find out the effect of different parboiling treatments on nutritional quality of rice.

Parboiled rice, prepared by pressure parboiling (Iyengar et al. 1980 - soaking paddy in water until it attains 18% moisture, followed by steaming at 15 lb/sq. in. for 20 min and subsequent shade drying to 10-12% moisture) and traditional parboiling (CFTRI 1969 - soaking paddy at 70±2°C for requisite time to attain 35% moisture, followed by steaming at atmospheric pressure for 15 min and drying to 10-12% moisture) were milled and ground into flour for incorporating into diet. Raw milled rice was used to constitute control diet and casein was used in the reference diet. The diets, prepared according to the method of Chapman et al (1959), were isocaloric with 6% protein and adequate provisions of vitamins and minerals. Diets with 6% protein levels were used because of the low protein content of rice. Murata et al (1978) have also reported that low protein containing samples cannot be run above dietary protein level of 5-7%. Weaning albino rats (24 days old), with mean weights of 26-28 g, were obtained from Department of Biochemistry of the University. There were 8 animals in each group (4 males and 4 females). The groups were so constituted that the average initial weights of all groups were the same. The animals were housed in aluminium cages and fed *ad libitum*. The rats of 24 days of age have been also used by other workers (Singh et al. 1992; Gopal et al. 1988). Equal number of male and female rats have been included to overcome the effect of sex on PER. The mean values are reported for PER estimations. Weighed amounts of diet were offered to rats every day. The food residues were collected, dried overnight at 110°C and weighed. The food consumption of individual rat in each group was recorded. The animals were weighed at weekly intervals for a period of four weeks and PER was calculated using the formula:

$$\text{Protein efficiency ratio} = \frac{\text{Gain in weight, g}}{\text{Protein intake, g}}$$

After 4 weeks, the animals were sacrificed and blood was drawn for determination of haemoglobin, packed cell volume, red blood cell count and differential leucocyte counts, according to methods given by Dacie and Lewis (1975). In addition, the liver, heart, kidney and spleen were collected and weighed. Statistical analysis was done according to methods given by Guenther (1964).

Data showing average feed and protein intakes by different groups are given in Table 1. Interestingly, the diet intake by group fed on casein diet was found to be least, thereby resulting in lowest

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TABLE 1. EFFECT OF DIFFERENT DIETS ON AVERAGE FEED CONSUMED, PROTEIN INTAKE BY RATS AND PROTEIN EFFICIENCY RATIO

Group	Average initial body weight of rats, g	Cumulative feed intake in 28 days, g	Total protein intake in 28 days, g	Total gain in weight after 28 days, g	Protein efficiency ratio
Control rice diet	26.46±2.58[1]	178.61[a]±8.99	10.71[1]±0.54	21.34[a]±4.40	1.98±0.4
Pressure parboiled rice diet	26.27±1.85	179.58[a]±10.18	10.77[a]±0.61	21.88[a]±3.19	1.89±0.6
Traditionally parboiled rice diet	26.44±3.62	162.30[b]±13.59	10.10[b]±0.82	18.87[ab]±4.44	1.86±0.4
Casein diet	26.44±1.35	123.39[c]±5.46	7.48[c]±0.33	16.18[b]±2.10	2.18±0.3
F. Ratio	N.S.	100.32*	100.30*	4.56*	N.S.
CD value	-	7.72	0.46	3.56	-

[1] Mean ± S.D. (Standard deviation) N.S: non-significant. Values with dissimilar superscripts vary significantly. C.D. = Critical difference. * Significant variation.

protein intake. Among the rice diets, feed intake was highest for group fed the diet based on pressure parboiled rice. Protein intake varied significantly among groups. However, protein intake for the control group and that for group fed on pressure parboiled rice did not differ significantly between themselves.

Data on weight gains (Table 1) by rats revealed significant variations between groups. Weight gains were significantly more for rats fed on diets based were not statistically significant.

Protein efficiency ratio (PER) did not vary (Table 2) significantly for different groups. However, PER value was highest for rats fed on casein diet (2.18), followed by diets based on raw rice (1.98), pressure parboiled rice (1.89) and traditionally parboiled rice (1.86), respectively. The PER values tended to decline with processing treatment, though non-significantly. Parboiled rice digests much slower

TABLE 2. EFFECT OF DIFFERENT DIETS ON ORGAN WEIGHTS AND HEAMATOLOGICAL ANALYSIS OF BLOOD OF RATS.

Attribute	Control rice diet	Pressure parboiled rice diet	Traditionally parboiled rice diet	Casein diet	F.Ratio	Critical difference value
Final body weight, g	49.10[a]±5.02	48.05[a]±2.52	46.65[a]±6.421	42.40[b]±1.54	3.12*	4.80
Spleen, g	0.15[a]±0.03	0.11[b]±0.01	0.12[b]±0.02	0.08[c]±9.91	14.96*	0.02
Liver, g	1.96[a]±0.26	1.72[b]±0.19	1.79[b]±0.18	1.35±0.09	5.46*	0.07
Kidney, g	0.55[a]±0.04	0.48[b]±0.04	0.46[b]±0.08	0.39[c]±0.02	11.51*	0.22
Heart, g	0.27[a]±0.03	0.26[a]±0.02	0.21[b]±0.02	0.21[b]±0.02	7.27*	0.03
Haemoglobin, g/100 ml blood	12.25[a]±0.25	11.38[a]±1.65	9.95[b]±1.21	12.34[a]±0.50	6.24*	1.31
Packed cell volume, mm/100 ml blood	36.08±3.19	34.06±4.35	29.94±3.78	34.53±4.31	N.S.	N.S.
Red blood cells, million/ml blood	5.23[a]±0.14	5.03[a]±0.40	4.55[a]±0.83	5.95[b]±0.47	5.89*	0.59
Total leucocyte count/ml blood	6050[a]±235	8038[b]±990	6363[c]±990	6813±815	14.40*	6.77
Differential leucocyte count/ml blood						
- Neutrophiles	29.00[a]±2.24	29.38[a]±3.16	39.25[b]±6.18	35.38[c]±2.5	11.90*	4.22
- Lymphocytes	68.63[a]±1.20	67.00[a]±3.91	57.75[b]±5.24	62.75[c]±2.44	16.88*	3.48
- Eosinophiles	1.25±1.00	1.00±0.71	1.75±0.66	1.88±0.70	N.S.	N.S.
- Monocytes	0.34±0.70	0.25±0.43	0.75±0.66	0.63±0.45	N.S.	N.S.
- Basophiles	-	-	-	-	N.S.	N.S.

Values with dissimilar superscripts vary significantly. * Significant variation.

on control and parboiled rice in comparison to rats fed on reference diet. Among three rice based diets, the weight gains were highest for diet based on pressure parboiled rice. However, the differences

than raw rice on account of lesser ease of penetration of enzymes in parboiled rice (Srinivasan and Venkatagiri 1939).

Data on absolute as well as % organ weights

for spleen, liver, kidney and heart are given in Table 2. Data revealed significant increases in final body weights for rats fed on all the three rice diets, as compared to those fed on casein. However, the three test diets did not vary significantly among themselves. Weights of different organs, from rats fed on reference diets, were significantly lower in most of the cases, in comparison to other groups. This could be due to smaller body weights as compared to other three groups. Values for haemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBC) were found to be within the normal limits (Table 2). Basophiles were absent. PCV, eosinophiles and monocyte counts varied non-significantly, whereas haemoglobin, RBC, TLC, neutrophiles and lymphocytes varied significantly among the groups.

Rice, among the cereals, has been reported to have relatively good amino acid pattern and its PER value varies from 1.38-2.56 depending on variety (Juliano 1972). In present study, the PER in range of 1.86-1.98 could be achieved for rice diets varying in treatments, with protein adjusted to 6%. PER tended to decrease with treatment, though the differences are statistically non-significant. The decrease in PER was more for traditional parboiling as it involved severe treatment. This might have been due to loss of certain water soluble protein fractions during the processing treatment, as reported by Anthoni Raj and Singarvadivel (1980), but the findings need further verification by carrying out a detailed study on this aspect.

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Studies on Microorganisms Associated with Prawn Pickle

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Microorganisms associated with prawn pickle, stored at ambient temperature (30±2°C), were studied for 270 days at regular intervals. A decrease in total viable count up to 60 days and a slight increase thereafter were observed. *Bacillus* spp. were the dominant group comprising more than 50% of the total population, followed by *Micrococcus* spp. The population of anaerobic spore-formers increased with the increase in storage period. All the bacterial isolates were positive for lipid hydrolysis, while only *Bacillus* spp. were able to liquefy gelatin. The initial bacterial flora were sensitive to low pH and higher salt levels, in contrast to higher salt and acid tolerance by the surviving bacterial flora during storage.

Keywords : Pickling, Prawn pickle, *Bacillus*, *Micrococcus*, Anaerobic spore-former, *Staphylococcus*.

Pickling is an ancient form of fish preservation. Pickled prawns are considered a delicacy and are generally expected to have a shelf-life of more than 6 months at ambient temperature, without any harmful bacteria being present (Chandrasekhar 1979). Several reports are available on various aspects on bacterial spoilage of prawn during storage, the preparation of pickled products and their quality changes during storage (Chandrasekhar et al. 1978; Chandrasekhar 1979; Thampuran and Gopa Kumar 1991; Chaudhari and Bose 1971). However, little information is available on the microbiology of pickled products during storage. Karunasagar et al (1989) have reported the microorganisms associated with spoiled prawn pickles. The present communication reports the result of a study on the microorganisms associated with the prawn pickle during storage at ambient temperature and their tolerance to various levels of pH and salt.

Frozen, peeled and undeveined, mixed prawns of the species *Parapenaeopsis stylifera* and *Penaeus indicus*, procured from local processing plant, were thawed and deveined. Prawn pickle was prepared from these mixed prawns using standard recipe and methodology developed by this Institute (Abraham and Jeyachandran 1992). The pickle was packed in clean dry bottles of 250 g capacity, sealed airtight and stored for a period of 270 days at ambient temperature (30±2°C). Before sealing, care was taken to prevent the exposure of meat and exclude the growth of aerobic moulds and yeasts for which a layer of oil was ensured at the top to cover the solids. Samples were drawn at monthly intervals for analyses.

Titratable acidity as % acetic acid and salt content of the pickled prawn were determined by AOAC (1975) methods. The pH was measured after homogenising 10 g meat in 50 ml distilled water. Total viable counts (TVC) and aerobic spore-formers (ASF) were enumerated on tryptone-glucose-yeast extract (TGYE) agar (Speck 1976). Anaerobic count (ANC) and anaerobic spore-formers (ANSF) were enumerated by MPN black tube technique using differential reinforced clostridial medium (Collins et al. 1989). Isolates on TGYE agar plates were picked, purified and identified up to generic level as per standard key (Lechavellier et al. 1980) and percentage composition calculated from the total population. The isolates were tested for gelatinolytic and lipolytic activities (Collins et al. 1989). Salt (sodium chloride) and pH tolerance of selected isolates were examined using nutrient broth (Salle 1961) with varied pH (2.8 to 10.0) and salt (0 to 16.5%) levels.

The pH of the prawn pickle ranged between 4.71 and 4.79, while the salt content ranged between 3.53 and 4.03% during the entire storage period. Titratable acidity, however, increased marginally in first 4 months and remained constant thereafter. The total viable counts decreased in first 60 days and then showed a slight increase (Table 1). The aerobic spore-formers comprised more than 50% of the viable bacterial count throughout the storage period. These are also reported to be dominant in fish pickles (Chandrasekhar et al. 1978). The counts of anaerobes and anaerobic spore-formers increased with period of storage. The air-tightness of pickle bottles might have resulted in a partially anaerobic conditions in the pickle to favour the growth of anaerobes. The ANC and ANSF

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TABLE 1. BACTERIOLOGICAL CHARACTERISTICS AND TITRATABLE ACIDITY OF PRAWN PICKLE

Storage, days	Counts/g				Bacterial composition,%			Titratable acidity, % acetic acid • ($x10^{-1}$)
	TVC	ASF	ANC	ANSF	*Bacillus* sp.	*Micrococcus* sp.	Gelatin liquefiers	
0	$1.6x10^3$	$9.0x10^2$	<2	<2	58.8	23.5	58.8	2.5
30	$5.5x10^2$	$4.5x10^2$	2	2	85.7	14.3	85.7	2.8
60	$1.0x10^2$	$6.0x10^1$	20	20	75.0	25.0	75.0	3.1
90	$1.2x10^2$	$6.5x10^1$	32	32	52.6	47.4	52.6	3.0
120	$1.0x10^2$	$8.0x10^1$	278	278	80.0	20.0	80.0	3.3
150	$1.2x10^2$	$9.0x10^1$	345	345	80.0	20.0	80.0	3.3
180	$2.0x10^2$	$1.0x10^2$	390	390	50.0	50.0	50.0	3.2
270	$4x4x10^2$	$2.7x10^2$	345	345	61.0	39.0	61.0	3.3

TVC = Total viable count, ASF = Aerobic spore-formers, ANC = Anaerobic count, ANSF = Anaerobic spore-formers. • Values are mean of four determinations. Standard deviation values ranged between ± 0.001 and ± 0.008. The pickle samples contained 17.7% of *Staphylococcus* spp., immediately after preparation and 94 to 100% of lipase producers throughout the storage.

counts were the same during storage. These observations suggest that the anaerobes present in prawn pickle are spore-formers. Occurrence of anaerobic *Clostridium perfringens* type organisms, in low numbers, in oil-based egg pickles (Swamy et al. 1978), and anaerobic spore-formers in commercial prawn pickles (Abraham and Jeyachandran 1993) have been well documented.

Immediately after preparation of the pickle, the population was dominated by *Bacillus* spp., followed by *Micrococcus* spp. and *Staphylococcus* spp. On subsequent sampling, no *Staphylococcus* spp. were encountered. Neither the *Bacillus* spp. nor the *Micrococcus* spp. followed a definite pattern during storage. Their percentage composition varied between 50 and 85 for *Bacillus* spp. and 14.3 and 50 for *micrococcus* spp. All isolates of the genera *Micrococcus* and *Bacillus* were found to show lipolytic activity, while only *Bacillus* spp. showed gelatinolytic activity (Table 1). Thus, the inherent microflora may bring about some changes in the protein and lipid components of the prawn pickle. The hydrolysis of lipids by microbial lipases have been reported to cause serious quality deterioration in seafoods (Khayat and Schwall 1983). Usha (1983) reported a rapid hydrolysis of groundnut oil, when used as a pickling recipe, by *Bacillus* spp.

TABLE 2. THE CHANGING BACTERIAL FLORA IN PRAWN PICKLE AS INDICATED BY LAG PHASE IN DAYS

Isolates	Acidophilic and halophilic nature of selected isolates			
	Immediately after pickling		90 days after pickling	
	pH 4.4	Salt, 10.5%	pH 4.4	Salt, 10.5%
Bacillus 1	4	5	1	2
Bacillus 2	4	3	1	3
Bacillus 3	ND	ND	2	2
Micrococcus 4	3	6	2	1
Micrococcus 5	3	5	2	1
Micrococcus 6	ND	ND	1	1
Staphylococcus 7	4	1	ND	ND
Staphylococcus 8	3	1	ND	ND

ND : Not done

The selected pickle bacterial flora showed longer lag phase at lower pH (4.4) and at higher salt (10.5%) levels immediately after pickle preparation, as compared to those of 90 days after pickling (Table 2). These observations reveal that the initial bacterial flora are sensitive to low pH and higher salt levels. The results of this study are in accordance with Karunasagar et al (1989). According to them, both acid and salt tolerant bacteria are responsible for spoilage of prawn pickle.

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Influence of Antioxidants on the Sensory Quality and Oxidative Rancidity of Frozen Edible Oyster

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Sensory acceptability and oxidative rancidity of antioxidant-treated edible oyster, frozen at -40°C and held under frozen storage (-18°C) condition, were investigated. The sensory acceptability declined steadily during storage and was accompanied by an increase in the oxidative rancidity of the product. Untreated and butylated hydroxy toluene (0.2% w/w) treated samples developed dark discolouration after two months of storage. Addition of clove powder (0.20% w/w) significantly reduced the oxidative rancidity, measured as thiobarbituric acid reactive substances, and improved the sensory acceptability of the product.

Keywords : Frozen edible oyster, Antioxidants, Butylated hydroxy toluene, Clove powder, Oxidative rancidity.

Lipid oxidation during frozen storage is one of the major causes of deterioration in the quality of processed bivalve meat (Ablett and Gould 1986). In our earlier paper, we reported the quality characteristics of freeze-dried edible oyster *Crassostrea madrasensis* (Preston) (Indra Jasmine et al. 1990). Attempts have also been made to reduce lipid oxidation through the use of antioxidants (Ablett et al. 1986; Jeong et al. 1990). It has been reported that oxidative deterioration of meat lipids can directly affect many quality characteristics such as colour, flavour, texture, nutritive value and safety (Pearson et al. 1983, Pawar and Magar 1967). The present study was aimed at comparing the sensory quality and oxidative rancidity of farmed edible oyster in control and antioxidant-treated samples.

Live farmed edible oysters, *Crassostrea madrasensis* (Preston), were collected from the oyster farm of Central Marine Fisheries Research Institute, Tuticorin. The oysters were depurated in running water for 24 h and shucked by placing in boiling water for 5 min. The meat was separated manually from shells, washed well and kept in iced condition until freezing. Before freezing, the oysters were treated differently. The first treatment was with 0.02% (w/w) butylated hydroxy toluene (BHT) as described by Jeong et al (1990). The second treatment was with 0.20% (w/w) fine clove (*Syzygium* sp.) powder. Finely powdered clove bud was directly added to the oyster meat, thoroughly mixed and packed. No preservatives were added (untreated) in one lot for comparison purposes.

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The treated and untreated oysters were packed (300 g/pack) in low density polyethylene (LDPE) filmic bags and frozen at -40°C for 90 min in contact plate freezer. The frozen blocks were packed in waxed cartons and stored at -18°C for 5 months. The samples in duplicate were periodically drawn and thawed in running water (30±1°C) for 60 to 90 min. Moisture and lipid contents were determined by AOAC (1975) methods. Oxidative rancidity, measured as 2-thiobarbituric acid (TBA) reactive substances, was determined by the modified method as described by Woyewoda and Ke (1979) and expressed as μ moles of melanoldehyde/kg oyster. The free fatty acid (FFA) was estimated by titrimetric method (Ke et al. 1976) and expressed as % oleic acid/kg fat. For sensory evaluation, the oyster meat samples were cooked in 2% sodium chloride solution for 5 min and served to a group of 6 to 7 semi-trained panelists. The sensory attributes such as colour, odour, taste, texture, flavour and overall acceptability were recorded using a 5 point Hedonic scale (excellent 5, good 4, fair 3, acceptable 2, and unacceptable 1) in the open laboratory. The scores for each attributes were pooled and mean sensory scores calculated.

ANOVA was used to determine the significance of difference among samples. A simple correlation was used to correlate (a) the mean sensory scores for overall acceptability scores (OAS) with TBA, FFA and storage period and (b) storage period with TBA and FFA for all samples (Snedecor and Cochran 1962). A linear regression of mean sensory scores for OAS and storage period was used to determine the shelf-life.

The moisture content of frozen oyster meat

samples fluctuated between 76.23% and 79.15%, while the total lipid content ranged between 2.37% and 2.46% during the storage. No measurable desiccation of oyster meat occurred throughout the storage period at −18°C. A progressive increase in FFA levels was observed with period of storage (P<0.01). The results presented in Table 1 are in agreement with those of Ablett et al (1986). The TBA values in treated and untreated samples showed an increasing trend (P<0.01), thereby indicating lipid oxidation during frozen storage. The TBA value was high (P<0.05) in the untreated, followed by BHT and clove-treated oyster meat samples. The results (Table 1) demonstrate that the antioxidant treatment had an impact on the stability of the oyster lipids, the sample treated with clove being more stable. Jeong et al (1990) reported that natural vitamin E acted effectively as an antioxidant on the lipids of the Japanese oyster, as compared to BHT during frozen storage. In this study, the clove powder was found to be an effective antioxidant and the oxidative rancidity was retarded significantly (P<0.05).

The OAS for the frozen oyster meat samples showed a decreasing trend with period of storage. A highly negative correlation existed between OAS and storage period (P<0.01). These results confirm the previous observations that the sensory acceptability of bivalves, held under frozen storage conditions, decline steadily (Ablett et al. 1986; Jeong et al. 1990). A significant difference in OAS (P<0.01) among samples was observed. The clove treated oyster meat was rated good compared to the other two samples. Since the colour of the clove-treated oyster meat was light brown, this attribute did not secure good scores. With respect to taste, texture and flavour, clove-treated sample scored high, followed by control sample (Table 2). Clove contains high amount of eugenol, an antioxidant, and a number of flavour enhancing components (Gopalakrishnan et al. 1990) and these might have improved the acceptability of the product. It was noticed that the untreated and BHT-treated samples developed dark discolouration after two months of storage. Such trend was also reported by Jeong et al (1990). The dicolouration observed in this case may be due to interaction between the hydrolysed products of lipids and

TABLE 1. FREE FATTY ACID (FFA) AND THIOBARBITURIC ACID (TBA) VALUES OF FROZEN STORED OYSTER MEAT

Storage, days	FFA, % oleic acid/kg fat			TBA, μ moles melanaldehyde/kg oyster		
	BHT-treated	Clove-treated	Untreated	BHT-treated	Clove-treated	Untreated
0	0.8	0.9	1.0	7.4	8.9	8.7
30	1.8	1.5	2.5	8.5	9.0	9.8
60	2.1	1.9	2.6	12.0	10.0	11.8
90	2.7	2.5	3.6	16.5	13.0	17.0
120	4.3	3.5	5.0	19.3	14.0	20.5
150	4.9	3.9	5.1	21.5	14.0	22.3
SD range	±0.00 to ±0.42	±0.06 to ±0.66	±0.00 to ±0.56	±0.15 to ±0.41	±0.02 to ±0.45	±0.13 to ±0.87

Values mean of four determinations, SD = Standard deviation.

TABLE 2. SENSORY EVALUATION OF FROZEN STORED OYSTER MEAT

Storage, days	Attributes																	
	Colour			Flavour			Odour			Taste			Texture			Overall acceptability		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0	4.5	3.8	4.5	4.3	4.8	4.0	3.8	4.8	3.8	3.5	4.8	4.0	3.8	4.8	4.4	4.1	4.4	4.2
30	4.5	3.5	4.5	3.8	4.5	3.8	3.8	4.8	3.8	3.5	4.6	3.8	3.8	4.3	4.0	4.0	4.3	4.1
60	4.0	3.5	4.0	3.6	4.3	3.8	3.8	4.6	3.6	3.4	4.5	3.8	3.8	4.2	4.0	3.8	4.1	3.9
90	3.8	3.2	4.0	3.4	4.2	3.5	3.4	4.5	3.6	3.3	4.2	3.8	3.8	4.0	4.0	3.6	3.9	3.9
120	3.8	3.0	4.0	3.3	4.0	3.3	3.2	4.3	3.6	3.2	4.0	3.6	3.6	4.0	3.8	3.5	3.8	3.7
150	3.4	3.0	3.8	3.2	3.6	3.2	3.0	3.5	3.4	3.2	3.6	3.4	3.5	3.5	3.5	3.3	3.5	3.4

A = BHT-treated B = Clove-treated and C = Untreated

protein components of oyster.

A significant (P<0.01) negative correlation was observed between TBA and OAS, and FFA and OAS for all the samples (Table 3). The results reveal that oxidative rancidity does play an important role in declining sensory quality and acceptability of frozen oyster meat. The clove-treated oyster meat was estimated to be acceptable for 278 days as compared to 235 and 237 days for BHT-treated and untreated samples, respectively, based on TVB-N levels (Balasundari 1992). However, based on the linear regression of OAS and storage period, all the samples were estimated to be acceptable for more than 12 months. Thus, the clove powder could be effectively used as an antioxidant in frozen oyster meat.

TABLE 3. CORRELATION COEFFICIENTS BETWEEN DIFFERENT PARAMETERS

Parameters	Correlation coefficient (r)		
	BHT-treated	Clove-treated	Untreated
TBA vs OAS	-0.990*	-0.937*	-0.933*
FFA vs OAS	-0.961*	-0.975*	-0.916*
OAS vs storage period	-0.989*	-0.990*	-0.971*
TBA vs storage period	0.989*	0.950*	0.962*
FFA vs storage period	0.980*	0.990*	0.975*

* P < 0.01

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Lactic Acid Bacteria : Edited by S. Salminen and A. von Wright, Published by Marcel Dekker Inc., 270, Madison Avenue, New York, NY 10016, USA, 1993; pp 456; Price US $ 165.00

Lactic acid bacteria (LAB) have an important role in food preservation. Besides, they are known for their therapeutic properties and beneficial health effects. The growing biotechnological processes in the developing world have opened up new vistas of research, covering technological aspects of LAB. These aspects are to be established, after a thorough study is undertaken on a large number of species and strains of this group of bacteria. On the basis of above-mentioned needs, there is requirement for a critical appraisal of the current developments in the study of LAB. It is to the credit of Dr. Seppo Salminen and Dr. von Wright, the internationally recognised experts, that a complete book on lactic acid bacteria has been edited and published as a reference book under the series of Food Science and Technology. The editors have put forth all their efforts to provide an impressive and much-needed reference book, illustrating the current developments of LAB, which are spread over 14 chapters and has 1300 literature citations. This has been made possible by the valuable contributions from 24 internationally recognised experts. A glance at the contents gives a glimpse of the different aspects of LAB in relation to foods and nutrition, which have been extensively covered in this book. It has been published by Marcel Dekker, Inc., using acid-free paper as per their tradition, in printing of books.

The chapter on classification and physiology of LAB, presented by Axelsson, highlights recent taxonomic revisions suggesting that LAB comprise the following:*Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus and Vagococcus*. The basis for classification such as morphology, mode of glucose fermentation, growth at different temperatures, configuration of the lactic acid produced, ability to grow at high salt concentrations of the lactic acid produced, ability to grow at high salt concentrations and acid or alkaline tolerance have been presented in detail. Besides, the chapter includes some of the additional characteristics such as fatty acid composition and motility used in classification. A new dimension added to this aspect of study has been the measurements of true phylogenetic relationships with rRNA sequencing, which has a role to play in classification. New tools for classification and identification of LAB have been brought into focus, of which, the most promising for routine use are nucleic acid probing techniques, partial rRNA gene sequencing, using the polymerase chain reaction and soluble protein patterns.

Mayra-Makinen and Bigret have given an in-depth knowledge about the various industrial uses of LAB, particularly those of mesophilic and thermophilic starters, which are used by dairy industry. Two problems namely, (i) to identify the corresponding genetic determinant of certain physiological characteristics and (ii) to solve regulatory problems and constraints, as far as genetically modified microorganisms are concerned, as put forth by the authors to the research community, are the highlights of this chapter. The stability of LAB in fermented milks, presented by Lee and Wong, emphasises the need to understand the factors determining the shelf-life of milk-based products, wherein the stability could be improved through product development and genetic manipulation of cells. This chapter covers the major LAB used in fermented milks and the factors affecting survival of cells in milk products. The chapter on LAB in cereal-based products by Salovaara presents the significance of lactic acid fermentations as an integral part of cereal food processes. In comparison to dairy processes, the research input on LAB in cereal food processes is nominal. In this background, the author has projected the need to carry out research on the traditional fermented cereal foods, so as to improve quality and attractiveness of these foods as economical and nutritious staple foods.

The potentiality of LAB in food preservation has been attributed to the production of antimicrobial substances. Davidson and Hoover have presented very pertinent information relating to chemical or biochemical properties, antimicrobial spectrum, factors affecting activity, mechanism of action, genetics of production and potential application of the various inhibitors produced by species of *Lactococcus, Pediococcus, Lactobacillus* and *Leuconostoc*. It is worth mentioning the aspect of concern, put forth by the authors, relating to the development of bacteriocin-resistant subpopulations of foodborne pathogens. The

potential impact of this resistance needs to be evaluated, prior to wholesale use of these compounds in foods. Considering the advances made in genetic engineering, Wright and Sibakov have provided a comprehensive presentation of genetic modification of LAB through the sections of plasmid biology, gene transfer, genetic recombination, DNA repair and gene cloning. Most of the information presented relates to *lactococci*. The authors have highlighted the need to document more information relating to *lactobacilli*.

The chapter on LAB in health and disease, by Salminen et al, presents a highly resourceful information about requirements of probiotic strains, mechanism of action of LAB as probiotics and studies on LAB and health. The section on influence of yoghurt-containing preparations on the side effects of radiotherapy adds a new dimension. The information on the metabolic activities of *lactobacilli* inhabiting GI tract, with particular reference to intestinal drug and cholesterol metabolism, presented by Lichtenstein and Goldin, is worth reading in the light of the present day health problems. A highlight of this chapter, has been the role played by fermented and non-fermented dairy products on plasma cholesterol. The chapter by Mikelsaar and Mändar brings into focus the current state of knowledge regarding the establishment, survival and stability of specific LAB in the human microbial ecosystem. Salminen et al. have attempted to provide an outline into the aspects concerning growth of intestinal *lactobacilli* and bifido-bacteria, in relation to carbohydrate substrates. In the light of the above beneficial effects of LAB, which have been very well accepted, the presentation given by Donohue et al. about toxicity of LAB, gives ample impetus to research work on toxicity studies in animal models for newer strains, especially those derived by biotechnological processes.

The subject of LAB as animal probiotics has been elaborately presented by Nousiainen and Setäta, with an emphasis on gut microflora in pigs and calves. An outstanding feature of this chapter has been the information relating to potential of action of LAB as biological performance enhancers. The proposed new idea of "mixed probiotics", based on a combination of strains and non-absorbable sugars, is an area for creative research. The rapid advancement in the field of biotechnology has focussed a lot of research attention on *Bifidobacterium*. It is to the credit of Ballongue, for presenting an extensive literature, highlighting important functional aspects such as physiology, metabolism, resistance to antibiotics, influence of ecological factors and probiotic role. In conclusion, the editors have projected very important future trends in research on LAB and product development, with emphasis on application of new molecular taxonomic criteria and industrial applications of molecular genetics research of LAB, relating to dairy, probiotics and antimicrobials.

This book, the first of its kind, devoted entirely to lactic acid bacteria is rich store-house of information about a rapidly changing and extremely important area of food and nutrition research. This volume, with latest literature citations, provides a better understanding about the potential of LAB in new applications and especially of their role in health and diseases. The book is indispensable to all those working with LAB and fermented foods as well as feed products and is an essential resource for food scientists and technologists. It will be an asset to possess this book in R & D Institutions, Universities, other organizations connected with foods as well as feeds and the Governmental agencies involved in developing regulatory policies for health products based on LAB. Finally, this book would serve as an important introduction to any student or scientist of food science and technology interested in these developments.

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Antimicrobials in Foods : Second Edition, revised and expanded : By P Michael Davidson and A Larry Branen, Published by Marcel Dekker, Inc., 270, Madison Avenue, New York, NY 10016, USA, pp 648, 1993; Price: US$ 195 (Price subject to change without notice)

The interest in antimicrobials and the role they play in the food industry is unquestionable. Despite the conflicting views on their safety, the antimicrobials will continue to be one of the most important classes of food additives, until some other equally potential method takes over their place. Keeping this in view, the revised and expanded book on *"Antimicrobials in foods"* is a timely and useful reference book for researchers, technologists, regulatory agencies and students working in the field.

All the 17 chapters appearing in the book are of high quality, giving up-to-date information on various aspects of antimicrobials. The authors have

exhaustively surveyed literature on every topic and have ably presented them in a very comprehensive manner. The format adopted for each chapter is excellent and highly acceptable to all classes of readers.

The chemical and physical properties of additives included in the book are brief and at the same time appropriate. The book also provides useful information, such as the antimicrobial spectrum, minimum inhibitory concentration, LD_{50} values and various applications of additives. The brief account of assay methods and a newly added chapter on "Chapter for Evaluation" will be of immense benefit to academics, industrial and regulatory researchers to standardize, compare and maintain uniformity in testing methods.

The chapter on the most common chemical preservatives like benzoic acid, sorbic acid, sulphur dioxide and parabens is exhaustive. The research history on nitrites has been presented in the chronological order, which will enable the reader to understand the conceptual changes that have taken place in their uses over the years. It will also help the researcher to plan the future work in the area. Nisin, which was only of academic interest about 10 years back, when the first addition of this book was published, has been given considerable importance in the revised edition. The chapter covers various aspects, including genetics, toxicity and legal aspects of nisin. Mention has also been made on the possibilities of modification of this peptide antibiotic for wider use by gene transfer technique in future.

As the search for natural antimicrobials is a continuing process, chapters on natamycin, bactderiocins and other naturally occurring compounds are timely and very appropriate. These developments will, of course, open more and more avenues for new generation antimicrobials.

In short, it is a book written by internationally renowned authors in the field, with special emphasis on developments in chemical antimicrobials.

The book is a must to the food processors, researchers and teachers, for it can be used as a reference book. The editors and the contributing authors deserve sincere appreciation for their efforts in bringing out this revised edition.

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Rice Process Technology: by S. Bandyopadhyay and N.C. Roy, Oxford & Publishing Co. Pvt. Ltd., New Delhi, 1992, pp : 204, Price : Rs. 125/-.

This book has been written as a text book to engineering students and practising engineers. Though books on rice technology are available in the market, an attempt made by the authors to write a text book from engineering point of view is a welcome step. The book is divided into several chapters viz., introduction, properties of rice, fundamentals on parboiling, parboiling processes, drying of paddy, milling of paddy and rice bran as well as rice bran oil. The authors rightly lay more emphasis on the parboiled rice, as it conserves not only the rice resources, but also the nutritional quality of the rice. This is yet another need-based attempt.

Though the book is profusely illustrated both with figures and tables, to make the students and practising engineers to easily grasp and understand the subject, a thorough reading of the text makes one think that the book was prepared in a haste. Information on the recent literature is scanty. The method of presenting the matter in three forms (text book, reference book and general book styles) at different places also mars cogency.

The lack of preparatory work has led to a lot of mistakes and misconception of the subject. The knowledge, made available on different aspects of rice processing technology during the past 20 years, is sparsely reflected in the book.

Errors in reporting, presentation of data, references, etc. are numerous. For example, Jayaraman has not carried out any work indicated in Fig. 3.4. The authors' name of ref. 20 in chapter 3 has been wrongly quoted. References 11 and 10 of chapter 3 are one and the same. The title of ref. 6 reads as 'Students of swelling and hydration' (p 38). Reference 2 of chapter 3 does not contain any basic work as reported in section 3.1.1. Similarly, reference 11 of chapter 2 does not contain the matter reported in p 33. Contents of tables 3.3 are not from the paper of Bhattacharya and Subba Rao (1966). References with Ghose as first author are not provided, though cited in Table 3.11 and Fig 3.13. References 25 and 26, just before equation 3.8, are not of Ghose et al. The uniform standard of classification suggested by the Government of India and the data given in Table 2.10 are different. Source for table 2.10 is not given.

The dissolution of oil globules during soaking (3.3.4) has been disputed as oil is immissible in water. First sentence of p 26 needs a change - foreign matters do not include varietal admixture and brokens. Section 2.5.3 says that colour is the more stable characteristic of milled rice. It is not correct. Last sentence of 2nd para of p 44 conveys that protein content drops in parboiling, whereas data in Table 3.2 show more protein in milled parboiled rice (p 41). To avoid confusion among students, it can suitably be edited. The values of Km in Table 3.10 are shown as km x 10^3 whereas in Illustration 3.1, as km x 10^{-3}.

It is stated on p 8 that colour is imparted to rice in traditional parboiling and can be improved by modern methods; but strong colour occurs in high temp. soaking (p 51, 3rd line). Signs of fermentation have also been seen in hot soaking process (4.1.3 (1) 5th line); whereas line 3 on p 173 describes hot soaking as improved method. It is further indicated that the fermentation due to longer period of soaking also imparts brown colour to rice (p 96 (2)). In practice, however, the colour of parboiled rice produced in traditional process is better. While describing hot water soaking (p 101), temperature of upto 75°C is indicated, whereas ideal temperature for avoiding bursting/colour (p 60) is 16-70°C. Page 52 indicates that, if a rice of low colour is desired, the soaking temperature should be below 70°C. Table 4.2 and 9th line of p 112 indicate a temperature of 65-70°C.

As per section 3.7, well soaked paddy contains 40-50% moisture db, whereas optimum moisture for parboiling as per references 6 and 27 as well as and Table 3.9 is 50-55% and it is not known as to why soaked paddy is to be heated to 105°C in parboiling. Further, it is indicated that there should be no loss of moisture from the grain during parboiling. What does it mean? It is a known fact that moisture is lost during gelatinization in some parboiling processes. There is no mention about pressure parboiling, sand parboiling and other techniques of parboiling developed in India. The statement 'tempering gives satisfactory gelatinization even with moisture less than optimum level (p 57) is not correct. Husk splits at 65°C and above (p 58, 12th line). It may be true as observed in reference 6. Does it mean that husk splits in all grains in CFTRI process? Further, items (a) and (c) of p. 58 convey different views on husk splitting. The definition of parboiling (3.1.3) gives the impression that the pretreatment is given to threshed paddy before taking to storage. But in practice, most of the paddy parboiled represents stored paddy. There is some confusion about gelatinization. P 38 says that gelatinisation is a mild form of chemical change, whereas first line of 3.3.1 says it is a physico-chemical change. Since performed gelatinization could be reversed, it can only be considered as a physical change. There is no alkali spreading and *cleaning* test (p 54). This reaction does not show distinctive difference between the raw and parboiled, as indicated. The alkali reaction test of Ali and Bhattacharya (1972) alone distinguishes raw and parboiled rice. It is indicated that the differentiation is recognized only after 24 h. It is not correct, as it could be recognised even at a shorter time. A quantitative alkali test is available.

Numerous direct and indirect objective tests exist for indicating tenderness and cohesiveness of parboiled rice. At least, a passing reference would justify section 3.5.3.

Drying of parboiled paddy does not begin at a temperature of about 100°C (p 142) as the handling, and conveying operation prior to its receipt at drying site dissipates the heat appreciably.

The account on the terms and steps of conditioning and tempering from p 145 through p 148 is confusing (indicated as without cooling, in other place indicated as tempering or cooling, in yet another place heat must be dissipated-spreading out in well-ventilated storage). An opportunity should have been provided to the engineering students to know something about the dryers developed in India (RPEC recirculatory dryer, cup and cone dryers, conduction drying, etc.)

In p 159, it has been indicated that hullers are of 250-500 kg capacity. Hullers with capacity of 1 tonne and more are also in operation. Contents of Tables 6.1 and 6.2 provide contradictory information about home-pounding. Last para of p 162 states that huller yields 6-8% less yield of rice, whereas table 6.4, which follows, does not show a loss up to 8%. All these require thorough editing. Versions in p 169 through 180 give confused concept of whitening, polishing and machines used (e.g., cone-shaped abrasive machines are not mounted horizontally (4th line, p 169 and 14th and 15th lines of p 170). Polishing machines are fitted with leather strips - the terms friction and abrasive whiteners, friction-type polisher, polishing machine. Para 3 of p 170 indicates that Indian condition permits polish only about 4%, whereas 6th line of p 163 indicates not more than 5% removal of bran.

In practice, private millers and public sector organisations (e.g., TNCSC) resort to more than 6% polish. Item 4 of p 174 says that 'Co 25' gives 23-25% of head rice in modern rice mill (it gives the impression that the performance of modern mill is poor for such varieties). Parboiled bran contains 17-18% moisture (p 182). Does it mean that the rice during milling contains over 20% moisture?

Chapter 7 has been written with reference to Indian scenario, prevailing some 15 years back. The potentiality for rice bran oil has been indicated as 3.5 lakh tonnes as against 8 lakhs tonnes. The actual production of rice bran oil in India now is nearly 4 lakh tonnes but indicated as 1.4 lakh tonnes. Techniques exist to pelletise parboiled rice bran without any additives or raw rice bran. The authors need not recommend the addition of raw rice bran with parboiled rice bran. Fourth para of p 201 indicates that importers specify sand silica contents of not less than 14% for extracted meal. It is wrong. Chapter 2 needs condensation and writing in text book style. The materials provided are confusing.

A lot of mistakes (spelling and words) have crept in throughout (e.g. *forward* (p viii), *nutrional Griest, scriptures, micelle unsaponifiable water*, polished rice *seeds*). In page 20, it is indicated that a 100 g protein of white rice can supply 0.084 mg thiamin, 0.026 mg riboflavin and 1.9 mg niacin. What does it mean? Few sentences are reproduced more than once. In first line of introduction, the botanical name of rice has not been properly printed.

On the whole, it is felt that the book requires a thorough checking, editing and revision. If issued in the present form, the engineering students and practising engineers would get a rather confused information.

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Toxicological Evaluation of Certain Veterinary Drugs Residues in Food : Published by International Programme on Chemical Safety (IPCS). The monographs prepared by Joint FAO/WHO Expert Committee on Food Additives (JECFA). Price : Sw. fr. 40 and for developing countries : Sw. fr. 28.

This book is toxicological monograph summarizing safety data on veterinary drugs. The first chapter comprises detailed information on toxicological data on four anthelminthic agents namely, flubendazole, ivermectin, thiobendazole and triclabendazole.

The second chapter deals with two antimicrobial agents namely furzolidone and nitrofural. The third chapter contains information on two production aids - bovine somatotropins and ractopamine. The fourth chapter is about trypanocideisometamidium.

For each drug, detailed information on the absorption, distribution, excretion and biotransformation in different animal species like rats, dogs, mice, rabbits, pigs, cattle, horses and goats is provided. In addition, data on acute, short term, long term toxicity studies in various animal species are included in each chapter. Special studies on genotoxicity, reproduction studies are also available for each drug. Information on safety and efficacy of certain drugs on children, adults and pregnant women is available. The book also provides important information on ADI values for the above veterinary drugs.

This book is very informative and useful for those engaged in veterinary drugs, government and food regulation agencies, industrial testing laboratories, toxicity testing laboratories, veterinary colleges and universities.

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Environmental Health Criteria 145 : Methyl Parathion Published by WHO 1993; p 244, Price 28 Swf : for developing countries Swf 19.6.

This book on the organophosphorus insecticide methyl parathion is published under the International Programme on Chemical Safety (IPCS). It consists of 244 pages and the contents are covered under 10 chapters. It broadly covers the following aspects. (i) Physico-chemical properties and analytical techniques, (ii) Environmental exposure, disinfestation and metabolism, (iii) Effect of methyl parathion on microorganisms, animals and humans and (iv) references.

The first two chapters of the book give an outline of the physico-chemical properties of the chemical and an extensive account of the analytical methods. The analytical methods deal with sampling, extraction, clean-up for residues from different sources such as plants, body fluids, soil, water, air and formulations. A detailed account of analytical instrumentation includes TLC, GLC, HPLC, spectrophotometers, polarography, and GC-MS. Chapters 3 to 6 deal with the environmental

exposure and distribution of methyl parathion in air, water, soil and its entry into the food chain. Biotransformation includes biotic, abiotic degradation, degradation in aquatic environment and bioaccumulation. Information on interaction of this chemical with the various physical, chemical and biological factors and its ultimate fate is also dealt within these chapters. The levels of methyl parathion and its metabolites in air, water, soil, food and organisms are also listed. The absorption, distribution and metabolic pathway, the various enzyme systems involved are also discussed in detail with special reference to mammals.

Effects of methyl parathion on microorganisms, aquatic and terrestrial plants and animals are discussed in chapter 7. The toxicity data ares summarised in a comprehensive table giving information on the species, lethal dose level, experimental conditions, test period and the storage at which it affects. Chapter 8 deals with the effect on experimental animals (*in vitro* and *in vivo*) and chapter 9 is on the exposure of general population, acute and long term effects and epidemiological studies.

The reference section is extensive and up-to-date (1991) having 625 references with the latest reference being that of 1991. At the end, the important aspects of treatment of OP poisoning in man is given as Annexure I.

The book undoubtedly will serve as a good reference and ready reckoner, It covers most aspects in relation to the environment and provides most of the important information in easy-to-use tables. The book is useful to decision makers, researchers and students.

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Quality Assurance in Tropical Fruit Processing: *By* A. Asker and H. Treptow. Published by Springer - verlag, Berlin Heidelberg. 1993, pp. 238, Price. Not mentioned.

This book on Quality Assurance in Tropical Fruit Processing is a very timely publication. The tropical fruits and their processed products are gradually finding an important berth in the international market. Throughout the world, people are becoming extremely quality conscious and the quality assurance in food products has become the order of the day. The book is written in nine chapters covering the following important topics 1) Quality Assurance Management, 2) Analytical Methods, 3) Physical Measurements, 4) Sensory Analysis, 5) Microbiological Analysis, 6) Water Control, 7) Sanitation Control, 8) Waste Disposal Control and 9) Assessment and Improvement of Quality.

The chapter Quality Assurance Management discusses covering personnel requirements, organisation and function, and layout and laboratory requirements. In this chapter, the author has highlighted the importance of qualification and experience requirement of the quality assurance manager and also indicated duties and the great responsibilities of the quality control personnel. The quality assurance manager should have the same status and authority as that of the production manager and should be responsible to the top management of the organisation. The functions of quality control dept. are given very lucidly in this chapter. Information given on the layout of the quality assurance laboratory and equipment requirement is very useful. The analytical methods are covered in the second chapter, determination/ estimation of almost all the constituents of fruit are mentioned and instrumental analysis is given wherever applicable. It is surprising that estimation of tanins/total phenolics is not given in this chapter, tanin being an important constituent of tropical fruit. Determination of peroxidase activity is also not mentioned in this chapter, which is also important for detection of enzyme inactivity in tropical fruit processing. Chapter 3 covers the physical measurement aspects viz. colour, consistency, texture etc. The sensory analysis in chapter 4 includes organisation of tests, statistical test, design and selection and training of panel members which are important from the practical point of view. Chapter 5 gives precise details of microbiological analysis, covering all aspects. Water control, an important aspect in fruit processing, which is often neglected, is discussed in a very comprehensive manner in chapter 6. The sanitation control and waste disposal control is given in chapters 7 and 8. Sanitation and waste disposal control are very important from the points of view of health and environment. These two aspects are often considered as not important from the quality assurance point of view. It is nice to see that these two aspects are very well covered in this book. The last chapter gives the assessment and improvement of quality, highlighting the importance of inspection, quality control, quality management, quality assurance management from the point of view of

ISO 9000 and quality improvement.

The book has two appendices. Appindix 1 gives references, recommended readings including Books and Journals. It would have been easier for the readers if the references would have been given at the end of each chapter. In the list of journals recommended, the name of the Journal of Food Science and Technology of the Association of Food Scientists & Technologists (India) should have been mentioned. Extremely useful information is given in Appendix 2, covering the names of equipment/ substances, Type/Brand and their suppliers.

There are several books available on the analysis of fruits and vegetables and their products, but this book covers everything in a very comprehensive manner. It may be pointed out that an additional chapter on assessment of physiological parameters such as respiration, ethylene estimation etc., which play a key role in quality assessment would have made this book more useful. Though this book is written only for tropical fruits, it will hold good for almost all the fruits and vegetables. This book in the present form is recommended as a text or reference book for the students, research workers and teachers in the field of food science and technology, in general, and post harvest technology of Horticultural crops in particular. This will also be important reference book for the chemists and quality control managers of food factories based on fruits and vegetables.

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Technology of Reduced-Additive Foods: Edited by J. Smith Blackie Academic & Professional - An imporint of Chapman & Hall, Wester Cleddens Road, Bishopbriggs, Glasgow G64 2NZ, 1993: pp 249.

The food industry for many years reacted to consumer demand for more appealing and convenient food products by using additives. More recently, the demands of consumers have grown to include still higher performance products, but with less additives. The industry has responded accordingly. There are often significant scientific and technical obstacles to be overcome to make a product with less additives. It is against these technical challenges that this book is intended to address.

Chapter 1 covers starter cultures in dairy products, meat products and bread. Development in starter culture technology and ways in which starter cultures are replacing traditional additives in foods has been detailed. Interesting additives as anti-staling agents have been described.

Chapter 2 deals with a review on new animal-derived ingredients. Developments in meat *surimi* will be very interesting for research workers.

Chapter 3 addresses new marine-derived ingredients. Use of chitosan to form very thin extremely strong films or membranes is an interesting feature.

Chapter 4 is about reduced-additive bread making technology. Effect of oxygen on particular production has been illustrated.

Chapter 5 is about Novel food packaging. The scope of avoidance of additives is covered first, followed by properties of packaging materials, and packaging processes. Interesting features are development of packaging materials which can work as oxygen and ethylene scavengers and inclusion of permethrin in polypropylene to prevent insect attack; production of micro porous films for maintenance of MAP; use of ethylene vinyl alcohol copolymer coextrusions for longer aseptically packed foods. A plastic bottle named OXBAR totally impermeable to oxygen; concept of heat treatment to milk powder to provide natural anti-oxidant and adhesive oxygen absorbers have been discussed.

Chapter 6 evaluates the processing environment, processing methods, use of various alternative preservatives in relation to HACCP (hazard analysis critical control points).

Chapter 7 lists variety of ingredient plants and food plants, including members of leguminosae, fruits and nuts, culinary herbs and spices, essential oils, gums and starches.

Supplementation with vitamin-E, carotenoids, vitamin-C like food supplements and effect on the food of the animals is the subject matter of Chapter 8.

Detailed review on reduced-additive brewing and wine making with particular emphasis on reducing the use of sulphur dioxide has been covered in chapter 9.

To sum up, this book contains contributions from the Western world on developments in additives. It will prove to be a vital asset to all R & D institutions, Universities and other organisations with interest in foods and processing of foods. It will be a very valuable addition to any Library.

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INDIAN FOOD INDUSTRY

A Publication of Association of Food Scientists and Technologists (India)

Contents of January-February 1994 Issue

ANNOUNCEMENTS

HUMAN RESOURCE DEVELOPMENT FOR EMERGING FOOD INDUSTRIES - A SEMINAR

From 900-1800 hrs, on Saturday, 11.06.1994, at Taj Residency, Bangalore-560 001.

The Indian food industry is poised for a tremendous growth as is witnessed by the setting up of manufacturing facilities by such multinationals as Coca Cola, Pepsi, Kelloggs, Pizza Hut, McDonalds, Wimpy, BSN, Unilever, Heinz, Wrigleys, Mars M&M and others. Simultaneously, established Indian food processors are also gearing up to acquire a greater market share for their products and preparing strategies to face competition from internationally established consumer food product brands.

The Centre for Processed Foods and the Association of Food Scientists and Technologists (I), Bangalore Chapter, have felt that it is an opportune time to conduct a seminar on Human Resource Development for Emerging Food Industries on Saturday, the 11th June 1994 in Bangalore. Eminent professional managers from the food processing and the food machinery-manufacturing industries have been invited to offer their long experience in dealing with the lack of availability, or unpreparedness, of industries in absorbing or adopting the newer emerging technologies that will aim at excellence in food product quality, service and delivery. This seminar should be of great significance to both educational institutions and food industries in developing a cadre of food scientists/technologists, engineers who will be able to meet the challenges of the future.

Registration fee is Rs. 100/- for members and Rs. 250/- for non-members. For details, please contact Dr. R.R. Mohite, Secretary AFST(I), Bangalore Chapter, C/o ERG, 42/1, Palgprove Road, Austin Town, Bangalore 560 047. Ph: 5589910/564338. Fax : 5561992/5580706.

Association of Food Scientists and Technologists will be conducting a one day Colloquium on "GATT - an opportunity or challenge for Indian Food Industry" on 10th June 1994 at IFTTC auditorium, CFTRI Campus, Mysore. The following eminent speakers from different fields of specialization are likely to make their presentation.

1. Sri A.V. Ganesan - Ex. Commerce Secretary, Government of India.
2. Sri. P. Murari - Ex. Secretary to President of India.
3. Sri N. Subbaram - Advisor, Patents, CSIR.
4. Secretary, Ministry of Food Processing, Government of India.
5. Sri Sharad Joshi - President, Shetkari Sangathana.
6. Dr. M. Surendra Patel - Ex. Patent Advisor, Government of India.

Members are invited to attend the colloquium and take part in the discussions.

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I, Shri G.A. Krishna, hereby declare that the particulars given are true to the best of my knowledge and belief.

Sd/- G.A. Krishna
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