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Determination of Vitamin A in Vanaspati by High Pressure Liquid Chromatographic Method Using U.V. Detector

T.N. MURTHI, V.D. DEVDHARA AND GEORGE JACOB
National Dairy Development Board, Anand — 388001, India

Received 9 December 1988; revised 28 June 1989

Vitamin A is extracted from a few mg of vanaspati with isopropanol and it is satisfactorily separated as a peak by a rapid liquid chromatographic (HPLC) method within 5 min. The extraction of vitamin A from the fat phase is complete and it can be determined by this method without further clean up and saponification procedures at as low as 3 I.U./g. The vitamin A contents of vanaspati samples were found to be 22 to 25 I.U./g.

Vanaspati (hydrogenated fat) which accounts for 20 per cent of all the vegetable fats consumed in our country, is nutritionally enriched by the addition of 25 I.U./g[1] vitamin A during the blending step of the manufacture from partially hydrogenated oils. Vitamin A is estimated by the colorimetric method which is based on the measurement at 620 nm of the unstable blue colour resulting from the reaction of vitamin A and antimony trichloride[2]. There are other tests methods based on ultra-violet absorption[3] and high pressure liquid chromatography[4,5]. In general, these test methods require hydrolysis or saponification of the sample. Methods that involve a multistep clean-up procedure make analysis of vitamin A very time consuming for routine quality control use. A simple and rapid method for the estimation of vitamin A is a requisite for the vanaspati industry to ensure a minimum level of 15 I.U./g at the retail outlet as per the Govt. regulations and the Indian Standard Specifications[6].

A simple and rapid high pressure liquid chromatographic (HPLC) method described here consists of extraction of vitamin A from a few milligrams of vanaspati sample with HPLC— grade isopropanol, injection of iso-propanol extract directly on to a Zorbax silica column and detection using U.V. detector.

Materials and Methods

Aliquots of a standard isopropanolic stock solution of vitamin A (as palmitate) were diluted so as to contain 90, 180 and 360 I.U. vitamin A per 100 ml isopropanol. Similarly, portions of, refined groundnut oil sample with known concentration of vitamin A were also diluted so as to contain 32, 15, 7 and 3 I.U. vitamin A per gram refined groundnut oil. From these oil samples, about 0.4 g material was weighed separately in a series of 25 ml volumetric flasks. The vanaspati samples were melted at 45°C before weighing. All these samples were shaken vigorously with 20 ml HPLC grade isopropanol for 4 minutes and allowed to stand for ten min to separate the phases. Optimum level of solvent required for the complete extraction of vitamin A from the fat phase is determined by shaking each time 0.4 g of oil sample with 5, 10, 15, 20 and 25 ml isopropanol. Simultaneously, isopropanol extract of pure refined groundnut oil sample was also prepared.

A Shimadzu HPLC, LC — 4A unit equipped with U.V. detector at 328 nm and isopropanol as mobile solvent at a constant flow rate of 0.5 ml/min was used for all analysis on a Zorbax silica column (25 cm × 4.6 mm SS). After stabilizing the column at a constant flow rate 10 μ l of standard solution containing 90 I.U. vitamin A per 100 ml isopropanol was injected.

Results and Discussion

After 5 min of injection of the above standard, a single sharp peak was obtained. The other standard solutions containing 180 and 360 I.U. of vitamin A per 100 ml isopropanol also gave a single sharp peak, the height (mm) of which was proportional to the concentration of vitamin A of the standard. Injection of 20 μl of isopropanol extract of the vanaspati and refined groundnut oil samples containing known levels of vitamin A gave exactly similar peak at the same retention time and at the same detector sensitivity (Fig.1). However, there are other small peaks before and after vitamin A which are rudiment at higher concentration level and somewhat prominent at lower concentration level of vitamin A of the extract from the fat phase (Fig.2). Vitamin A peak can be easily identified from these small peaks and its height is measured particularly, at lower concentration

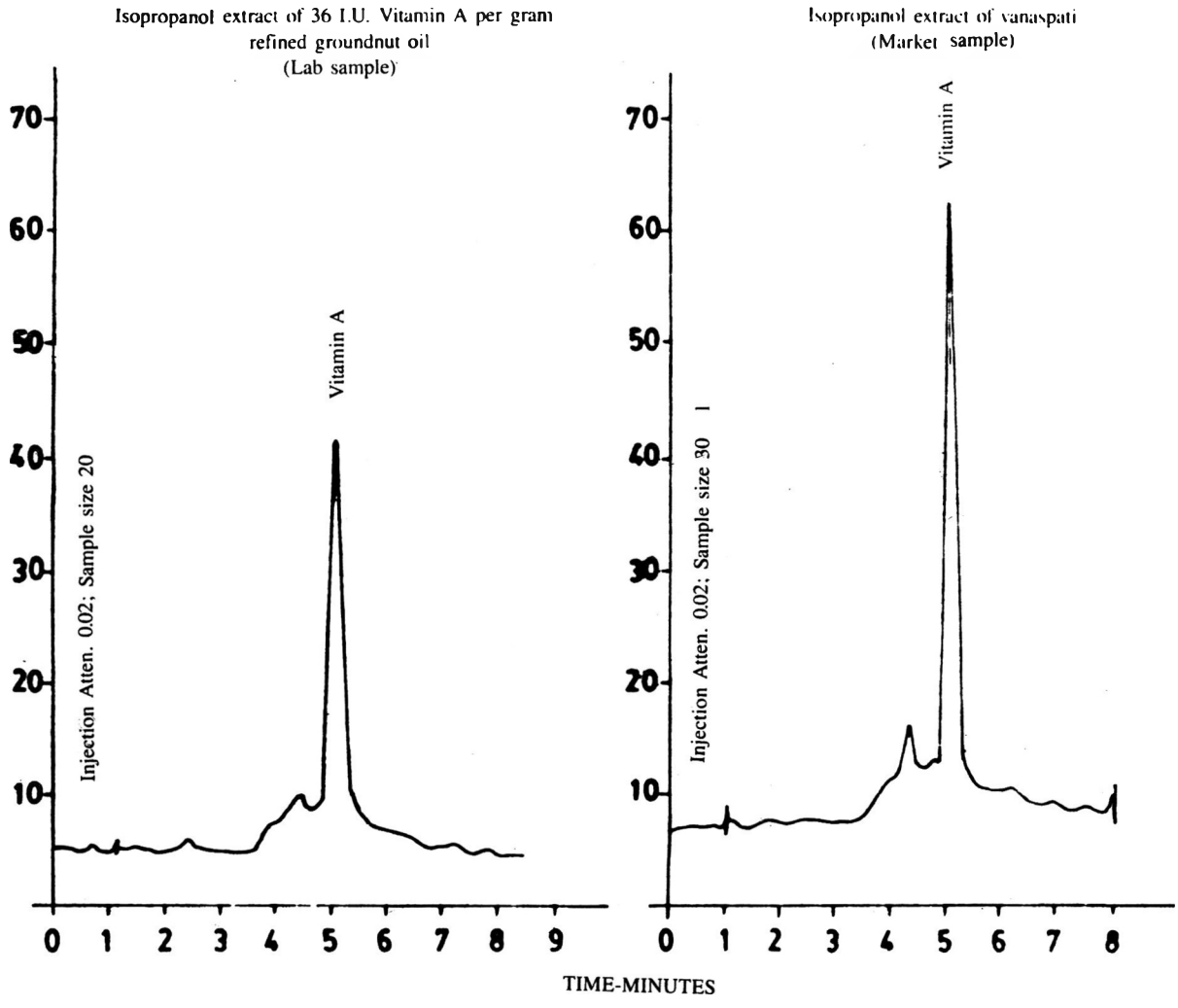

Fig.1. U.V. Determination of vitamin A; isopropanolic extraction of refined groundnut oil containing known levels and vanaspati samples.

(Fig.2) by drawing a base line from the starting and ending points of the vitamin A peak. Pure solvent (blank) and isopropanol extract of pure refined groundnut oil did not give any peak (Fig.2). Thus, it is confirmed that the peak separated under these chromatographic conditions corresponds to that of vitamin A palmitate.

However, initially, it is essential to adjust the operating conditions such as flow rate, sample size, injection sensitivity and chart speed to get a reasonable sharp peak for Vitamin A. In this investigation, a constant flow rate of 0.5 ml/min, a sample volume of 10 to 30 μ l, sensitivity at 4 and a chart speed of 5 mm/min was used to get reproducible results for the standard and the sample. Oil to solvent ratio of 1:50 (wt/vol) resulted in a complete extraction of vitamin A from the oil phase. It is important to limit the size of the oil or vanaspati sample to a few milligrams (300 to 400 mg) in 20 ml isopropanol not only for the complete extraction of vitamin A from the sample but also to save the column (capacity of the column used is 30 μ l of the extract) from over loading. Vitamin A in isopropanol extract or standard solutions tend to undergo deterioration after 8 hr at ambient conditions and after 48 hr under refrigeration. Therefore, the extracts were chromatographed without much delay and within 30 min after the extraction.

A calibration curve of vitamin A concentration against their peak heights as determined by the HPLC was drawn and based on the standard curve, the concentration of vitamin A in refined groundnut oil samples was calculated. Thus, refined groundnut oil (lab) containing 30, 15, 7 and 3 I.U./g vitamin A gave 32, 15, 7 and 3 I.U. respectively as calculated from the standard curve. Therefore, extraction of vitamin A from the oil phase is complete with isopropanol and gives values which are comparable with the known levels. The vanaspati samples (market) by this method contained 22 to 25 I.U./g.

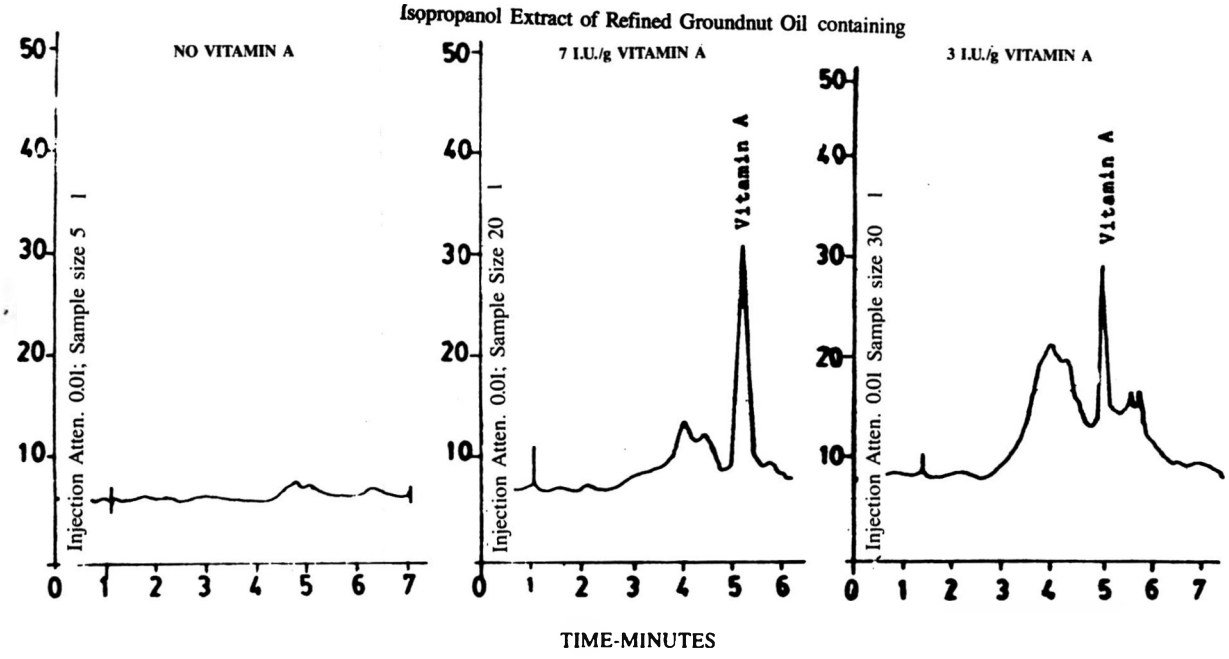

Fig.2. U.V. determination of vitamin A : isopropanolic extraction of refined groundnut oil containing known levels.

The concentration of vitamin A (I.U./g) in oil samples were also verified by the following equation.

$$\text{Vitamin A} = \frac{A \times C \times D}{B \times S}$$

where A is the peak height of the sample, B is the peak height of the standard. C is the concentration of the standard in I.U./ml, D is the final volume of the sample and S is the sample weight in grams.

Thus, by this HPLC method, a satisfactory separation of vitamin A from the isopropanol extract of vanaspati sample was effected in 5 min on a Zorbax silica column followed by U.V. detection (as low as 3 I.U./g) without further clean up and saponification procedures.

References

1. *The Prevention of Food Adulteration Act*, 1954, 9th Ed., Eastern Book Company, Law Publishers and Book Sellers, Lalbagh, Lucknow, 1988, 131.
2. Subramanyam G B and Perrish D B, Colorimetric reagents for determining vitamin A in feeds and foods, *J ass Off Analyt Chem*, 1976, **59**, 1125.
3. Dann W J and Evelyn K A, The determination of vitamin A with photo-electric colorimeter, *Biochem*, 1938, **32**, 1008.
4. Thompson J N Hatina G and Maxwell W B, High performance liquid chromatographic determination of vitamin A in margarine, milk, partially skimmed milk and skimmed milk, *J Ass Off analyt Chem*, 1980, **63**, 894.
5. Autzenuller K Pilz J and Tosche R, Fast determination of vitamin A palmitate in margarine by HPLC, *Fette: Seifen.Anstichm, 1979*, **81**, 40.
6. *Specification for Vanaspati* (First Revision) IS:10633 — 1986. Indian Standards Institution, New Delhi, 1987.

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Amylase and Protease Production by *Bacillus subtilis:* Studies on Improvement of Strain and Medium

S.N. KADREKAR AND G.B. RAMASARMA
Research Laboratories,
Raptakos, Brett & Co. Ltd., Worli, Bombay — 400 025, India

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Resting cells of a local strain of *Bacillus subtilis* were subjected to repeated treatment with the mutagenic agent, N-methyl-N'-nitro-N-nitrosoguanidine (NTG), with a view to developing improved mutant strains for production of α-amylase and protease. A simplified agar-plate procedure was developed for screening the mutants. An improved strain, NTG-121, was isolated; it produced on liquid culture medium under stationary conditions 17.7 FIP units of amylase activity and 0.66 FIP units of protease activity per ml of broth compared to 10.61 and 0.36 units respectively produced by the parent strain. Further treatment of growing cultures of mutant NTG-121 with the same mutagenic agent resulted in mutants producing more of protease (1.57 units per ml) but less of amylase (14.7 units per ml). Addition of corn-steep liquor, mineral salts mixture (Ca, Mn, Zn, PO_4), starch and casein to the liquid culture medium in which strain NTG-121 was grown, increased the production of amylase and protease to 24.7 and 2.67 FIP units respectively per ml of broth.

Amylases and proteases are widely used in food, pharmaceutical and other industries[1,2]. Some of these enzymes are commercially produced from bacterial fermentations, especially with *Bacillus subtilis*. High-yielding strains of this organism are obtained by screening isolates from different sources and further improved by inducing mutations[3–5]. However, the published literature provides very little information of value on the procedures employed in developing the commercially used strains or the actual enzyme yields obtained[6]. Despite recent avances in genetic engineering, the older procedures of mutagenesis and random screening are still considered to be cost-effective and reliable methods of strain improvement[7].

The purpose of the present study was to develop improved mutant strains from a locally isolated strain of *Bacillus subtilis* and to study the effect of adding different nutrients to the culture medium on the production of α-amylase and protease by the selected mutant strain.

Materials and Methods

Bacterial culture: After a preliminary screening of three different cultures of *B. subtilis*, a local isolate obtained from the Indian Agricultural Research Institute, Delhi, was selected for these studies. The culture was maintained on solid culture medium (*vide infra*).

Mutagenic agent: N-Methyl-N'-nitro-N-nitrosoguanidine (Fluka, W. Germany).

Media ingredients: Bacto peptone, tryptone, yeast extract and agar (Difco Laboratories, U.S.A.):
Beef extract (Hi-Media Laboratories, Bombay)
Casein hydrolysate (Priya Chemicals, Bombay)
Corn-steep liquor (Anil Starch Products, Ahmedabad)
Soluble casein (E. Merck, W. Germany)
Soluble starch (Glaxo Laboratories, Bombay)
Maltose (E. Merck, W. Germany)

Solid culture medium: Peptone 6.0 g, tryptone 4.0 g, yeast extract 3.0 g, bef extract 1.5 g, glucose 1.0 g and agar 20.0 g in 1000 ml, pH 7.0: test tubes containing 10 ml portions of the medium were sterilised at 121°C (15 lb steam pressure) for 20 min, cooled and stored in refrigerator.

Liquid culture medium: It was of the same composition as the solid culture medium but with the omission of agar; 50 ml or 25 ml of the medium were placed in 250 ml conical flasks and sterilized similarly.

Cell suspension for mutagenic treatment: Ten ml of a 24 hr culture of the strain grown in 50 ml of liquid culture medium at 37°C was centrifuged at 3000 r.p.m. for 10 min and the cells were aseptically washed three times with sterile normal saline; the washed cells were resuspended in 10 ml of sterile saline and suitably diluted in sterile buffer solution so that the final suspension had an optical density corresponding to a concentration of 2×10^8 cells per ml.

Mutagenic treatment: To 9 ml of the cell suspension in 0.1M phosphate buffer of pH 7.2 placed in each of five test tubes was added 1 ml of sterile solution containing graded amounts 0 (control), 150, 500, 1000 and 1500 mcg) of the mutagen, N-methyl-N'-nitro-N-nitrosoguanidine (NTG). During exposure to the mutagenic agent, 1 ml of the suspension was aseptically withdrawn at intervals of 10, 20, 30, 40 and 60 min.

Selection of mutants: Each treated sample was aseptically centrifuged at 3000 r.p.m. for 10 min and the cells were washed three times with sterile saline. The washed cell suspension was serially diluted in sterile saline so that 0.1 ml of the suspension, when plated in solid culture medium containing 2 per cent of soluble starch or 1 per cent of casein, gave 3 - 5 colonies per plate on incubation at 37°C for 72 hr. The plates were visually examined and the colonies were counted in order to calculate the percentage of survivors. Two or 3 colonies showing the widest zones of starch or casein hydrolysis (*vide infra*) were isolated from the treated plates and streaked on nutrient agar slants; after incubation for 24 hr, the slants were stored in refrigerator.

The ability of each strain to produce α -amylase and protease in a liquid medium was studied using the following procedure. A 24 hr growth of the strain in 50 ml of liquid culture medium was centrifuged at 3000 r.p.m. for 10 min and the cells were suspended in 10 ml of sterile saline; 1.25 ml of this suspension was inoculated into 25 ml of sterile liquid medium contained in a 500 ml conical flask. After incubation under stationary conditions for 72 hr at 37°C, the cells were removed by centrifuging and the clear broth was used for estimating the enzyme activities by the F.I.P. methods[8]. One F.I.P. unit of amylase activity is contained in that amount of the standard preparation that, under the conditions of the assay, decomposes starch at an initial rate such that one micro-equivalent of glucosidic linkage is hydrolysed per min. One F.I.P. unit of protease activity is contained in that amount of the standard preparation that, under the conditions of the assay, hydrolyses casein at an initial rate such that there is liberated per min an amount of peptides not precipitated by trichloroacetic acid that gives the same absorbance at 275 nm as one micromole of tyrosine.

Results and Discussion

Mutagenic studies: The procedure described in the literature[4,9] to screen the treated cells for amylase or protease production consisted of isolating the individual cells, plating them on solid nutrient medium containing, 1 per cent soluble starch or 0.5 per cent soluble casein and after incubation spraying the plates with dilute iodine solution or dilute hydrochloric acid so that the clear zone around each colony could be measured; the cells corresponding to colonies with widest zones were selected as the most promising mutant strains. In the present study, this two-step procedure was greatly simplified by incorporating in the solid medium 2 per cent of soluble starch or 1 per cent of soluble casein which imparted sufficient opacity to the medium so that the clear zone of hydrolysis could be measured directly without the need for spraying with iodine solution or hydrochloric acid and the cells from the selected colony could be transferred to slants. This procedure was successfully applied in picking out the more productive mutant strains from the treated cells for further evaluation by broth culture.

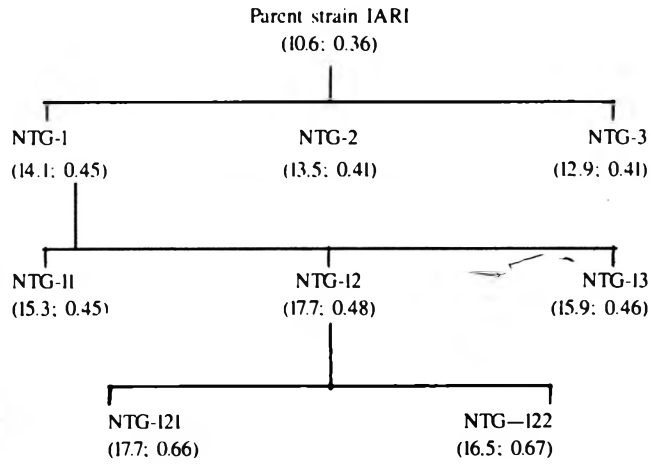

Fig 1. NTG treatment on resting cells of *B. subtilis*. The parenthetical values represent F.I.P. units of α -amylase and protease activities, respectively, per ml of broth.

It was observed that, on treatment with NTG at a concentration of 1000 and 1500 mcg per 10 ml for 30 to 60 min, the mortality rate of the cells was about 80 per cent and some of the surviving cells proved to be better producers of amylase activity. By repeated treatment with NTG, a series of mutants, designated as NTG-1, NTG-12 and NTG-121, were obtained. The results of evaluation of enzyme production by the parent and mutant strains are shown in Fig. 1. As the amylase production seemed to have reached a plateau with NTG—121, it was not subjected to any further treatment under the same conditions. However, in view of the reported[10] use of growing cultures of *Neurospora crassa* for mutagenic treatment, this procedure was tried on the mutant strain NTG-121 for possible improvement. Actively growing cells of the mutant were inoculated into 10 ml of the liquid culture medium containing graded amounts (50, 100, 200, 300,

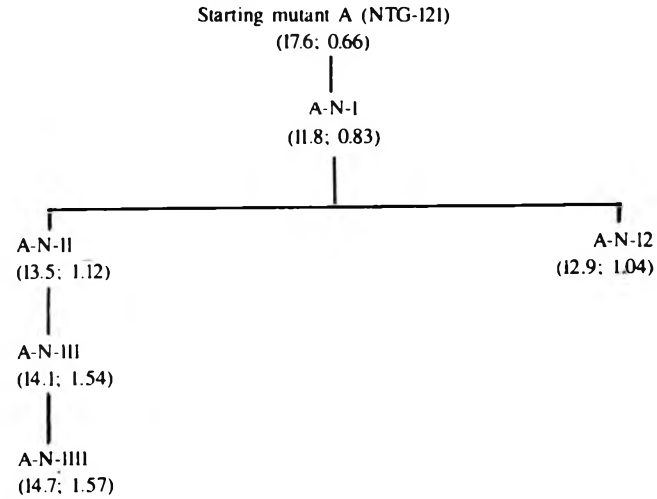

Fig 2. NTG treatment on growing cultures of *B. subtilis*. mutants. The parenthetical values represent F.I.P. units of α-amylase and protease activities, respectively, per ml of broth.

TABLE 1. EFFECT OF ADDITIONAL NUTRIENTS ON ENZYME PRODUCTION BY MUTANT NTG-121

Medium No.	Composition of medium	Enzyme activity (F.I.P units/ml broth)	
		α -Amylase	Protease
A	Liquid culture medium	18.0	0.71
B	A with casein hydrolysate in place of peptone & tryptone	15.9	0.56
C	B + 0.2% corn steep liquor	17.4	0.70
D	C + salt mixture*	18.3	1.02
E	D + 1% soluble casein	17.5	1.21
F	D + 1% soluble starch	20.8	2.09
G	F + 1% soluble casein	20.8	2.43
H	G with peptone & tryptone in place of casein hydrolysate	22.1	2.48
I	H + 1% maltose	24.7	2.67

*0.1% of each of $CaCl_2$ (anhydr.), $MnSO_4 . 4H_2O$, $ZnSO_4 . 7H_2O$ and KH_2PO_4.

500, 1000, 1500, 1600, 1700, 1800, 1900 and 2000 mcg) of NTG and incubated at 37°C for 24 hr. The tube containing 1.6 mg of NTG per 10 ml showed a minimum of growth. The mutant isolated from this tube was tested for enzyme production by the methods described earlier. By repeated treatment under similar conditions, successive generations of mutants were obtained. For the purpose of screening the mutants, use was made of solid culture medium containing 1 per cent of casein since mutagenic treatment of growing cultures resulted in mutants with higher protease production. The results presented in the Fig. 2 show that these mutants produced lower levels of α-amylase but much higher levels of protease. Thus, the fourth generation mutant A-N-1111 was able to produce more than double the amount of protease compared to the starting strain NTG-121.

Improvement of culture medium for enzyme production: The primary interest of this part of the work was in the production of α-amylase. Therefore, the mutant strain NTG-121 was selected for studies on improvement of the culture medium. The plan of the study was to start with a basal medium B having the same composition as that of the liquid culture medium A described earlier except that the less expensive casein hydrolysate was substituted at 1 per cent level for 0.6 per cent of peptone and 0.4 per cent of tryptone. This medium B was successively supplemented with a variety of nutrients and the resulting media were designated as C, D etc. The results presented in Table 1 were in line with those reported by earlier workers[1,2,11], namely, increased enzyme production on the addition of nutrients such as peptone, corn-steep liquor, maltose and mineral (Ca, Mn, Zn, PO_4) salt mixture. When 1 per cent of casein alone was added as a possible inducer, there was only a slight increase in the production of protease. With the addition of 1 per cent of soluble starch alone, protease production increased by 65 per cent while amylase production increased only slightly. On the other hand, when both starch and casein were added together (medium G), there was a further increase in protease production; the reason for this increase is not apparent.

Compared to the yields under stationary culture conditions with medium I (24.7 and 2.67 units per ml of amylase and protease activities respectively), shake-flask culture yielded much higher levels of enzyme production, namely, 27.4 and 4.08 units per ml of amylase and protease respectively. This suggests that submerged aerobic fermentation would be preferable for large scale production of the two enzymes using the mutant strain NTG-121.

Acknowledgement

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References

1. Fogarty W M and Kelly C T, Amylases, amyloglucosidases and related glucanases, in *Microbial Enzymes and Bioconversions*, by Rose A H (Ed.), Academic Press, London 1980, 144.
2. Aunstrup K, Proteinases, in *Microbial Enzymes and Bioconversions*, by Rose A H (Ed.), Academic Press, London, 1980, 80.
3. Higerd T B Hoch J A and Spitzizen J, Hyperprotease-producing mutants of *Bacillus subtilis*, *J Bact.*, 1973, **112**, 1026.
4. Yoneda Y and Maruo B, Mutation of *Bacillus subtilis* causing hyperproduction of α-amylase and protease and its synergistic effect, *J Bact*, 1975, **124**, 48.
5. Bailey M J and Markkanen P H, Use of mutagenic agents in improvemnet of α -amylase production by *Bacillus subtilis*, *J appl Chem Biotech*, 1975, **25**, 73.
6. Ingle M B and Erickson R J, Bacterial α-amylases, in *Adv appl Microbiol*, 1978, **24**, 257.
7. Rowlands R T, Industrial strain improvement: mutagenesis and random screening procedures, *Enzyme Microb Technol*, 1984, **6**, 3.
8. Ruyssen R and Lauwers A (ed.), *Pharmaceutical Enzymes*, Scientific Publishing Co, Gent (Belgium), 1978, **74**, 83; British Pharmacopoeia, 1988, H.M. Stationery Office, London, pp A 178-9.
9. Hopwood D A, The isolation of mutants, in *Methods in Microbiology*, 3A, by Norris J R and Ribbons D W (Eds), Academic Press, London, 1970, 363.
10. de Serres F J and Malling H V, in *Chemical Mutagens: Principles and Methods for Their Detection* by Hollaender A (Ed.), Vol. 2, Plenum Press, New York, 1971, 2 315.
11. Gandhi A P, Studies on the production of -amylase by *Bacillus subtilis* growing in the batch and chemostat cultures, *J Fd Sci Technol*. 1982, **19**, 177

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Factors Affecting Scutellum Retention in Milled Rice

G.N. RAJU, M.K. BHASHYAM AND T. SRINIVAS*
Discipline of Cereal Science and Technology
Central Food Technological Research Institute, Mysore - 570 013, India

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The frequency of grains with scutellum in the 14 varieties of milled rice varied from 12 to 86%. In the high scutellum retention (SR) varieties, a cementing material was present between the epithelial layer and endosperm cells. In low SR varieties, this material was absent. SR in milled rice increased with a decrease in the initial moisture of raw brown rice, by parboiling, and occurred more with Satake than McGill polisher. Brokens in the brown rice prior to milling was associated with low SR in milled rice.The thiamine content of milled rice with scutellum was more (124 to 138 μg/100 g) than that without scutellum (80 to 84 μg/100 g). High SR variety retained more thiamine than the low SR variety even after washing. Scutellum retention in milled rice is thus nutritionally beneficial and can be enhanced by improving varietal and processing factors.

Milling is an important operation in rice processing to remove the fibrous bran which covers the surface of the grain and to make it more palatable and digestible. During this process, partly or fully the nutritionally rich aleurone and subaleurone layers as well as the germ are also eroded causing a substantial nutritional loss in the milled grain[1,2]. Attempts are being made in several directions to minimise these losses. Some of the methods suggested are giving low polish[1,2], parboiling the rice[3,4] and developing grains with uniform distribution of nutrients[5,6].

In rice, the germ is present on the ventral side of the proximal end of the grain[7]. The embryonic axis (EA) present on the peripheral side consists of the dormant shoot (plumule) and root (radicle) along with their associated structures. During milling operation, most of the EA is removed due to its characteristic surface position. The scutellum which accounts for nearly 50 per cent by weight of the germ[8], is located below the EA in a depression and is, thus more protected than the latter. Depending on the extent of its adhesiveness and on other milling conditions, it may be retained partially or fully or removed totally during milling. The scutellum accounts for 50 per cent of the thiamine present in the brown rice and contains substantial amounts of proteins, fat and other important minerals[8-10]. Its retention in the milled rice is, therefore, of great nutritional importance.

The present study was designed to generate information on the extent of varietal variation in scutellum retention (SR), chemical and histological peculiarities and the methods by which maximum economic benefits are achieved by reducing loss of scutellum in milled rice.

Materials and Methods

Sample preparation: Paddy was harvested at a moisture content of 20-24 per cent and shade-dried to about 13 per cent. For the study of the effect of moisture content on SR, separate samples were shade-dried to moisture levels ranging from 15.8 to 9.8 per cent. All of them were stored in air-tight containers at 5°C until milled. Moisture content was estimated as per the method of Indudhara Swamy *et al.*[11].

Scutellum retention in the milled rice: Paddy was shelled in a McGill sheller No.2 and brown rice obtained. Duplicate samples of 150 g of brown rice were progressively milled to obtain approximately 2, 4, 6 and 8 per cent polished rice in a McGill Mill No.2 using a standard abrasion pressure (2 lb load). Samples of about 10 g milled rice were withdrawn for detailed study at each degree of milling. About 100 whole grains in duplicate samples were randomly selected from each sample of milled rice and scored for the presence or absence of scutellum. The visibility of scutellum was improved by staining the lipids with Sudan IV. A magnifying lens was used for accurate judgement regarding presence or absence of scutellum.

Cracked grains and milling breakage: Samples from the varieties were collected at optimum (20 to 24 per cent moisture) as well as advanced stages of maturity (16 to 17 per cent moisture). They were shade-dried to a moisture of about 13 per cent. Percentage of cracked grains in the samples was estimated by using a paddy crack detector[12]. Milling breakage in rice was estimated by the method of Raghavendra Rao *et al.*[13].

*To whom all correspondence should be addressed.

Parboiling: Parboiled rice was prepared as per the method of Kurien *et al.*[14].

Proportion of germ and scutellum in rice: The germ in the brown rice was dissected by a razor blade and the scutellum in the milled rice by a chiselled needle. The proportion of germ by weight was estimated by collecting one gram of germ from a random sample of brown rice in duplicate and that of scutellum in randomly selected 100 milled grains in duplicate.

Histology of rice germ: Ten micron thick microtome sections of rice were prepared[15,16]. They were stained with 1 per cent aqueous solution of methylene blue and 1 per cent erythrosin-B in alcohol.

Effect of milling machines on SR: McGill mill No. 2 (a metal roller friction type) and Satake laboratory grain testing mill (an abrasive type of mill with an emery disc) were used for this study. About 150 g of brown rice was milled in each kind of mill until a desired polish was achieved. About 100 whole grains in duplicate were randomly selected and scored for SR.

Chemical analysis: Protein content was estimated in the brown rice samples by microkjeldahl method. Oil content of germ and bran was estimated by Soxhlet method using petroleum ether 60-80°C[17]. Thiamine content was estimated by Thiochrome method[5,18] in brown rice and in C and E type grains to represent grains with and without scutellum respectively. For estimating thiamine content in washed rice samples. 5 g of milled rice in duplicate with 2 volumes of water was taken in a beaker and stirred for 60 sec. The water was drained out, and the sample thus recovered was dried at room temperature until it attained its original moisture before estimation.

Results and Discussion

On the basis of the proportion of the germ retained in the milled rice samples, five types of grains were identified which were designated as A,B,C,D and E (Fig.1). At each stage of milling, grains of A, B and C categories were treated with scutellum and those of D and E type without scutellum.

Among the 14 varieties studied, the degree of SR was highly variable. At 6 per cent milling, it was highest in 'Sitabhog' (86 per cent) and lowest in 'Vani' (12 per cent) and intermediate in other varieties (Table 1). The factors associated with SR could be broadly classified into two categories, viz., those which were heritable and varietal, or physical and environmental. In the 10 varieties listed in Table 1, the germ and grain weights, protein content of brown rice and oil content of germ were not related to SR. The oil content of bran (including germ) showed an inverse relation with SR ($r=-0.669^*$).

The histological studies by light microscopy showed that in rice the scutellum is made up of parenchymal cells of irregular shape, similar to those observed by Santos[19] and is connected to the endosperm by means of an epithelial layer (Fig.2a). The presence of a scutellar epithelium has been observed in rice, wheat and maize. In rice, the cells of eoithelium were elongated and appeared like fingers (Fig. 2a) similar to those observed by Pomeranz[20] in wheat. He observed that the ends of the finger-like epithelial cells were impregnated with a cementing material, which was responsible for the tight adherence of the germ to the endosperm in wheat. MacMasters[21], Mahadevappa and Desikachar[22] and Bechtel and Pomeranz[23] have observed crushed cell mass, devoid of protoplasm, between the endosperm and scutellar epithelium in rice. The presence of

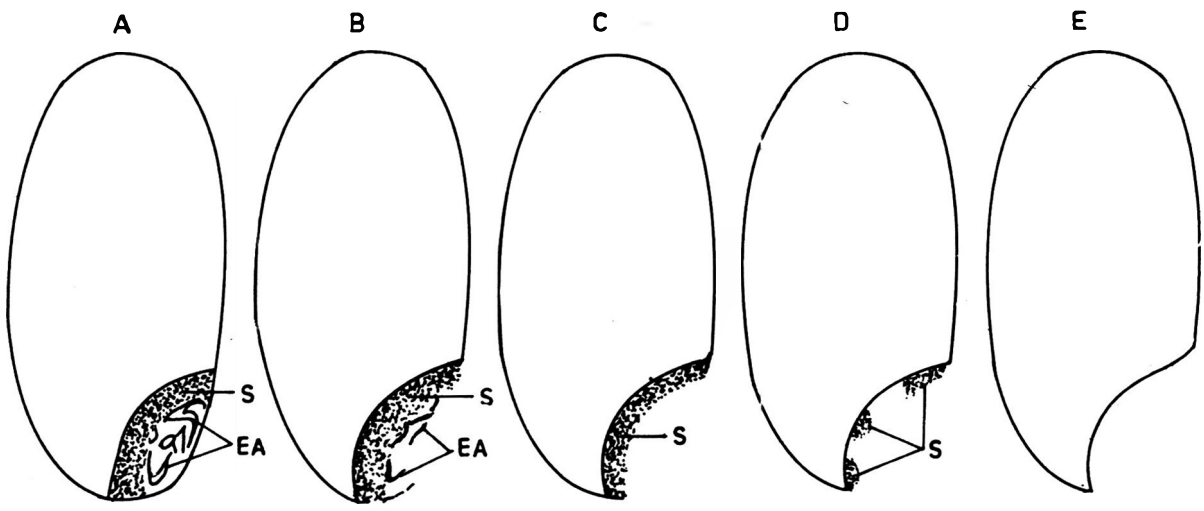


Fig. 1. Scoring criteria for scutellum retention in milled rice. A - Brown rice with intact embryonic axis (EA) and scutellum (S) = Germ; B — Polished rice (Stage 1) EA partially removed and S intact; C — Polished rice (Stage 2) EA completely removed and S intact; D — polished rice (Stage 3) EA completely removed and S partially removed; E — Polished rice (Stage 4) Germ completely removed.

TABLE 1. SCUTELLUM RETENTION (SR) IN MILLED RICE AND OIL CONTENT IN BRAN AMONG RICE VARIETIES

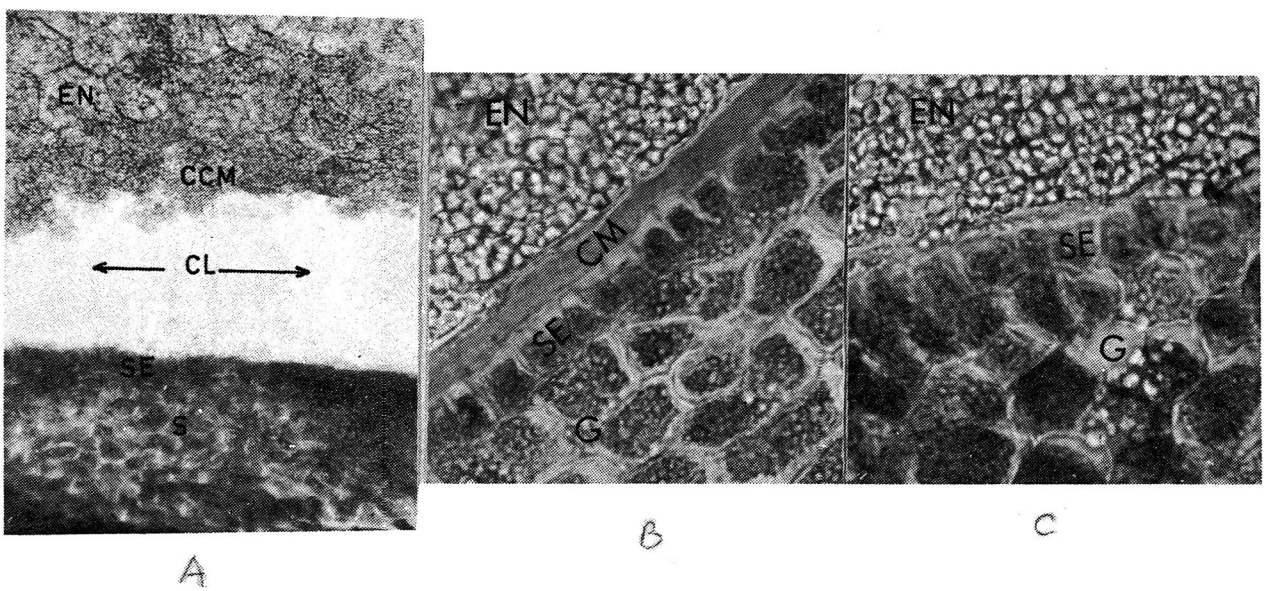
Variety	Grains with SR in 6% milled rice (%)	Oil content in bran (%)
Sitabhog	86	20.6
Mangala	84	22.6
KMP-41	71	21.5
KMP-39+	68	ND
Intan	64	23.2
Shastikasali	64	21.4
Lagore	62	21.7
S701	54	23.7
IRAT-10	52	23.3
S199	49	23.5
ES-18+	45	21.4
ADT-27+	31	20.6
Madhu+	23	ND
Vani	12	24.6

+ Not included in the statistical analysis (full data were not available)
ND Not done
SR vs Oil content. r = −0.699 (Significant at 5% level)

these crushed, structurally defective cells, also termed as fibrous zone was shown to be responsible for the easy separation of the germ from the endosperm[23]. MacMasters[21] postulated that these amorphous cells form an adherent layer between the germ and the endosperm and offer resistance to germ removal.

In all the high SR varieties, in which the proportion of grains with scutellum ranged from 50 to 86 per cent, a cementing material (CM) adjacent to the epithelial layer towards the endosperm was thick and prominent (Fig. 2b), whereas in the low SR varieties the CM appeared to be absent (Fig. 2a). This suggests the possible role of CM in the tight adherence of scutellum in high SR varieties. Wolf *et al.*[24] have suggested that the variation in hemicellulose content at the scutellar region must be responsible for the variation in adherence of scutellum in maize. In rice, the formation of a cleavage line in the region between the epithelium and the endosperm in a low SR variety ('Vani') is shown in Fig.2c. The apparent absence of CM possibly causing easy cleavage is also evident in the Figure.

The germ is removed whole or in pulverised form during milling operation. So, it is rather difficult to determine the proportion of germ in the form of scutellum left in the milled grain. A preliminary study has, however, indicated that the proportion of scutellum in the C type grains of the 6 per cent milled rice was between 0.58 and 1.81 per cent by weight (Table 2). This constituted about 24.2 to 39.3 per cent of the total germ weight and was roughly proportional to the original germ weight in different varieties. The literature value of 50 per cent scutellum in the germ[8] was estimated in the brown rice. The slightly lower values we obtained appear to be pertinent since they are estimated in the milled rice in which



Figs. 2a and 2b. Light micrographs of Longitudinal sections (L/S) of rice grains at the attachment point between endosperm and germ in low (a) and high (b) scutellum retention varieties. (a) Epithelial layer without cementing material. (b) Epithelial layer with cementing material Endosperm (EN), Scutellar Epithelium (SE), Cementing material (CM) and Germ (G), (× 780).

Fig. 2c. Light micrograph of L/S of rice grain showing Endosperm (EN) Coenocytic cell mass (CCM), Cleavage line (CL), Scutellar Epithelium (SE) and Scutellum (S), (× 195).

TABLE 2. WEIGHT OF GERM AND SCUTELLUM RETAINED IN 6% MILLED RICE

Variety	Germ content (%)	Scutellum wt as % in milled 'C' type rice	Scutellum as % of germ
Sitabhog	4.6	1.81	39.3
Sukanandi	3.6	1.20	33.3
Shastikasali	3.0	0.89	29.7
S701	3.2	0.84	26.7
Mangala	2.4	0.58	24.2

the peripheral portion of the scutellum is likely to be lost during milling due to abrasion.

The moisture content prior to milling showed an inverse relation with SR in the two varieties. The SR increased from about 50 to 70 per cent when the moisture was decreased from 15 to 10 per cent (Fig. 3).

The effect of parboiling was studied in 6 varieties (Fig.4). SR increased in 'Vani', 'ES-18', 'IRAT-10' and 'S199' in 6 per cent milled rice on parboiling. In 'Intan' and 'Lagore' in which the SR was high its further increase was small. A similar trend was noticed in the effect of polishing machines on SR. In 'Vani' the scutellum retention increased from 12 to 51 per cent in raw rice and 35 to 68 per cent in parboiled rice (Fig.5) when the Satake grain testing mill (an emery abrasion type which produces relatively milder abrasion than the McGill polisher) was used against a metal roller friction type Mcgill polisher[25]. This beneficial effect of Satake polisher over McGill polisher was not found in the high retention variety, 'Intan'. These results indicated that the loss of scutellum in milling can be reduced either by making the bondage strong by hydrothermal treatment, such as parboiling, or by milling under low abrasive conditions. This advantage is more pronounced in varieties in which the scutellum attachment is relatively weak as in Vani than in varieties in which scutellum attachment is tight.

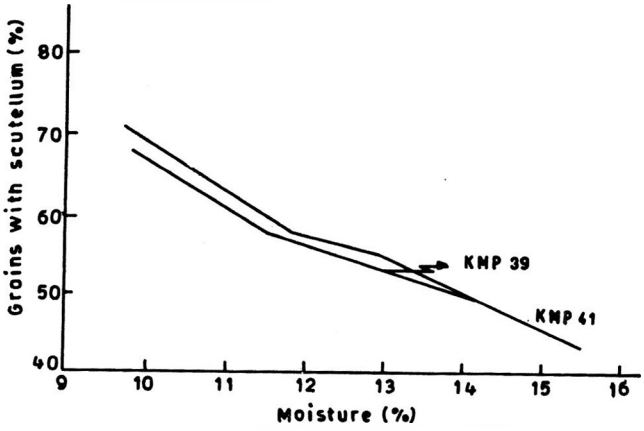


Fig. 3. Effect of moisture content of brown rice on scutellum retention in milled rice.

The effect of degree of milling on SR was studied in 10 rice varieties. In the low SR varieties, such as 'Vani', 'Madhu', etc., the rate of removal of scutellum was faster than those

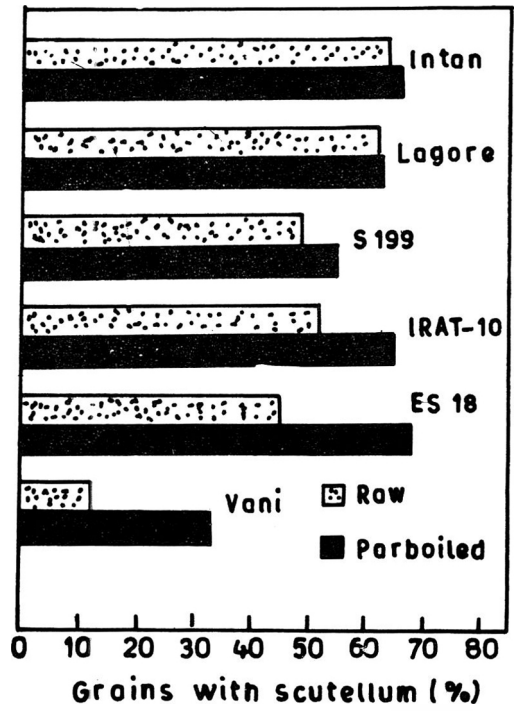


Fig. 4. Effect of parboiling on scutellum retention in milled rice of different varieties.

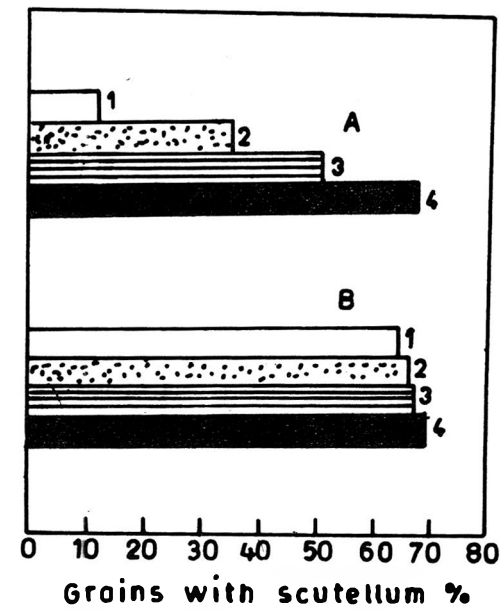


Fig. 5. Effect of polishers on scutellum retention in milled rice A — Vani, B — Intan.

1. Raw milled
2. Parboiled milled

McGill Polisher

3. Raw milled
4. Parboiled milled

Satake Polisher

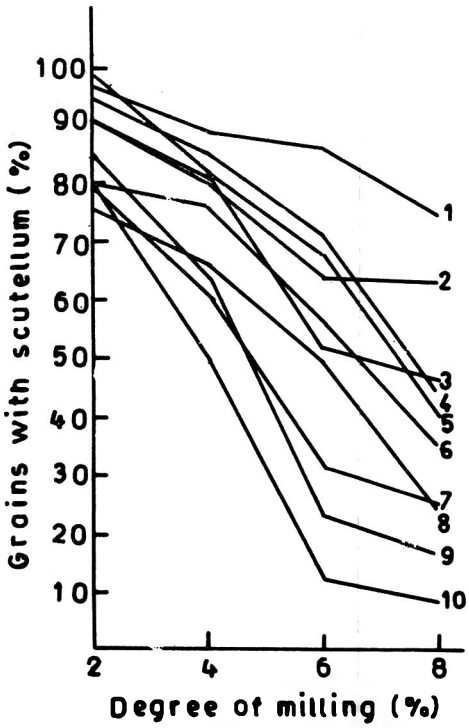


Fig. 6. Effect of degree of milling on scutellum retention in milled rice. 1. Sitabhog, 2. Intan, 3. IRAT-10, 4. KMP — 41, 5. KMP-39, 6. S701, 7. ADT-27, 8. S199, 9. Madhu and 10. Vani.

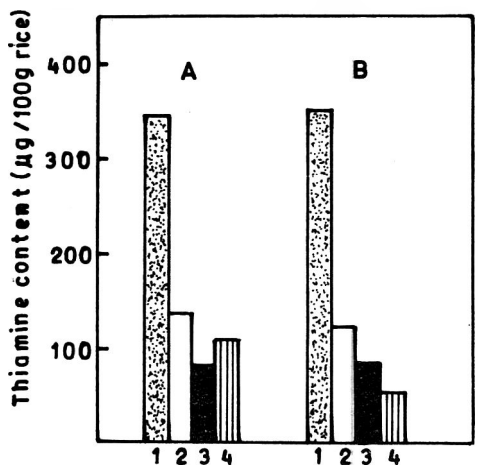


Fig. 7. Effect of scutellum retention on thiamine content of rice
A: Intan B: Vani
1. Brown rice,
2. Milled rice with scutellum (C type grain),
3. Milled rice without scutellum (E type grain) and
4. Washed milled rice.

of high SR varieties and in such varieties the loss of scutellum could be minimised by giving a low polish to the rice, preferably less than 4 per cent (Fig.6).

The effect on SR of brokens produced during milling due to preformed cracks in the late harvested samples was studied in two rice varieties. In the high retention variety, 'Intan', samples having no cracked grains gave very little breakage (2 per cent) during milling and retained high proportion of scutellum (64 per cent), whereas, in the same variety those which contained 70 per cent cracked grains gave about 71 per cent breakage, bringing the SR down to 28 per cent. In the low retention variety, Vani, the decrease in the SR due to high percentage of cracked grains (40 per cent) and breakage (67 per cent) was from 23 to 16 per cent. This clearly indicates the adverse effect of brokens on SR especially in varieties possessing a high tendency to retain scutellum in the milled rice.

Although extensive work has been done on the physiological,[26-28] histological[11,27,29] and chemical[9,10,30] aspects of scutellum in cereals, the nutritional significance of scutellum in rice has not received the attention it deserved. This may be due to its minuteness and also appears to be due to its inevitable loss during most of the milling operations. Intan and Vani as brown rice, 6 per cent milled with and without scutellum showed a thiamine content of 340, 138, 80 and 353, 124, 84 µg/100g respectively. The thiamine retained in milled rice after washing was 108.5 and 50.9 µg/100g in Intan and Vani (Fig.7) indicating the greater resistance of high SR variety against washing losses as compared to low SR variety.

The study has shown that the retention or removal of scutellum during milling is primarily a function of the degree of its adherence to the endosperm by a cementing layer. Other factors which ensure high SR in milled rice include lower moisture and crack free conditions of the paddy prior to milling, parboiling, use of polishing equipment which employs a relatively mild abrasive force to debran and giving low polish especially to the low SR varieties. Varietal selection, optimum harvesting and controlled drying of paddy are the beneficial approaches for increasing SR in milled rice. It is shown that even a portion of scutellum retained in the milled rice can considerably enrich thiamine content in human diet. There seems to be an excellent scope for improving the thiamine status of the milled rice by breeding high SR varieties.

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References

1. Bradbury J H Collins J G and Pyliotis N A, Methods of separation of the major histological components of rice and characterisation of their protein by amino acid analysis, *Cereal Chem*, 1980, **57**, 133.
2. Subrahmanyan V Narayan Rao M and Swaminathan M, Nutritive value and keeping quality of husked, under milled and milled raw rice, *Bull CFTRI*, 1955-56, **5**, 329.
3. Simpson I A, The distribution of thiamine and riboflavin in rice grains and of thiamine in parboiled rice, *Cereal Chem*, 1951, **28**, 479.
4. Subbarao P V and Bhattacharya K R, Effect of parboiling on thiamine content of rice, *J Agric Fd Chem*, 1966, **14**, 479.
5. Swaminathan M S Naik M S Kaul A K and Austin A, The choice of

strategy for the genetic upgrading of protein properties in cereals, millets and pulses, *Plant Fd Human Nutr*, 1972, **2**, 119.

6. Kennedy B M Schelstracte M and Tamai K, Chemical, physical and nutritional properties of high protein flours and residual kernel from the over milling of uncoated milled rice IV Thiamin, riboflavin and pyridoxine, *Cereal Chem*, 1975, **52**, 182.
7. Juliano B O, in *Rice : Production and Utilization*, by Luh, B.S., (Ed.), AVI Pub. Co. Inc. Westport, CT. 1980. 403.
8. Hinton J J C, The distribution of Vitamin B in the rice grain, *Brit J Nutr*, 1948, 2, 237.
9. Tanaka K Ogawa M and Kasai Z, The rice scutellum: Studies by scanning electron microscopy and electron micro probe x-ray analysis, *Cereal Chem*, 1976, **53**, 643.
10. Tanaka K Ogawa M and Kasai Z The rice scutellum II A comparison of scutellar and aleurone electron dense particles by transmission electron microscopy including energy dispensive X-ray analysis, *Cereal Chem*, 1977, **54**, 684.
11. Indudhara Swamy Y M Ali SZ and Bhattacharya K R, Hydration of raw and parboiled rice and paddy at room temperature, *J Fd Sci Technol*, 1971, **8**, 20.
12. Srinivas T and Desikachar H S R, A simple inexpensive device for detecting and estimating cracks in intact paddy grains, *J Fd Sci Technol*, 1973a, **10**, 197.
13. Raghavendra Rao S N Narayana M N and Desikachar H S R, Studies on some comparative milling properties of raw and parboiled rice, *J Fd Sci Technol*, 1967, **4**, 150.
14. Kurien P P Radhakrishnamurthy R Desikachar H S R and Subrahmanyan V. Effect of parboiling on the swelling quality of rice, *Cereal Chem*, 1964, **41**, 16.
15. Johansen D A, *Plant Microtechnique*, McGraw-Hill Book Co., Inc, New York, 1940.
16. Srinivas T and Desikachar H S R, Factors affecting the puffing quality of paddy, *J Sci Fd Agric*, 1973b, **24**, 883.
17. *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, D.C. 1970. 11th Edn.
18. McRoberts L H, Report on the determination of thiamine enriched flour. Comparison of thiochrome methods, *J Ass of Analyt Chem*, 1954, **37**, 757.
19. Santos J K, Morphology of the flower and mature grain of Philippine rice, *Philippine J Sci*, 1933, **52**, 475.
20. Pomeranz Y, Grain structure and end-use properties, *Fd Microstructure*, 1982, **1**, 107.
21. MacMasters M M, Important aspects of kernel structure, *Trans ASAE*, 1962, **5**, 247.
22. Mahadevappa M and Desikachar H S R, Some observations on the histology of raw and parboiled rice, *J Fd Sci Technol*, 1968, **5**, 72.
23. Bechtel D B and Pomeranz Y, Implications of the rice kernel structure in storage, marketing and processing: A review, *J Fd Sci*, 1978, **43**, 1538.
24. Wolf M J MacMasters M M and Seckinger H L, Composition of cementing layer and adjacent tissues as related to germ-endosperm separation, *Cereal Chem*, 1958, **35**, 127.
25. Chakravarty A and De D S, *Post Harvest Technology of Cereals and Pulses*, Oxford and I.B.H. Pub. Co., Bombay, 1981, 213.
26. Macleod A M and Palmer G H, The embryo of barley in relation to modification of endosperm, *J Inst Brew*, 1966, **72**, 580.
27. Swift J G and O'Brien T P, The fine structure of scutellum during germination, *Aust J Biol Sci*, 1972, **25**, 469.
28. Daussant J Miyata S Misui T and Akazawa T, Enzymic mechanism of starch breakdown in germinating rice seeds XV Immunochemical study on multiple forms of amylase, *Plant Physiol*, 1983, **71**, 88.
29. Bechtel D B and Pomeranz Y, Ultrastructure of the mature ungerminated rice (*Oryza sativa*) caryopsis, The germ, *Amer J Bot*, 1978, **65**, 75.
30. Dikeman E. Bechtel D B and Pomeranz Y, Distribution of elements in the rice kernel determined by X-ray analysis and atomic absorption spectroscopy, *Cereal Chem*, 1981, **58**, 148.

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Preliminary Studies on the Effect of Drying Temperature and Time on the Concentration of Dimethyl Sulphide in Sorghum Wort

GEORGIANA N. ANICHE AND HILARY NWOKEDI
Department of Applied Microbiology and Brewing, Anambra State University of Technology, P. M. B. 01660, Enugu, Nigeria.

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Samples of sorghum malt germinated for 5 days were dried at 50, 60 and 70°C for 24 hr. Samples were collected at 4 hourly intervals, mashed using 3 stage decoction method and dimethyl sulphide content in head space of samples was determined by a gas chromatographic method. Dimethyl sulphide levels decreased as drying temperature and time increased.

The importance of dimethyl sulphide (DMS) in the flavour of lager beer has led to much work on the role of precursors of DMS in malting and brewing[1,2]. The principal source of dimethyl sulphide in the absence of serious wort infection appears to be a heat labile precursor containing S-methyl methionine (SMM)[3,4]. Recently, however, it has been observed that DMS can be produced in beer made from worts which contain no significant concentration of precursor.[5]

In a malting trial carried out by some workers[6], it was found that the greater the moisture content of the germinating grain and the higher the germination temperature, the greater was the DMS precursor content. In the drying process, increasing the drying temperature caused increasing decomposition of DMS precursor and this decomposition continued during kilning. Also, the level of dimethyl sulphide decreased as the kilning temperature increased.[7] Narziss *et al.*[6] had reported that decoction mashing process produced wort with lower dimethyl sulphide than infusion mashing. Wilson and Booer[1] reported that increasing the boiling time and temperature led to the decrease of dimethyl sulphide in wort due to the chemical decomposition of SMM to free dimethyl sulphide which was lost by volatilisation. Anness[8] found that all DMS present in beer originates ultimately from thermal decomposition of SMM, a compound synthesized during the germination of barley. It has also been suggested that yeast can synthesize SMM and that metabolic breakdown of this compound was responsible for some of the DMS produced during yeast fermentation.[9]

Most brewing industries in Nigeria now use malted sorghum in lager beer production. No report exists in literature on the effect of malting and wort production parameters on the dimethyl sulphide content of sorghum wort or beer. The results reported in this paper are concerned mainly on the effect of drying temperature and duration on the DMS content of sorghum wort.

Materials and Methods

A sorghum variety, 'Ll499,' obtained from the Institute of Agricultural Research, Samaru, Zaria, was used. The sorghum grain was analysed for one thousand kernel weight, moisture content. germinative energy, germinative capacity and water sensitivity.[10] The fat, protein and nitrogen contents were also determined.[11]

Sorghum sample (1kg) was steeped in tap water for 24 hr. The water was changed at 6 hourly intervals. At the end of 24 hr, the grains were drained and heaped in a plastic container to conserve heat of germination. After 24 hr, the grains were spread evenly on a wet jute bag. They were germinated at 20 ± 1°C for 5 days and water was sprinkled on the germinating grains as the need arose.[12] At the end of germination, the green sorghum malt was divided into three portions. One portion was dried at a temperature of 50°C, the other portion was dried at 60°C and the third portion was dried at 70°C. Samples (20g) were collected from each portion at 4 hourly intervals. Rootlets were removed from the malted sorghum samples and ground finely in a Waring blender.

The different sorghum malt samples were mashed in a 250 ml beaker using three stage decoction process.[12] The resulting wort samples were filtered and analysed for dimethyl sulphide (DMS) content.[13]

Results and Discussion

The result of the analysis of the sorghum grain is shown in Table 1. The effect of drying temperature and time on the concentration of dimethyl sulphide is shown in Fig. 1. The concentration of dimethyl sulphide decreased as the drying time increased, with the lowest concentration being got at 24 hr of drying. This agrees with the work of Narziss *et al.*[6] who found that increasing the drying time of barley malt increased the decomposition of DMS precursor with the

TABLE 1. ANALYSES OF SORGHUM GRAIN

Moisture content (%)	8.26
Nitrogen (%)	1.67
Protein (N × 6.25) (%)	10.44
Germinative energy (%)	95
Germinative capacity (%)	98
One thousand kernel wt (g)	26.21
Fat "as is" (%)	3.20
Fat "dry basis" (%)	3.50
Water sensitivity	0

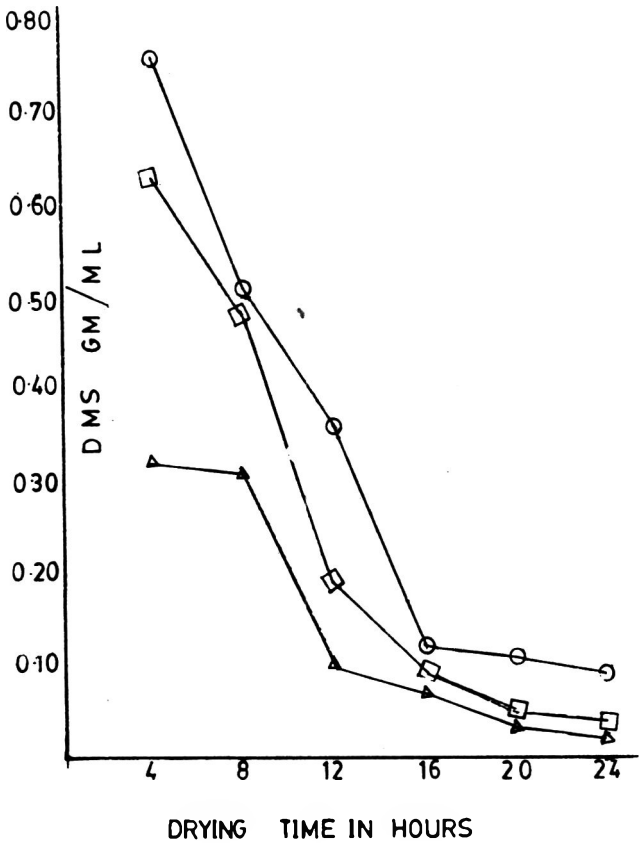

Fig. 1. Effect of drying temperature and time on Dimethyl Sulphide (DMS) concentration O, sample dried at 50°C; □ sample dried at 60°C; △ sample dried at 70°C.

resultant decrease in DMS level. The highest level of DMS was got at a drying temperature of 50°C while the lowest level was got at 70°C. This result agrees with the work of Nakajima and Narziss[7] who found that DMS levels in barley malt decreased as kilning temperature rose. This can be explained by the fact that high temperature leads to the chemical decomposition of S-methyl methionine which is a DMS precursor to free dimethyl sulphide which is then lost by volatilisation[8]. Sorghum malt is usually dried at 50°C to conserve the enzymes in the malt[12]. From the present study it is evident that the highest concentration of DMS was in sorghum malt dried at 50°C. Anness[8] has reported that in the mashing process, SMM is extracted from malt and is driven off during wort boiling. Therefore, regulation of DMS concentration in sorghum wort can then best be achieved by variation of the length and severity of wort boiling.

In conclusion, this study shows that sorghum malt contains dimethyl sulphide. Since dimethyl sulphide plays an important role in the flavour of lager beers, it is important that the conditions of sorghum malt drying are controlled to regulate the presence of DMS in sorghum wort.

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References

1. Wilson R J H and Booer C D, Control of the dimethyl sulphide content of beer by regulation of the copper boil, *J Inst Brew*, 1979, **85**, 144.
2. Dickenson C J, Relationship of dimethyl sulphide level in malt, wort and beer, *J Inst Brew*, 1979, **85**, 235.
3. Anness B J Bamforth C W and Wainwright T, The measurement of dimethyl sulphoxide in barley and malt and its reduction to dimethyl sulphide by yeast, *J Inst Brew*, 1979, **85**, 346.
4. Dickenson C J, Identification of dimethyl sulphide precursor in malt, *J Inst Brew*, 1979, **85**, 329.
5. Booer C J and Wilson R J H, Synthesis of dimethyl sulphide during fermentation by a route not involving the heat-labile DMS precursor of malt, *J Inst Brew*, 1979, **85**, 35.
6. Narziss L, Miedaner H and Bourjau T, The effect of malting and wort production parameters on the content of dimethyl sulphide and its precursors, *Brauwissenschaft*, 1979, **32**, 62 (*J Inst Brew Abstr*, 1979, **85**, 46.)
7. Nakajima S and Narziss L, The effect of kilning conditions on the dimethyl sulphide content of wort and beer, *Brauwissenschaft*, 1978, **31**, 145 (*J Inst Brew Abstr*, 1979, **85**, 46).
8. Annes B J, The role of dimethyl sulphide in beer flavour, *EU Brew Conv Monograph, VII Flavour symposium Copenhagen*, 1981, 135 (*J Inst Brew Abstr*, **89**, 107).
9. Annes B J, The reduction of dimethyl sulphoxide to dimethyl sulphide during fermentation, *J Inst Brew*, 1980, **86**, 134.
10. *Recommended Method of Analysis* Institute of Brewing, 1986.
11. *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington D.C., 1977.
12. Okafor N and Aniche G N, Preliminary investigations on brewing of lager beer from sorghum *Brew Distil Int*, 1980, **10**, 32.
13. Dickenson C J, Determination of dimethyl sulphoxide in malt, wort and beer, *J Inst Brew* 1981, **87**, 16.

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Temperature Dependence of the Soaking and Cooking Rate of Faba Bean (*Vicia faba* L.) Dal

M.B. BERA, K.L. SAHU, S. MUKHERJEE*, M. BARGALE AND Y.K. SHARMA
Department of Food Science and Technology, J.N. Agricultural University,
Jabalpur, M.P., India

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The rate equation involved two parameters i.e. hydration constant (ß) and diffusion constant (D) and their dependence on temperature has been studied. The hydration constant increased with temperature, while the diffusion constant increased with the increase in temperature and initial moisture content. Similarly, activation energy required during soaking decreased with increase in the initial moisture content. Diffusion related activation energy did not show any significant relationship with the initial moisture content. Various empirical equations developed for the prediction of the rate of moisture absorption, hydration constant, and diffusion constant are reported. The correlation coefficient (r = 0.8 − 0.9) of these empirical equations are found to be highly significant.

Dry beans are usually hydrated prior to cooking. While cooking, physico-chemical changes take place in the products leading to softening of the product, which can be easily consumed. The effect of hydration rate on the quality of the product as well as various processes for preparing quick-cooking dry bean is reported in the literature.[1-3]

Very little information is available on the hydration and cooking rate of Faba bean (*Vicia faba* L.) which is recently being introduced in India for cultivation. Faba bean is a legume crop of high yielding in nature and contains 20-25 per cent protein, which indicates its potential use in Indian diets as protein source. Therefore, present investigation was undertaken (1) to study the effect of temperature and initial moisture content on hydration rate, and (2) to study the effect of degree of hydration on the rate of softening by cooking as measured by INSTRON Universal testing machine. The rate of moisture absorption during soaking plays an important role in reducing the soaking/cooking time. To achieve this, the knowledge of saturation moisture content value is essential, which indicates the upper limit of moisture absorption by particular grain/material without any physico-chemical change in the material[4]. The rate of water uptake by the grain is directly proportional to the difference of saturation moisture content and the moisture content of the grain at any given time i.e.

$$dM/d\theta \propto (Ms - M) \quad \ldots\ldots\ldots\ldots (1)$$

$$\text{or} \quad dM/d\theta = \beta (Ms - M) \quad \ldots\ldots\ldots\ldots (2)$$

Where, Ms is the initial saturation moisture content, M is the initial moisture content, β is the hydration constant.

Rate of moisture absorption is very much influenced by the temperature of soaking medium which can be represented by Arrhenius law:

$$\beta = Ae^{-E/RT} \quad \ldots\ldots\ldots\ldots (3)$$

Where, A = constant having same unit as the hydration constant, E = activation energy, kcal/kg mole, R = Universal gas constant, kcal/kg mole, T = absolute temperature, °K.

Water absorption in dry legumes is a complex process of diffusion with swelling. In addition, there are two distinct resistances to diffusion, the seed coat and the cotyledons.[5] The diffusibility of water in solid is estimated by studying sorption kinetics.[6] The sorption kinetic method is based on the assumption that the adsorption and desorption rate follows the unsteady-state diffusion equation through the solid sample, the surface resistance to mass transfer is assumed to be negligible, the diffusion is assumed to take place from both flat surfaces of the sample. The solutions of the unsteady-state (transient) diffusion equation for constant diffusivity are available in graphical form for the basic shape of slab, infinite cylinder and sphere.[6] They are similar to the solution of the Fourier equation for unsteady state condition. For each geometric shape, the concentration ratio is given as a function of Fourier number, Dt/L^2, where D is the diffusivity, t is the time, and L is the slab half thickness, or the radius for the cylinder or sphere.

In the sorption Kinetics method, the fractional uptake or loss of the diffusent in the sample (m/m_e) is plotted versus (t/L_c^2½. Here, m is the water adsorbed after time t, m_e is the

Present address: *Department of Post-Harvest Process & Food Engineering, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.) India.

equilibrium value at infinite time. The approximate relation for solving unsteady state diffusion equation after considering appropriate initial and boundary condition gives the following expression[6].

$$D = \frac{0.049}{(t/L^2)½} \quad \ldots\ldots\ldots\ldots (4)$$

Where, D is the diffusion constant, m^2/s and $(t/L^2)½$ time corresponds to half equilibriums, $m/m_e = 0.5$. The diffusivity of various compounds depends on the temperature, and the following forms of Arrhenius equation has been applied.

$$\frac{d\ln D}{dt} = E_D/RT^2 \quad \ldots\ldots\ldots\ldots (5)$$

Where E_D is the energy of activation for diffusion.

Materials and Methods

Faba bean variety ('JV-2') dal was obtained from the Department of Post-harvest Process and Food Engineering, JNKVV, Jabalpur. The initial moisture content of the dal was adjusted to 12.0, 20.0 and 30.0 per cent (d.b.). Ten gram of the sample was taken in pyrex boiling tube and 100 ml of distilled water was added to it. The pyrex boiling tube was immersed in a thermostatically controlled water bath fixed at a predetermined temperature i.e. 25, 40, 60 and 80°C. After the predetermined soaking time (15, 30, 45, 60, 120 and 180 min) the dal sample was removed from boiling tube. The data on change in radius (r) and moisture content of the sample were determined. Similarly in another set of experiment the presoaked dal sample (2, 4, 6 and 24 hr) at room temperature (25°C ± 1) was cooked at 98°C ± 1 in the pyrex boiling tube for varying length of time (15, 30, 45, 60 and 120 min). After completion of cooking, the sample was analysed for its textural properties using INSTRON-1104 (UK) Universal testing machine.

Textural properties: The texture of dal was determined in terms of the load required for particular depth of compression of cooked dal. The cross head travel was fixed for 3 mm depth compression of cooked dal. The chart speed (500 mm/min) was also fixed. Average load of ten replications for each set of sample was noted. The textural property was expressed in terms of degree of cooking (Kg force/g. grain).

Results and Discussion

The variation of the moisture contents of Faba bean dal at various temperature and time levels during soaking are shown in Fig 1. It is seen that at higher temperature the rate of moisture absorption ($DM/d\theta$) was higher compared to the low temperature. To reach a moisture content (100 g. H_2O/100 g. dry matter) at 25°C, Faba bean dal took 90 min while at 80°C it took only 30 min. It appears, therefore, that temperature gradient during soaking was the main driving force in moisture absorption.

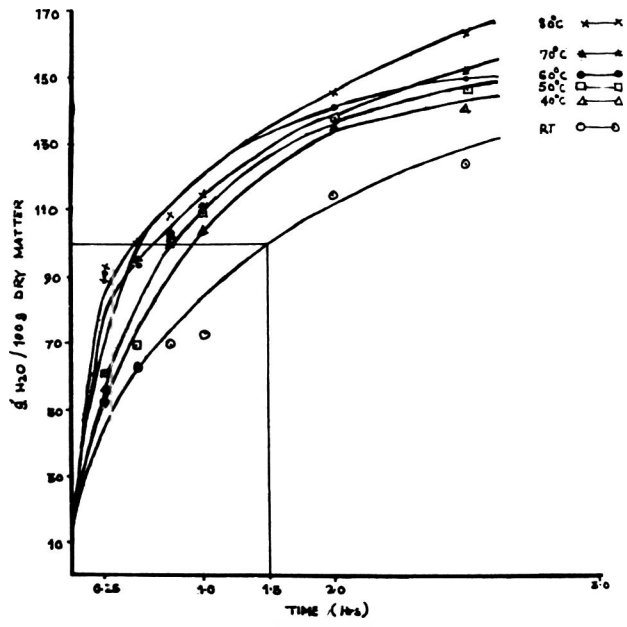

Fig. 1 Water absorption during soaking at various temperatures.

The saturation moisture content (Ms) was essential to investigate the soaking rate equation (Eqn. 2). The values of Ms at different initial moisture contents (12, 20 and 30 d.b.) and temperatures are given in Fig.2. Saturation moisture contents were 67.5, 77.5, 79.5 and 83.5 per cent (d.b.), respectively at 25° (RT), 40°, 60° and 80°C. It has been

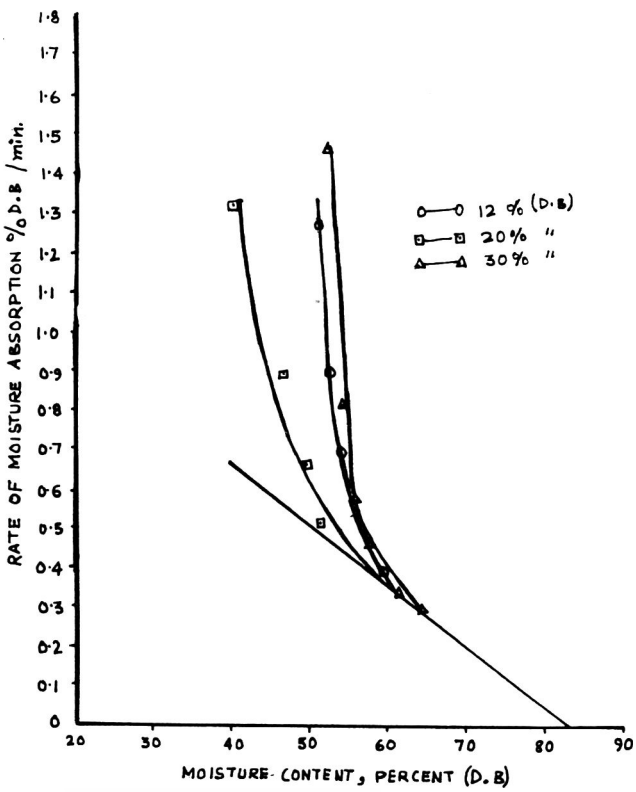

Fig. 2. Rate of moisture absorption v/s moisture content at soaking temperature of 80°C.

TABLE 1. EFFECT OF TEMPERATURE AND INITIAL MOISTURE CONTENT ON VALUES OF HYDRATION CONSTANT (β = 1/MIN), DIFFUSION CONSTANT (D = M^2/S)

Initial moisture content (%d.b.)	Temperature							
	25°C		40°C		60°C		80°C	
	β	D × 10^{-7}	β	D × 10^{-7}	β	D × 10^{-7}	β	D × 10^{-7}
12	0.072	3.88	0.079	4.71	0.180	5.50	0.211	5.68
20	0.046	5.56	0.067	5.50	0.094	6.44	0.101	6.62
30	0.114	5.83	0.141	6.621	0.189	6.80	0.200	7.42

TABLE 2. FUNCTIONAL RELATIONSHIP BETWEEN HYDRATION CONSTANT, DIFFUSION CONSTANT AND TEMPERATURE AT VARIOUS LEVELS OF INITIAL MOISTURE CONTENT

Initial moisture content (% d.b.)	Hydration constant (β)	Correlation coefficient (r)	Diffusion constant (D)	Correlation coefficient (r)
12	$\ln\beta$ = 5.1512 — 4664.03/RT	0.94	$\ln D$ = 14.5699 - 1.0366 × 10^{-4}/RT	0.75
20	$\ln\beta$ = 2.2555 — 3134.95/RT	0.94	$\ln D$ = 13.1044 — 783.78/RT	0.90
30	$\ln\beta$ = 1.65114 — 2249.65/RT	0.98	$\ln D$ = 13.2992 — 593.41/RT	0.92

observed that values of Ms increased with the increase in temperature.

Saturation moisture content related to each temperature was used to plot rate of moisture adsorption ($dm/d\theta$) versus (Ms-M) and the resulted curve showed a straight line. The slope of the straight line gave the constant (hydration constant). The hydration constant at each temperature and different levels of initial moisture content are presented in Table 1. The correlation coefficient (r) values for each empirical equation are also presented in Table 2. The highly significant values of correlation coefficient indicated that in general more than 80 per cent of the total variation of the observed data could be explained by these empirical models. When the values 'β were plotted against various levels of temperature and initial moisture content, it resulted in a straight line, which again confirms that rate of water absorption was temperature dependent. A linear relationship between the hydration constant (β) and reciprocal of absolute temperature (1/T) on semilog scale showed that the data fitted well to the equation 3. The slope of the straight line was used to calculate the activation energy (E). The activation energy ($\bar{E}$) values were 4664.03, 3134.95 and 2249.65 kcal/kg mol. °K, respectively at 12, 20 and 30 per cent (d.b.) moisture content. It was observed that the value of ($\bar{E}$) decreased with increase in initial moisture content i.e. higher the initial moisture content of Faba bean dal lesser will be the energy requirement during soaking. The functional relationship between hydration constant (B) and reciprocal of absolute temperature (1/T) from equation (3) are given in Table 2.

The diffusion constant (D) during soaking at various levels of temperature was determined using equation (4). In this values of m/m_c versus ($\sqrt{t/L^2}$) were plotted in the graph and values of $(t/L^2)_{0.5}$ were obtained and used for calculating values of D. The values of diffusion constant at each levels of temperature and initial moisture content are presented in Table 1. It is seen that diffusion constant increased with increase in initial moisture content and temperature. Since diffusion constants were also found to be temperature dependent, a linear relationship between diffusion constant and reciprocal of absolute temperature (1/T) on semilog scale, was obtained. The slope of the straight line of the curve was used to calculate diffusion related activation energy. These energies ($\bar{E}_D$) were 1.0366 × 10^{-4}, 783.78 and 593.41 kcal/kg mol. °K respectively at 12, 20 and 30 per cent (d.b.) moisture content. The functional relationship between diffusion constant (D) and reciprocal of absolute temperature (1/T) from equation (5) are given in Table 2. These empirical equations (Table 2) can be used to predict hydration constant and diffusion constant of Faba bean dal at a temperature range (25 - 80°C). In general, these empirical equation could explain more than 80 per cent of the total variation in the observed data.

Cooking characteristics: Faba bean dal sample initially soaked for 2, 4, 6 and 24 hr, and cooked at 98 ± 1°C (cooking temperature) for 15, 30, 45, 60 and 120 min, showed that degree of cooking (kg force/g. grain) decreased with increase in time of cooking i.e., dal became softer.

The initial moisture content of the dal before cooking influenced the degree of cooking with greater intensity than the time of cooking. For optimum cooking of any grain, the grain should have saturation moisture content. Hence, Faba bean dal at 12 per cent (d.b.) initial moisture content having obtained a saturation moisture content (67.5 per cent d.b.) at 98°C and its related hardness data or degree of cooking

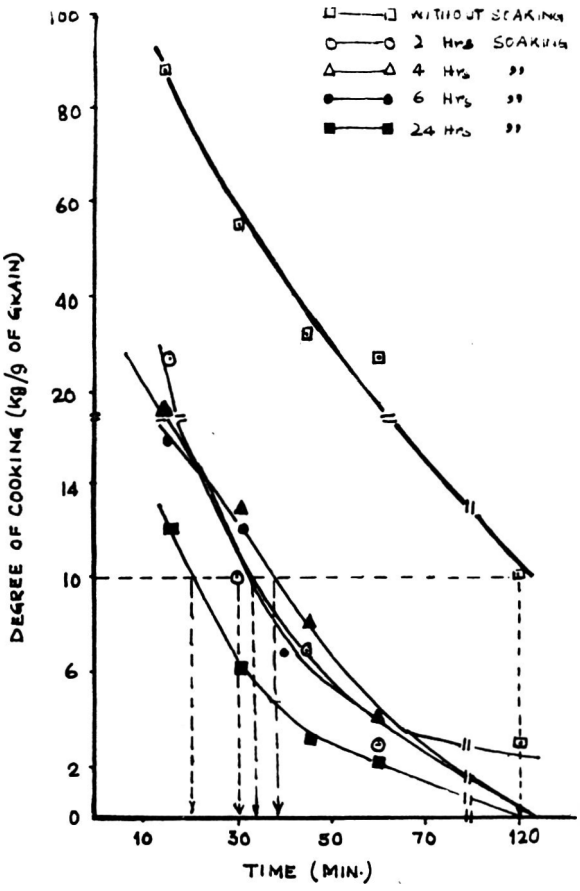

Fig. 3. Effect of soaking on degree of cooking of Faba bean dal at 98°C.

(10 kg. force/g. grain) was taken as basis for calculating the time required by Faba bean dal to reach optimum cooking level (Fig.3). It has been observed that Faba bean dal took 20 min. for 24 hr soaked sample while without soaked sample (control) took 120 min to attain optimum degree of cooking (10 kg. force/g. grain). The cooking time of Faba bean dal when compared with other conventional dal, i.e. pigeon pea (110 min), green gram (93 min), black gram (143 min), chickpea (157 min) and soybean (300 min)[7], revealed that Faba bean dal takes less time to cook in comparison to soybean, chickpea and black gram.

It is therefore, concluded that soaking and cooking rate parameters are dependent on temperature and initial moisture content. The determinations of soaking and cooking rate and data on rate parameters may be used in the design of large scale cooking equipments.

References

1. Rockland L B , Heinrich J B and Doruback H J, Recent progress on the development of new and improved quick-cooking product from Lima and other dry bean, *In Report of the Tenth Dry Bean Research Conference, Davis, C.A.*, 1970.
2. Quast D G and de Silva S D, Temperature dependence of the cooking rate of dry legumes, J Fd Sci, 1977, **42**, 370.
3. Quast D G and de Silva S D, Temperature dependence of hydration rate and effect of hydration on the cooking rate of dry legumes, *J Food Sci*, 1977, **42**, 1299.
4. Kulkarni S D and Bal S, Saturation moisture content of paddy grains and its dependence of initial moisture content and temperature of soaking, *J Agric Engg*, 1986, **23**, 99.
5. Binder L R and Rockland L B, Use of automatic recording shear press in cooking studies of large dry time beans (*Phaseolus lunatus*) *Fd Technol*, 1964, **18**, 127.
6. Crank J, *The Mathematics of Diffusion*, Oxford Univ. Press, 1975, 2nd Edn, 424.
7. *Annual report in soybean product utilization project*. SPUP/CIAE. Centra Institute of Agricultural Engieering, Bhopal (M.P.), 1985.

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Evaporative Cooling Storage of Potatoes in Two Model Storage Structures

M. V. RAMA, H. KRISHNAMURTHY AND P. NARASIMHAM
Post-Harvest Technology Laboratory, Area of Fruits, Vegetables & Plantation Crops,
Central Food Technological Research Institute, Mysore - 570 013, India.

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Two model storage structures of evaporative cooling (EC) were designed, fabricated and their relative performance in curtailing weight losses and sprouting during storage of potatoes was evaluated in June/July and October/November months. System I, is a metallic chamber without perforations of the evaporative surfaces for restricting ventilation, which maintains a RH of 90 ± 5%, and system II is a weld mesh chamber (2.5 cm^2 mesh) with the evaporative sides covered with wet cloth to provide free movement of evaporatively cooled air, which maintains a RH of 85 ± 5%. Both the systems reduced the physiological losses in weight of potatoes during storage compared to those stored at ambient conditions (20-30°C, RH 40-80%). However, the system I has recorded less physiological weight loss than the system II.Both the systems maintained the temperature of air inside the EC closer to wet bulb temperature. Storing potatoes pretreated with sodium salt of naphthalene acetic acid (NAA) containing fungicide, Benlate each at 1000 p.p.m. concentration, in the EC containers had slightly reduced sprouting compared to untreated tubers and eliminated spoilage.

Shrivelling due to water loss is one of the serious problems encountered during storage of potatoes under tropical ambient conditions. Since refrigerated storage is expensive, in recent years, there is a shift in the emphasis from cold storage to other alternative storage systems. Evaporative cool storage is one such alternative system that is being explored in the country. During 3 months storage under EC conditions, weight loss of 9 per cent as against 15.1 per cent in country store was reported[1]. The present investigation employing two systems of evaporative cooling containers was carried out to assess (i) their efficacy in maintaining temperature close to wet bulb with high relative humidity inside EC chamber and (ii) the weight losses, sprouting and spoilage of potatoes during storage.

Materials and Methods

Description of the E.C. chambers: The system I used in this study was the same as described in an earlier publication.[2] The system II resembles system I in all respects except that the outer metallic chamber was replaced by a weld mesh (mesh size 2.5 cm^2 size) basket. The weld mesh basket is covered with wet gunny cloth, the latter did not obstruct ventilation. The top tray used in this system (to serve as reservoir of water to keep the gunny cloth remain wet) was devoid of vents. The performance of these two structures loaded with potatoes was studied in June/July and October/November months.

Potatoes were given a post-harvest dip treatment for 15 min in an aqueous solution of sodium salt of naphthalene acetic acid at 1000 p.p.m., containing 1000 p.p.m. benlate.[3] After surface drying, the tubers were filled into a smaller weld mesh (2.5 cm^2 mesh size) baskets and stored inside the E. C. systems. Untreated tubers (dipped in water and surface dried) were used as controls. The untreated and treated tubers (each 7½ kg) were separated from each other. Treated and untreated potatoes stored in gunny bags at ambient conditions (22-35°C, RH 50-80 per cent) served as controls.

Ten tubers, each from untreated and treated lots were marked, and distributed in the respective lots of potatoes held in the EC chambers and in gunny bags kept at ambient. They were weighed individually at the commencement of experiment and at regular intervals subsequently to determine the physiological loss in weight. The temperatures of air inside the evaporative cool chambers were recorded with probes of a. six channel digital temperature indicator. The core temperature of the tubers placed at the center of the E.C. chamber was determined with a sterile needle probe pierced into the geometrical center of the tuber and the relative humidity of the air inside the E.C. chamber was worked out by recording the wet bulb temperature by fixing, a wick immersed in water, to the probe kept inside the chamber. Ambient temperatures were recorded periodically with a wet and dry bulb thermometer and from their difference RH was obtained. The experiments were terminated when the evaporation of water was reduced due to change of season. At the end of the experiment, the number of sprouted tubers, the fresh weight of sprouts and number of spoiled tubers were recorded.

Results and Discussion

Temperature fluctuations: During June and July, the daily fluctuations in temperatures of ambient air between 9 a.m. and 5 p.m. indicated that the dry bulb temperature fluctuated between 24.5 and 28.3°C, while the wet bulb temperature

fluctuated between 21.5°C and 22.5°C. As against this, the temperature of air inside the EC chamber (System I) fluctuated between 22.2 and 23°C while the temperature of potato core fluctuated between 22.0 and 22.5°C. The core temperature of potato during the afternoons was even less than that of ambient wet bulb temperature mainly because of the raise in the wet bulb temperature of ambient air, and due to the differences in the specific heats of air and potato. (Table 1).

Similarly, during the October and November months, between 9 a.m. and 5 p.m. the dry bulb temperature of ambient air fluctuated between 24.5 and 30.5°C, while the wet bulb between 21.0 and 22.5°C. In the E.C. storage System I, the air temperature was between 23.2 and 24.5°C; and in System II it was between 21.0 and 22.5°C. The temperature variation in System II was less than that in System I, probably because of free movement of air. The relative humidity of the ambient air during this period fluctuated between 45 and 72.0 per cent, while in System I it was between 85 and 95 per cent, and in System II between 80 and 88 per cent. The lower RH inside the System II was probably because of free air movement (Table 1).

Physiological loss in weight and sprouting of potatoes: From the data presented in Table 2, both in summer and winter months, the potatoes stored in System I have lost around 1 per cent of their weight in 30 days period while in System II (employed during October/November months only) has lost around 1.5 per cent as against a loss of around 2.5 per cent at ambient conditions for the same period of storage. Under Mysore ambient conditions, since the fluctuations in R. H. and temperature are within narrow range, the loss in weight of potatoes stored at ambient conditions was around 2.5 per cent per month. However, as Varma and Sukumaran[1] in their studies noted the losses in weight at ambient conditions in North India during summer would be far higher. Since the relative humidities of the air maintained in System I are between 85 and 95 per cent, with a loss in weight around 1 per cent per month, it can be expected that this System will considerably reduce the weight losses to around 5 per cent in 3 months storage. It may also be noted that the treatment, as expected did not show any effect on weight losses.

Sprouting and spoilage of potatoes: The data on sprouting

TABLE 1. DAY TIME FLUCTUATIONS@ IN TEMPERATURE AND RELATIVE HUMIDITY OF AMBIENT AIR AND IN THE E.C. STORAGE SYSTEMS DURING STORAGE OF POTATOES

Storage method	Time of recording				
	9 a.m.	11 a.m.	1 p.m.	3 p.m.	5 p.m.
Rabi crop (June-July — 30 days)					
a. Ambient air:					
Dry bulb	24.5	26.5	28.3	27.9	26.8
Wet bulb	21.5	22.0	22.4	22.5	22.0
b. In EC System I:					
Potato core	22.0	22.0	22.1	22.2	22.5
Air	22.2	22.5	22.8	23.0	22.8
Kharif crop (Oct.-Nov. — 48 days)					
a. Ambient air:					
Dry bulb	24.5	27.5	28.5	30.5	28.7
Wet bulb	21.0	22.0	22.2	22.5	22.2
RH (%)	72.0	59.5	54.5	45.0	52.0
b. In System I.					
Air temp.	23.2	23.3	23.5	24.0	24.5
RH (%)	88	95	93	90	85
c. In System II:					
Air temp.	21.0	21.3	22.0	22.5	22.2
RH (%)	88	85	83	82	80

@ Mean values for the storage period.

TABLE 2. PHYSIOLOGICAL LOSS IN WEIGHT, SPROUTING AND SPCILAGE OF POTATO TUBERS IN STORAGE

Parameter	Ambient		E.C. storage	
	Untreated	Treated*	Untreated	Treated*
Physiological loss in wt (%)				
Kariff crop (June/July, 30 days)	2.57±0.71	2.44±0.51	1.10±0.25[1]	0.90±0.47[1]
Rabi crop (Oct.-Nov. 48 days)	4.14±0.30	4.03±0.36	1.52±0.17	1.68±0.31[1]
			2.60±0.25	2.20±0.20[2]
Mean loss in 30 days	2.58	2.48	1.02	0.98[1]
			1.62	1.38[2]
Sprouting@				
Sprouted (%)	100	84	100	84
Fresh wt of sprouts (g/100 tubers)	86.1	21.6	70.1	17.1
Spoiled tubers (%)	4.5	0	0	0

*Treated with NAA and Benlate
(1) System I; (2) System 2.

@Sprouting and spoilage data are for EC. system 1, during June/July experiment. Sprouting and spoilage data not recorded for Oct/Nov. experiment as the experiment was closed early as winter started.

of potatoes (Table 2) indicate that both under ambient and E.C. storage conditions, a slight reduction in sprouting was observed in NAA treated tubers compared to untreated. The fresh weight of sprouts shows that NAA treatment has significantly reduced sprout growth and was equally effective in curtailing sprout growth both under ambient and E.C. storage conditions. Only untreated tubers stored at ambient conditions showed spoilage of 4.5 per cent.

Thus the results presented in this paper show that E.C. chamber (System I) maintains 1 to 2°C above that of ambient wet bulb temperature together with high relative humidity (90 ± 5 per cent) thus enabling to reduce weight losses to around 1 per cent per month. The System II with perforations on the evaporative surface not only maintains temperatures similar to System I but also maintains lower RH, probably because of the free air circulation. The System I, which is based on the wet bulb principle has been successfully employed in the storage of apples and oranges,[2] based on the same principle, an EC storage chamber of 50 kg capacity has been designed, fabricated for home scale preservation of fruits and vegetables in rural and urban areas to meet the need of low and middle income groups.

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References

1. Varma S C and Sukumaran N P, Potato storage and processing in *Proceedings of Indo-Holland Potato Seminar May 20-21, 1986, New Delhi*, Published as Technical Communication No.1, P 86-95, by the National Horticultural Board, Gurgaon-122001.
2. Habibunnisa, Aror E and Narasimham P, Extension of the storage life of fungicidal waxol dip treated apples and oranges under evaporative cooling storage conditions, *J Fd Sci Technol* 1988, **25**, 75.
3. Rama M V and Narasimham P, Control of potato (*Solanum tuberosum* L.) sprouting by sodium naphthyl acetate during ambient storage, *J Fd Sci Technol*, 1989, **26**, 83.

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Effect of Extrusion Process Variables on Microstructure of Blends of Minced Fish and Wheat Flour

SUVENDU BHATTACHARYA*, H. DAS AND A.N. BOSE
Department of Agricultural Engineering, Indian Institute of Technology,
Kharagpur — 721 302, India

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The effect of the extrusion process variables, such as length/diameter ratio (L/D) of the extruder, composition of feed temperature of extrusion, and screw speed, on extrudate microstructure, was studied. The extrudates were produced from blends of wheat flour and partially dried low-cost marine fishes through a single-screw, variable-length extruder-cooker, with a barrel bore of 25 mm. Examination of the extrudates under scanning electron microscope revealed that higher L/D ratio, and incorporation of higher amount of fish in the extruder feed yielded a product with organized fibrous microstructure. These products can be used as an analogue for fish or meat. Expanded fish snacks, on the other hand, could be generated by use of lower L/D ratio or feed ratio. Increase in temperature (100-140°C) helped the texturization process by fibre formation whereas the effect of screw speed (30-90 r.p.m.) on extrudate microstructure was not clear.

Thermoplastic extrusion combines the heating of food products with the act of extrusion to create fabricated, cooked and shaped products of varying texture by using a wide range of ingredients such as plant[1,2] (oilseeds, cereals, etc.), microbial[3,4] and animal sources[5-7]. The current variety of extruded products is highly impressive[8]. Extrusion cooking can produce a chewy, structured extrudate which has been found suitable as a meat extender and is used after rehydration by the school lunch programme in different countries[9]. The low cost fish, particularly of marine source may also be used to have meat analogues[10] or expanded fish snacks[11] or crackers[12] such that a better utilization of marine resource, which is at present under-utilized in nature, may find a proper use[13,14].

The problems associated with all fabricated extruded products lie in the creation of texture. Apart from the different physical, and mechanical methods of texture measurements and sensory assessments[15] of the extruded products, the microscopic examination of the same is also considered to be a good tool for examination of its texture[16] as food texture is a result of microstructure, which, in turn, depends upon the physical forces on chemical components[17].

The process variables like length of extruder, composition of feed, moisture content of feed, temperature of extrusion, screw speed, pressure rise during extrusion etc. have significant effects on product texturization[18,19] and microstructure[20]. Kitabatake *et al*[21] found that long barrel extruder helped in texturization which proved that length of extruder could play a vital role in product texture and should be considered as a process variable.

In our previous study[22], we have reported that extruded products are nutritionally well acceptable. The present paper describes the effect of the extrusion process variables over the microstructure of extrudates obtained through the extrusion-cooking in a single screw cooking extruder.

Materials and Methods

Wheat flour: Refined wheat flour (locally called *maida*) was screened through a 100 mesh sieve to remove clumps, or foreign materials, if any. The proximate composition (Table 1) of wheat flour was obtained by AOAC methods[23].

Fish: Bombay Duck (*Harpodon nehereus*), a low-cost marine fish was collected in good condition and was brought under ice. The head, fins, tail and visera were discarded, and

TABLE 1. PROXIMATE COMPOSITION OF BOMBAY DUCK FISH MUSCLE AND WHEAT FLOUR

	Fish	Wheat flour
Moisture (%)	89.72 ± 1.96	14.04 ± 0.72
Protein* (%)	6.78 ± 0.58	10.27 ± 0.87
Fat (%)	0.86 ± 0.11	0.84 ± 0.20
Ash (%)	2.07 ± 0.33	0.72 ± 0.09
Carbohydrate (%) (by diff)	0.57	74.13

* N × 6.25 for fish and N × 5.7 for wheat flour
The values are the mean ± standard deviation of 5 observations.

**Present address*: Discipline of Confectionery and Convenience Foods, Central Food Technological Research Institute, Mysore — 570 013, India.

the fish was cleaned with tap water before slicing into pieces of thickness less than 10 mm. These pieces were partially dried at 60-65°C in a tray dryer (S.D. Instruments and Equipments, Calcutta) for 3-4 hr, to attain a moisture content that was a little lower than 40 per cent. The raw fish muscle was also analyzed for its proximate composition[23].

Extruder: A single-screw, variable length, cooking extruder with a barrel bore of 25 mm was developed[24] in the course of the present study. This stainless steel extruder could be adjusted at three different lengths (8, 12 and 16 times the internal diameter of the barrel) by use of different screws and interchangeable barrel sections. The ratio of the channel depths at the start of the feed and metering section was 3. The length of the feed-transition section of the screw was fixed at 20 cm, whereas the length of the metering section was made variable at 0, 10 or 20 cm (corresponding to L/D = 8, 12 and 16, respectively). The extruder was provided with electrical heaters fitted externally to the barrel and was equipped with automatic control devices for temperature. Provision was made to measure temperature and pressure of the food dough during extrusion. The clearance between the flight of screw and barrel was 0.2 mm. The helix angle and pitch (measured horizontally) of the extruder screw was 22° and 28 mm, respectively.

Preparation of feed for extrusion: The semi-dried fish were ground in a laboratory grinder; then a calculated amount of wheat flour was added, and mixed well to obtain a homogeneous feed. The amounts of fish and wheat flour were adjusted in such a way that their respective solid ratios were 1:1, 2:1 or 3:1. Care was taken to avoid lumping. The moisture content of the feed was then adjusted at 35 per cent by adding a little tap water, and mixed for another 5 min. Each lot was packed in double-walled polyethylene pouches, and allowed to equilibriate overnight at 4-5°C in a refrigerator. Before extrusion, the feed was allowed to come to ambient temperature (28-30°C) and remixed for 2 min after checking its moisture content.

Extrusion: The blend of fish and wheat flour was then passed through the specially developed, variable length food extruder. using a die of length 21.5 mm, and diameter 8 mm. The process variables for the extrusion of fish/wheat-flour blends were the L/D ratio (8, 12 and 16), feed ratio of fish and wheat flour (1:1, 2:1 and 3:1); temperature of extrusion (100, 120 and 140°C), and screw speed (30, 60 and 90 r.p.m.). Thus, in all 81 combinations were possible. The number of replications was three.

Microstructure of extrudate: The extruded rods were cryofractured by liquid nitrogen immersion technique[25]. The cross and longitudinal sections of the cryofractured sections were lyophilized to make them free of moisture. The extruder feed (moisture content 35 per cent) were directly lyophilized. Silver paste was used to mount the samples on the study. The samples were coated with gold in vacuum using a sputter coater and exam.ned at 25 kV with PSEM Scanning Electron Microscope. (Model No. 500, Phillips, Holland).

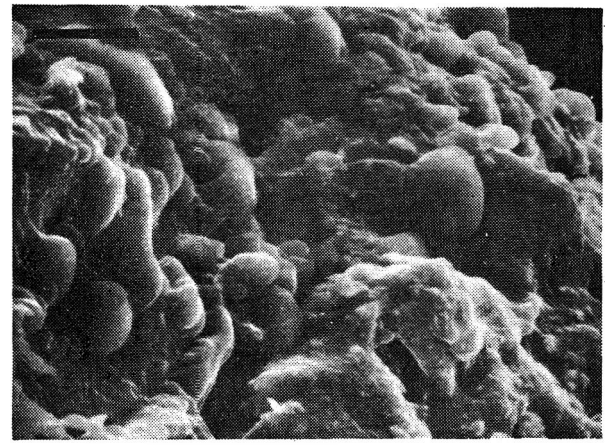

Fig. 1. SEM photomicrograph (800×) of extruder feed (prior to extrusion) having fish and wheat flour solid ratio of 2:1
Scale bar 10μ

Results and Discussion

Feed microstructure: Fig. 1 shows the SEM micrograph of the extruder feed just prior to extrusion (feed ratio = 2:1). Fused masses and loosely bound spherical bodies are visible in this micrograph.

Effect of L/D ratio: Fig. 2A, 2B and 2C are the SEM microphotographs (50×)of the cross-sections of extruded rod, produced at varying L/D ratios at 8, 12 and 16. In Fig. 2A, surface is full of cracks, holes, pits and pores but to a lesser extent in Fig 2B and 2C. Fig. 3A, 3B and 3C are the respective higher magnification (300×) and inclined (20°) views of Fig. 2A, 2B and 2C, respectively. Practically no pseudo-crosslinking or interfacial adhesion is observed in Fig. 3A, whereas, Fig. 3B shows some of them. Fig. 3C shows some fibres as if they are emerging out of the extruded rod and is perpendicular to the plane of cross-section. Thus, Fig 3C shows fibre formation in a particular direction as a result of extrusion with longer (L/D=16) screw.

Fig. 4 and Fig. 5 are the cross-sectional views of product, obtained from the smallest and largest screw (L/D=8 and 16, respectively). A crack in Fig. 2 A is focussed at higher magnification (1000×) as has been seen in Fig. 4. The cracks usually vary in length (may be upto few hundred microns) and are usually observed at the time of extrusion with low L/D ratio due to insufficient binding power at the time of extrusion. It is also observed that small pores are greater in number and are fairly distributed throughout the body whreas big pores are few and located near the boundary only (Fig. 4 and Fig. 5) which prove the biomodal distribution of pores. The possible explanation may be that a few small pore channel aggregate to form a big pore before the superheated steam leave the extrudate due to flash off. In Fig. 5, the big pores apparently seem to be expanded and cracked

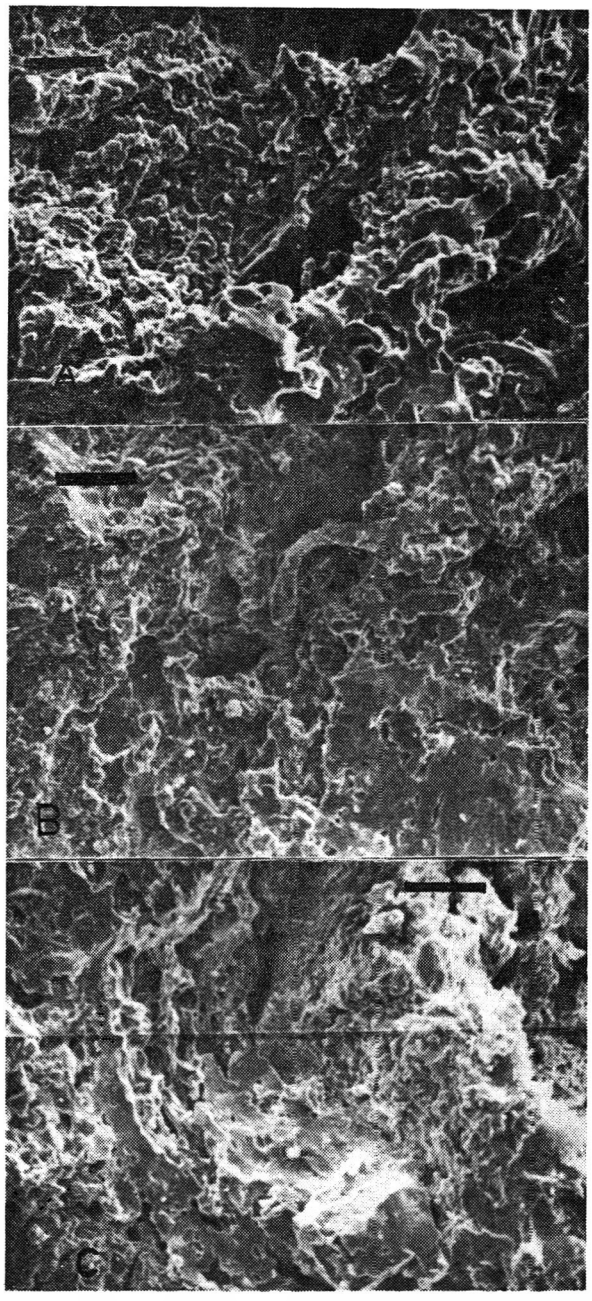

Fig. 2. Cross-sectional low magnification (50×) SEM views of extruded rod (extruded at 120°C, 60 rpm and 1:1) having L/D ratio of A) 8 B) 12 and c) 16

Scale bar 100 μ

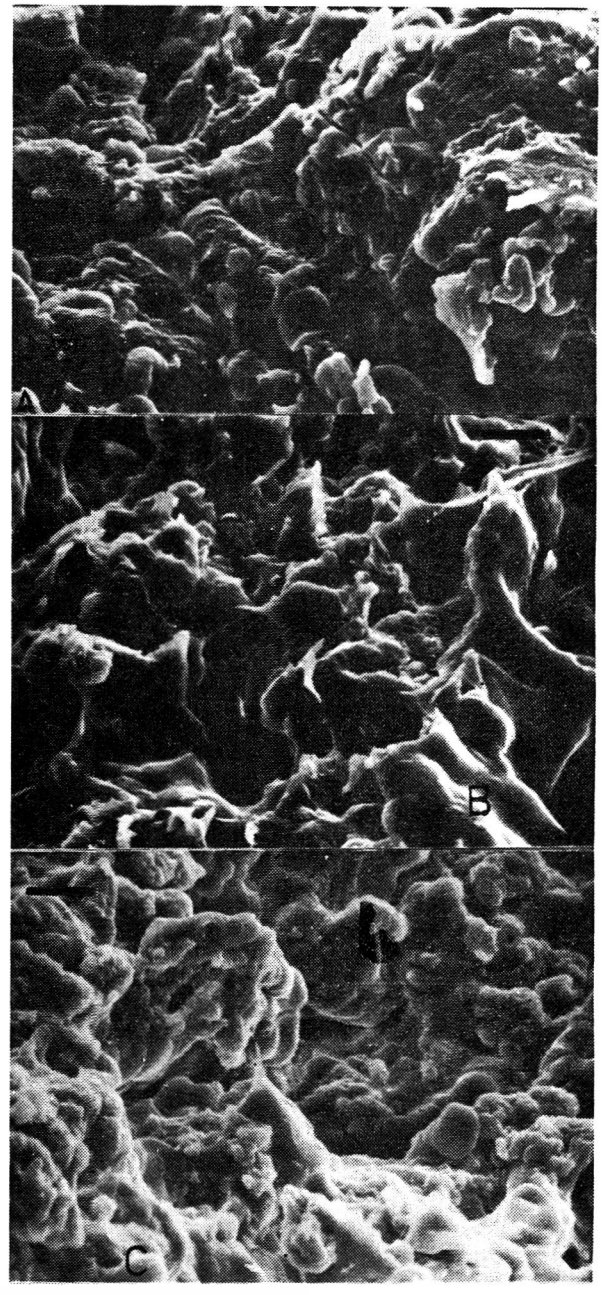

Fig. 3. Cross-sectional higher magnification (300×) SEM views of extruded rods (extruded at 120°C, 60 rpm and 1:1) having L/D ratio of A) 8 B) 12 and C) 16.

Scale bar 20μ

vacuole. The possible explanation may be that while releasing the superheated steam, the surrounding region expands and suddenly the superheated steam creates an opening to leave the extrudate under ambient pressure and thus, a cavity is formed. After releasing the steam, the starch embedded in protein matrix is quickly solidified[9] and retains partially open-cell structure and the yield is a typical expanded low density product. These products may find its use as fish snacks/crackers and consumption is possible after deep oil frying.

Fig. 6 A, 6 B and 6 C are the longitudinal views of the samples shown in Fig. 2 A, 2 B and 2 C, respectively. Fig. 6 A shows pores, cracks and holes with little fibrillation. Fig. 6 B is more compact with signs of initiation of short fibre formation but Fig. 6 C definitely shows the fibers (F) that are

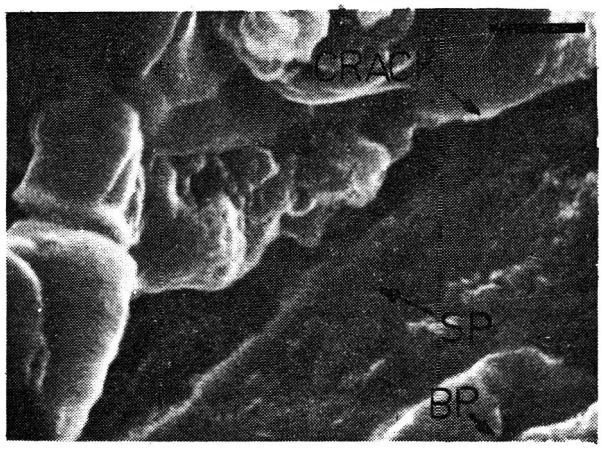

Fig. 4. Cross-sectional SEM view (1000×) of extruded rod (extruded at 120°C, 60 rpm, 1:1, L/D=8) showing crack, small (SP) and big (BP) pores.

Scale bar 10μ

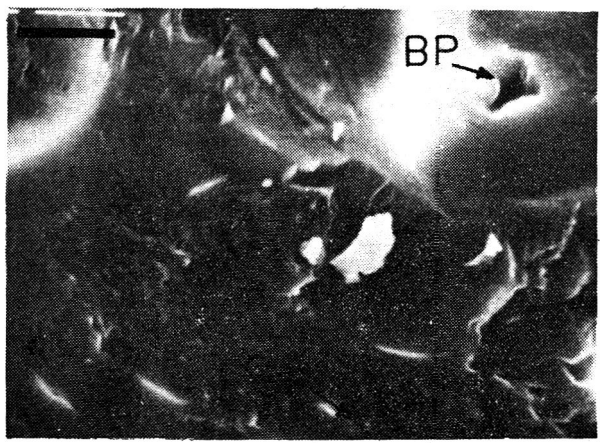

Fig. 5. Cross-sectional SEM view (1000×) of extruded rod (extruded at 120°C, 60 rpm, 1:1 L/D=16) showing small (SP) and big (BP) pores.

Scale bar 10μ

strengthened by fibrils (FB). The later photomicrographs indicate fibrosis and show that cleavage planes and fiber alignment planes are parallel though some cleavage plans are across several fibers. These findings tally with the results obtained by Neumann *et al* [26]:

Effect of feed composition: Fig. 7 A, 7 B and 7 C respectively denote the SEM cross-sections of extruded rods with feed comprising of fish and wheat flour solids in the proportion of 1:1, 2:1 and 3:1. The protein contents for the feeds are about 40, 51, and 61 per cent (dry basis), respectively. Fig. 7 A seems to have less compacted structure and loosely bound particles are visible. Fig. 7 B shows a little more homogeneity and short fibres are visible whereas Fig. 7 C represents a homogeneous product. Fig. 8 A, 8 B and 8 C are the SEM views of the longitudinal sections of the samples shown in Fig. 7 A, 7 B and 7 C, respectively. Though some structural networks and fibre formation are noticed in Fig. 8 A and 8 B the Fig. 8 C clearly indicates a high density texturized product and fibres are directed in a single direction only i.e. along the line of flow. It may be possible that higher amount of protein in extruder feed (as in the case of feed ratio of 3:1), the extent of fibre formation is favoured with increased

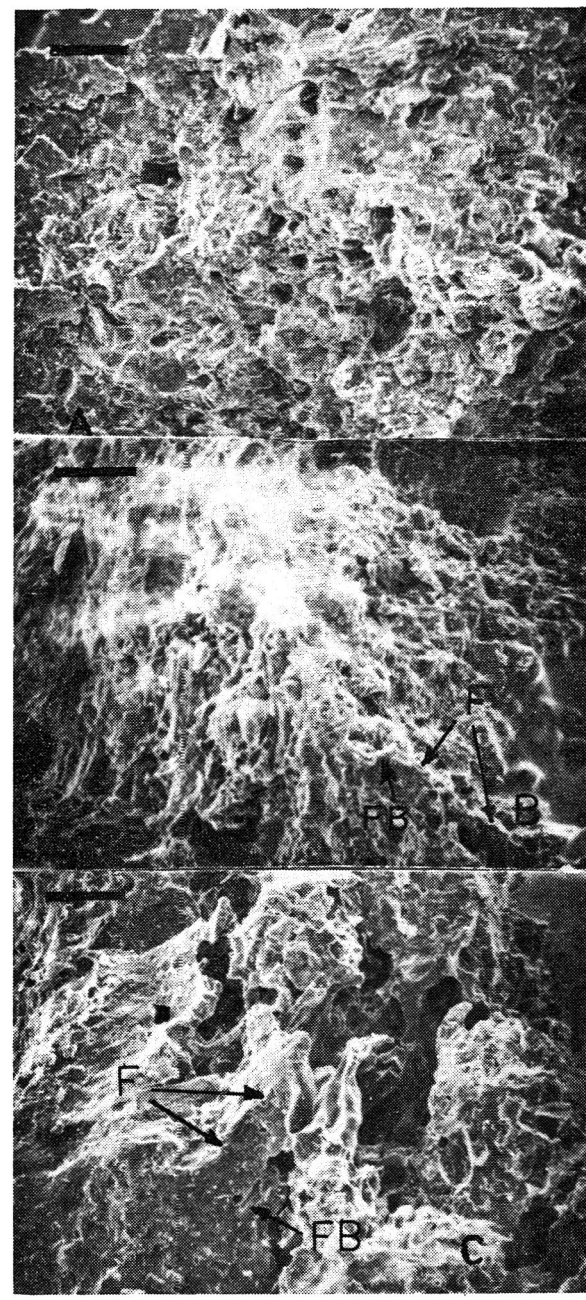

Fig. 6. Longitudinal low-magnification (50×) SEM views of extruded rod (extruded at 120°C, 60 rpm, 1:1) with the extruder having L/D ratio of A) 8 B) 12 and c) 16 showing fibre (F) and fibrils (FB).

Scale bar 100μ

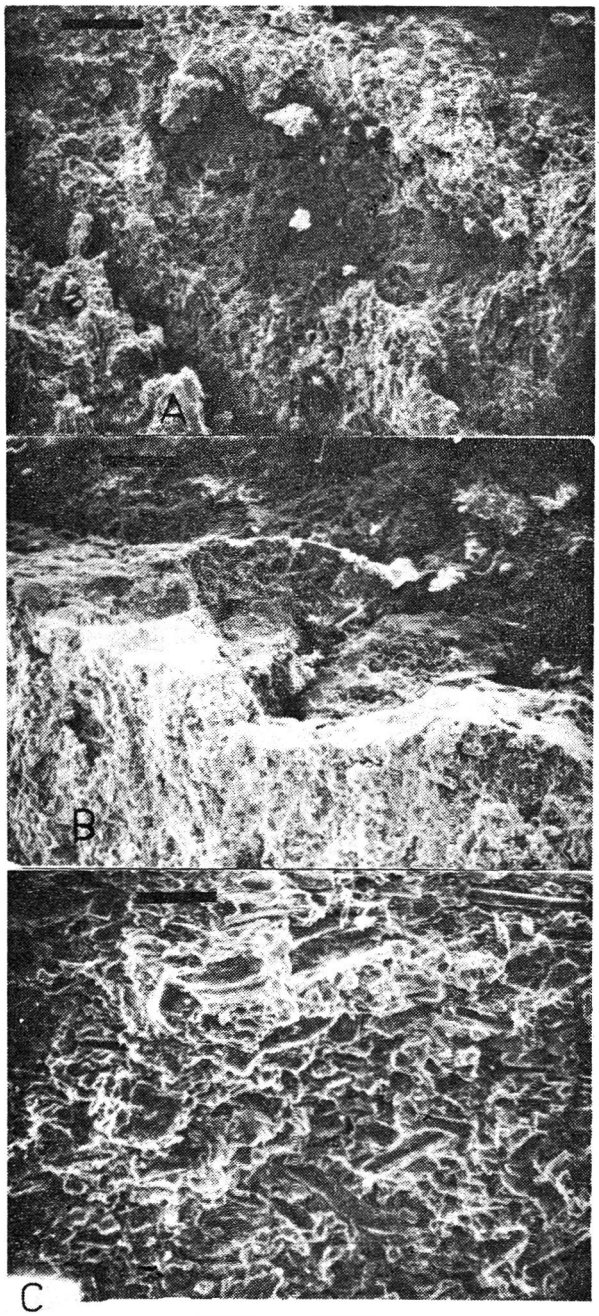

Fig. 7. Cross-sectional SEM views (50×) of extruded rods (extruded at 100°C, 60 rpm and L/D=12) with different feeds having fish and wheat flour solid ratios of A) 1:1 B) 2:1 and C) 3:1.

Scale bar 100μ

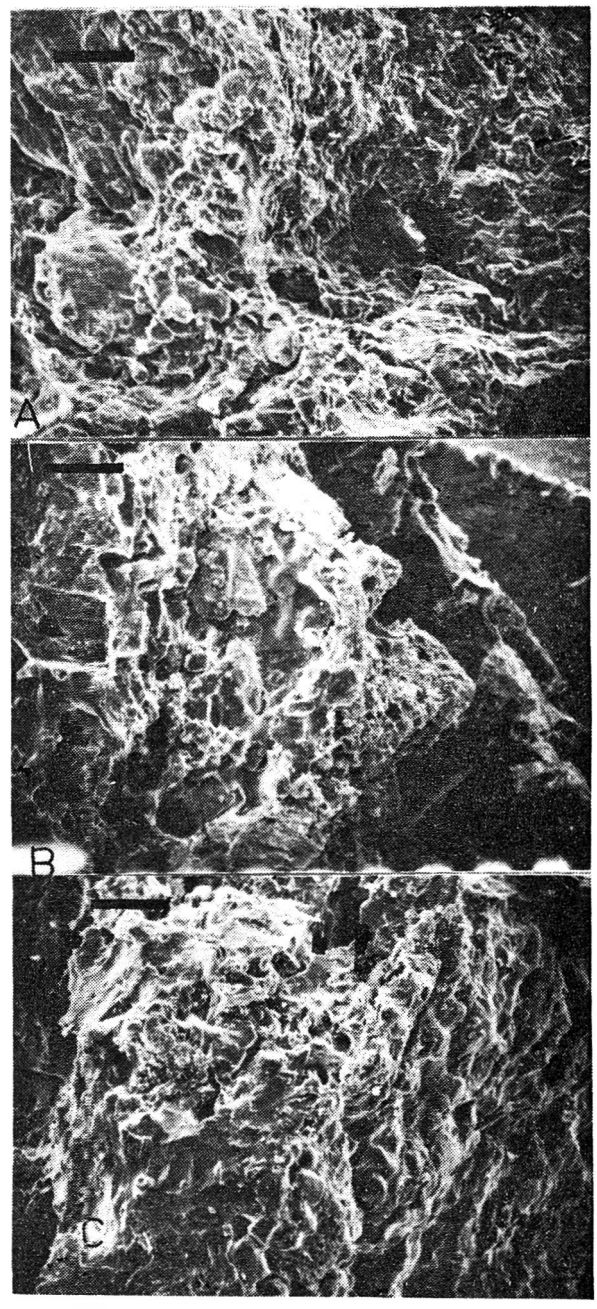

Fig. 8. Longitudinal SEM views (50×) of extruded rods (extruded at 100°C, 30 rpm and L/D=12) with different feeds having fish and wheat flour solid ratios of A) 1:1 B) 2:1 and C) 3:1

Scale bar 100μ

protein level and, consequently it favours the formation of big unbroken fibres compared to short brokens (as with feed ratio of 1:1) .

Effect of temperature of extrusion: Fig 9 A, 9 B and 9 C are the photomicrographs, extruded at temperatures of 100, 120 and 140°C, respectively. Extrusion temperature is found to affect the extrudate microstructure. Fibrillation is visible in Fig. 9 A and 9 B but typical oriented coarse fibres are only seen in Fig. 9 C. The rise in temperature during extrusion creates extensive protein denaturation and thus the fibre formation is affected producing a textured mass. These fibres are formed by the reaggregation and realignments of denatured protein subunits.

Fig. 10 is the magnified (800X) view of the rectangular area

in Fig. 9C. The proteinaceous fibre strands are apparently found to be 'cemented' with starchy materials. The tilted (20°) and still higher magnified (1600X) view of Fig. 10 i.e. Fig. 11 shows that two strands of fibre are found to be fused together to form the cross-link. These cross-links are stronger than starch cross-links. It may be possible that fused carbohydrate behaves as the cementing or cohesive of filling material for proteinaceous fibre strands.

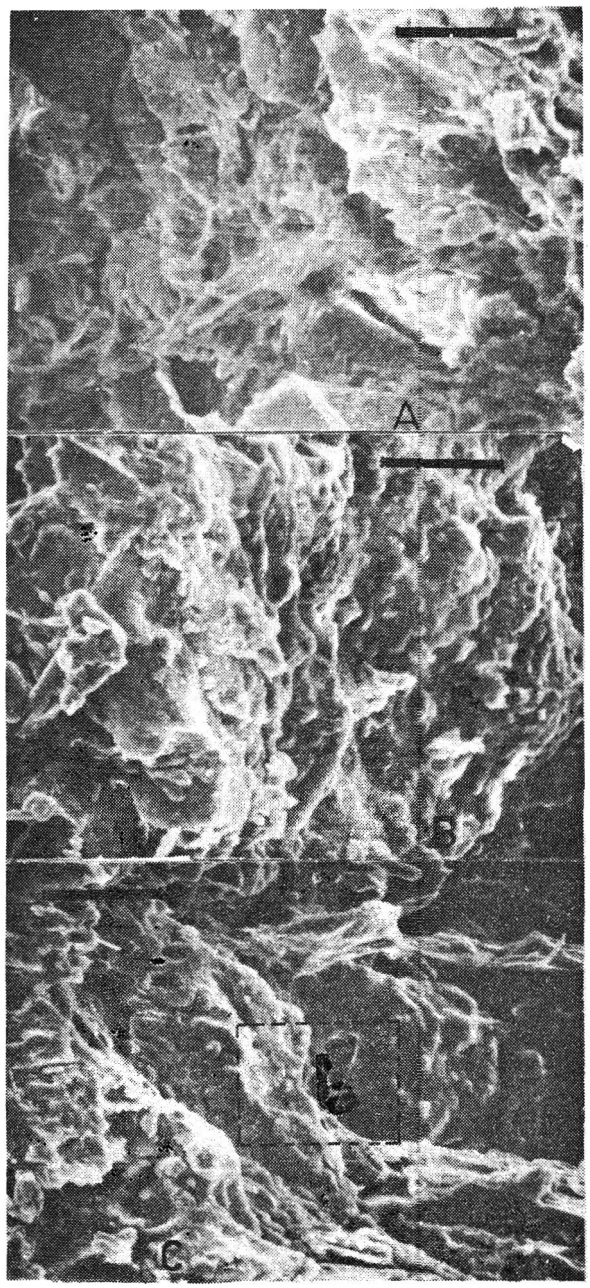

Fig. 9. Longitudinal SEM views (100×) of extruded rod, extruded at A) 100°C B) 120°C and C) 140°C with L/D=12, 60 rpm and feed ratio = 2:1.

Scale bar 40μ

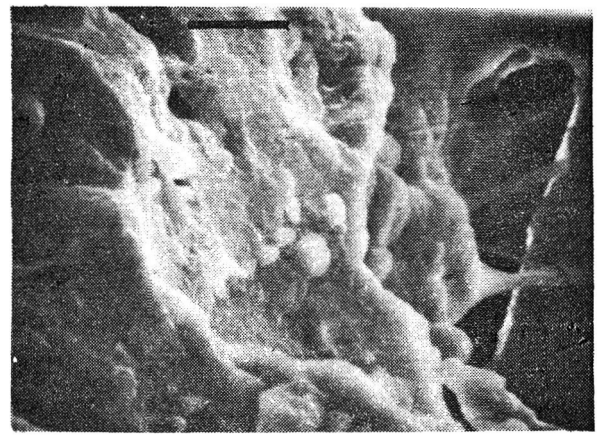

Fig. 10. Magnified (800×) SEM view of the rectangular area in Fig. 9.

Scale bar 10μ

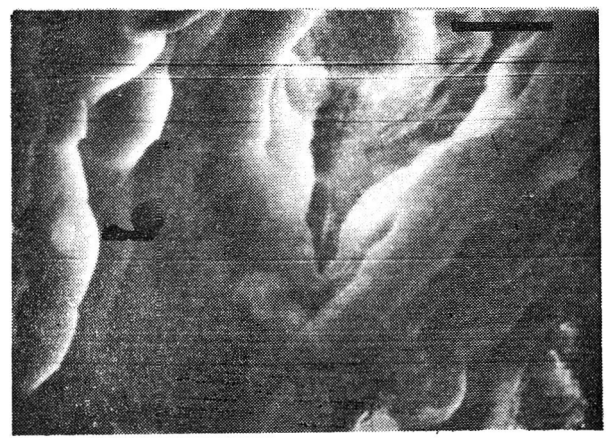

Fig. 11. Higher magnification (1600×) SEM titled (20°) view of the sample shown in Fig. 9.

Scale bar 5μ

Effect of screw speed: In the present investigation, it is difficult to draw a definite conclusion regarding the effect of screw speed on extrudate microstructure.

Acknowledgement

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References

1. Harper J M, *Extrusion Of Foods*, CRC Press, Inc, Florida, 1981, Vol.I, 2.
2. Kinesella J E, Texturized proteins: fabrication, flavouring and nutrition, *Crit Rev Fd Sci Nutr*, 1978, **10**, 147.
3. Lai C S Davis A B and Hoseney R C, The effect of a yeast protein concentrate and some of its components on starch extrusion, *Cereal Chem*, 1985, **62**, 293.
4. Lai C S Davis A B and Hoseney R C, Effect of whole yeast and various

fractions on some properties of extruded starch, *Cereal Chem*, 1985, **62**, 423.

5. Kristensen K H Gry P and Holm F, Extruded protein-rich animal by-products with improved texture, in *Thermal Processing And Quality Of Foods* by Zeuthen P *et al* (Eds), Elsevier Applied Science Publishers, London, 1984, 113.
6. Mandigo R W, Restructing of muscle foods, *Fd Technol*. 1986, **40** (3), 85.
7. Lee C M, Fabricated seafood products: Surimi manufacturing and fabrication of surimi-based products, *Fd Technol*, 1986, **40**(3), 115.
8. Industrial applications of extrusion cooking (present or potential), in *Thermal Processing And Quality of Foods* by Zeuthen P *et al* (Eds). Elsevier Applied Science Publishers, London, 1984, 282.
9. Smith O B and Ben-Gera I, The application of high temperature short time extrusion cooking in the food industry, in *Food Process Engineering* by Linko P *et al* (Eds), Applied Science Publishers Ltd., London, 1980, Vol.I, 726.
10. Suzuki T *Fish and Krill Protein: Processing Technology*, Applied Science Publishers Ltd, London, 1981, 120.
11. Maga J A and Reddy T, Coextrusion of carp (*Cyprinus carpio*) and rice flour, *J Fd Proc Pres*. 1985, **9**, 121.
12. Murray B P Stanley D W and Gill T A, Improved utilization of fish-protein: Co-extrusion of mechanically deboned salted minced fish, *Can Inst Fd Sci Technol J*, 1980 **13** 125.
13. Regenstein J M, The potential for minced fish. *Fd Technol*, 1986, **40**, 101.
14. Grantham G J, Minced fish technology: A review, *FAO Fish Tech*, 1981, Paper No. 216, 72.
15. Linko P Colonna P and Mercier C, High-temperature short-time extrusion cooking, in *Advances in Cereal Science and Technology*. American Association of Cereal Chemists, Inc, St Paul, Minnesota, Vol.IV, 145.
16. Kazemzadeh M Aguilera J M and Rhee K C, Use of microscopy in the study of vegetable protein texturization, *Fd Technol*, 1982, **36**, 111.
17. Stanley D W, Chemical and structural determinants of texture of fabricated foods, *Fd Technol*, 1986, **40**, 65.
18. Lawtor J W, Davis A B and Behnke K C, High-temperature short-time, extrusion of wheat gluten and a bran-like fraction, *Cereal Chem*, 1985, **62**, 267.
19. Aguilera J M, Rossi F Hiche E and Chichester C O, Development and evaluation of an extrusion texturized peanut protein, *J Fd Sci*, 1980, **45**, 245.
20. Owusu-Ansah J, Van de Voort F R and Stanley D W, Textural and microstructural changes in corn starch as a function of extrusion variables, *Can Inst Fd Sci Technol J*, 1984, **17**, 65.
21. Kitabatake N, Megard D and Cheftel J C, Continuous gel formation by ETST extrusion-cooking: Soy proteins. *J Fd Sci*, 1985, **50**, 1260.
22. Bhattacharya, S, Das H and Bose A N, Effect of extrusion process variables on in-vitro protein digestibility of fish-wheat flour blends, *Fd Chem*. 1988, **28**, 225.
23. *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, D C, 1980, 13th Edn.
24. Bhattacharya S, *Development of a variable length single screw extruder and study of the product and extrusion characteristics of fish-wheat flour blend*, 1987, Ph.D. Thesis, Indian Institute of Technology, Kharagpur.
25. Wolf W J and Baker F L, Scanning electron microscopy of soybeans and soybean protein products, *Scanning Electron Microscopy*, 1980, **3**, 621.
26. Neumann P E, Jasberg B K and Wall J S, Uniquely textured products obtained by coextrusion of corn gluten meal and soy flour, *Cereal Chem*, 1984, **61**, 439.

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Physico-Chemical Changes During Preparation of Plum Juice Concentrate

M.A. WANI*, S.P.S. SAINI AND G.S. BAINS**
Department of Food Science and Technology,
Punjab Agricultural University, Ludhiana — 141 004, India.

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Pulp and juice from three plum varieties were subjected to concentration in a glass evaporator at 50-60°C under vacuum of 27-30 inches of mercury. The plum pulp could not be concentrated beyond 26°Brix, while the juice was done to 73°Brix. An increase in total soluble solids, total solids, reducing sugars, browning and viscosity was observed with negligible decrease in acidity and pectin during concentration process.

Preparation of fruit juice concentrates is one of the best and latest methods of utilization of fruits during glut season. Fruit juice concentrations of orange, passion fruit, guava and banana have been studied by some workers[1-5]. But stone fruits like apricots, cherry and plum juice have not received similar attention. Only Aulich[6] has been able to effect the concentration of plum puree. Though fruit juice concentrate industry at present is in infancy in India, the need to develop it is obvious because of extensive cultivation of this crop in Jammu and Kashmir and Himachal Pradesh. Present studies were undertaken to study changes during manufacture of plum juice concentrate in glass vacuum evaporator.

Materials and Methods

Three plum varieties namely, 'Alubukhara', 'Santa-Rosa' and 'Satsuma' were selected for the study. Pulp was extracted by cold and hot break extraction processes.

Cold break process: After sorting, the fruits were washed in cold water and were as such passed through stainless steel pulper (Raylon India) fitted with 1/32" mesh. Extracted pulp was immediately heated in steam jacketted kettle held at 82°C for 2 min hot filled in 650 ml bottles, crown corked and pasteurized in boiling water for 40 min till further processing.

Hot break process: It involved mashing of plums with 4 per cent of added water to facilitate heating, crushing and extraction. Crushed plums were boiled in stainless steel steam-jacketted kettle for 3 min. The pulp was obtained in similar way as discussed above.

Concentration: Concentration of plum pulp/juice was carried out in a stationary type glass evaporator at 50-60°C having capacity of 1.5 under vacuum of 27-30 in. of mercury. The concentrator could evaporate 0.67 kg of water per hour and theoretical energy consumption was 0.7 kg steam per 0.67 kg of water evoporated. It was single stage, single effect batch type concentrator.

Physico-chemical analysis: Analysis of fresh juice, pulp and their concentrates were carried out. Total soluble solids, acidity, pH and sugars were determined by standard AOAC[7] methods. Total solids were determined gravimetrically. As described by Ranganna[8] pectin was determined as calcium pectate by modified Caree and Haynes method and Stormer viscosity was used to determine viscosity of samples taking distilled water as standard reference. Extent of browning was determined by taking known quantity of samples (2g) which was extracted with 20 ml of 60 per cent ethanol. Absorbance of browning was measured at 440 nm using Baush and Lomb Spectronic-20 Spectrophotometer.

Results and Discussion

Physico-chemical changes which occurred during concentration of pulp/juice of 'Alubukhara', 'Santa-rosa' and 'Satsuma' varieties of plum are shown in Tables 1-6 respectively. The results reveal that soluble solids and total solids increased with concentration of pulp as well as juice. A slight increase in pH in early stage of concentration was observed followed by constant pH at elevated level of evaporation, showing remarkable buffering capacity of plum pulp/juice. The pH of pulp/juice concentrates ranged between 3.04 and 3.12.

A slight decrease in acidity was observed during later stages of concentration. The average decrease in acidity was 0.22 and 0.13 per cent in cold extracted pulp/juice and in hot extracted plum pulp/juice concentrate respectively on dry matter basis. According to Bauman[9] the slight decrease in acidity may be due to loss of volatile acids. There was an increase in reducing sugars during concentration and per cent

Present address: *PHT Div. SKUAST Shalimar Kashmir
**Food Science and Technology Dept. PAU, Ludhiana, Punjab, India.

TABLE 1. PHYSICO-CHEMICAL CHANGES DURING CONCENTRATION OF ALBUKHARA PLUM PULP

Parameter	Cold Pressed			Hot Pressed			
Total soluble solids (°Brix)	14	17	20	11	14	17	20
Total solids (%)	14.7	17.9	21.2	11.8	15.0	18.2	21.4
Total sugars (as dextrose %)	5.6 (38.1)	6.82 (38.1)	8.04 (38.1)	5.0 (42.4)	6.36 (42.4)	7.7 (42.3)	9.07 (42.4)
Reducing sugars (as dextrose %)	4.8 (32.7)	6.0 (33.5)	7.7 (36.3)	4.9 (41.5)	6.3 (42.0)	7.14 (42.1)	9.0 (42.2)
Titrable acidity (as malic acid %)	1.67 (11.4)	2.01 (11.2)	2.34 (11.0)	1.54 (13.1)	1.95 (13.0)	2.37 (13.0)	2.77 (12.9)
Pectin (as calcium pectate %)	1.37 (9.3)	1.65 (9.2)	1.96 (9.2)	1.37 (11.6)	1.74 (11.6)	2.09 (11.5)	2.44 (11.4)
Colour (OD at 440 nm)	0.18	0.20	0.20	0.17	0.21	0.26	0.28
Stormer viscosity (sec)	13	18	23	10	17	19	22

Figures in parenthesis indicate values on dry matter basis.

TABLE 2. PHYSICO-CHEMICAL CHANGES DURING CONCENTRATION OF SANTA ROSA PLUM PULP

Parameter	Cold Pressed				Hot Pressed			
Total soluble solids (°Brix)	13	18	22	26	11	15	18	22
Total solids (%)	13.8	19.2	23.4	27.7	11.6	15.8	18.9	23.2
Total sugars (as dextrose %)	7.0 (50.7)	9.74 (50.7)	11.9 (50.8)	14.05 (50.7)	5.9 (50.9)	8.03 (50.8)	9.61 (50.8)	11.80 (50.9)
Reducing sugars (as dextrose %)	6.0 (43.5)	8.36 (43.5)	10.2 (43.5)	12.2 (44.0)	4.3 (37.1)	5.9 (37.3)	7.2 (38.1)	8.95 (38.6)
Titrable acidity (as malic acid %)	1.67 (12.1)	2.27 (11.8)	2.8 (12.0)	3.30 (11.9)	1.66 (14.3)	2.25 (14.2)	2.69 (14.2)	3.28 (14.1)
Pectin (as calcium pectate %)	0.88 (6.37)	1.21 (6.3)	1.46 (6.2)	1.73 (6.2)	0.88 (7.6)	1.20 (7.6)	1.40 (7.4)	1.75 (7.5)
Colour (OD at 440 nm)	0.20	0.26	0.30	0.32	0.18	0.19	0.19	0.22
Stormer viscosity (sec)	0.20	24	26	28	19	23	25	28

Figures in parenthesis indicate values on dry matter basis.

increase depended upon variety and processing treatment (Tables 1-6). These changes in reducing sugars were attributed to the inversion of non-reducing sugars. Pectin content of pulp/juice decreases slightly during concentration process, being more at intial stage of concentration. Average decrease in pectin content was 0.10 and 0.14 per cent in hot and cold break extracted pulp/juice concentrates respectively on moisture free basis (Tables 1-3). Negligible losses in pectin contents during concentration seem to be caused by break down of pectic substances.

After reconstituting the concentrates to the original strength it was found that absorbance of brown colour extract increased with increased concentration. But rate of browning in case of hot break extracted pulp/juice concentrate was higher, showing the predominance of Maillard's reaction.

A highly significant increase in viscosity during final stage of concentration particularly of juice concentrates was observed (Tables 4-6). Increase in viscosity was negligible during initial stage of concentration but drastic increase was observed, when soluble solids level reached beyond 50 per cent. No major difference in viscosity could be observed in pulp concentrate because plum pulp could not be concentrated beyond 26°Brix so as to project changes in the viscosity.

It was concluded from the present investigation that plum pulp could be concentrated upto 26°Brix, while as juice could be concentrated upto 72°Brix successfully in laboratory model glass vacuum evaporator. Thus, the fruit can successfully be

TABLE 3. PHYSICO-CHEMICAL CHANGES DURING CONCENTRATION OF SATSUMA PLUM PULP

Parameter	Cold Pressed			Hot Pressed		
Total soluble solids (°Brix)	10	15	17	10	13	16
Total solids (%)	10.8	16.2	18.4	10.6	13.9	17.1
Total sugars (as dextrose %)	5.8 (53.7)	8.7 (53.7)	9.88 (53.7)	5.7 (53.8)	7.48 (53.8)	9.20 (53.8)
Reducing sugars (as dextrose %)	4.9 (45.4)	7.4 (45.7)	8.8 (47.8)	4.69 (44.2)	6.1 (44.9)	7.9 (46.2)
Titrable acidity (as malic acid %)	1.25 (11.6)	1.86 (11.5)	2.10 (11.4)	1.30 (12.3)	1.69 (12.2)	2.06 (12.0
Pectin (as calcium pectate %)	0.95 (8.8)	1.4 (8.6)	1.60 (8.7)	1.0 (9.4)	1.29 (9.3)	1.59 (9.3)
Colour (OD at 440 nm)	0.20	0.26	0.31	0.20	0.22	0.27
Stormer viscosity (sec)	15	18	19	14	17	18

Figures in parenthesis indicate values on dry matter basis.

TABLE 4. PHYSICO-CHEMICAL CHANGES DURING CONCENTRATION OF ALUBUKHRA PLUM JUICE

Parameter	Cold Pressed					Hot Pressed				
Total soluble solids (°Brix)	12	17	36	51	73	10	20	40	60	70
Total solids (%)	10.2	17.2	36.5	51.9	73.3	10.6	21.3	42.5	63.8	76.4
Total sugars (as dextrose %)	5.0 (41.0)	7.0 (41.0)	14.9 (41.0)	21.19 (41.0)	30.3 (41.0)	4.6 (43.4)	9.23 (43.3)	18.4 (43.3)	27.68 (43.4)	33.15 (43.4)
Reducing sugars (as dextrose %)	4.6 (37.7)	7.0 (38.4)	14.0 (38.4)	20.0 (38.5)	30.0 (38.6)	4.2 (39.6)	8.49 (39.9)	16.89 (39.7)	25.8 (40.4)	31.3 (41.0)
Titrable acidity (as malic acid %)	1.67 (13.7)	2.37 (13.7)	5.0 (13.7)	7.09 (13.7)	10.16 (13.6)	1.6 (15.1)	3.22 (15.1)	6.38 (15.0)	9.6 (15.0)	11.48 (15.0)
Colour (OD at 440 nm)	0.21	0.21	0.24	0.26	0.29	0 20	0.23	0.24	0.29	0.31
Stormer viscosity (sec)	5	6	6	37	137	5	5	11	37	165

Figures in parenthesis indicate values on dry matter basis.

TABLE 5. PHYSICO-CHEMICAL CHANGES DURING CONCENTRATION OF SANTA-ROSA PLUM JUICE

Parameter	Cold Pressed					Hot Pressed				
Total soluble solids (°Brix)	11	22	38	60	72	10	20	35	60	70
Total solids (%)	11.3	22.6	39.1	61.6	74.3	10.3	20.5	35.7	61.0	73.4
Total sugars (as dextrose %)	6.0 (53.1)	12.0 (53.1)	20.8 (53.1)	32.68 (53.1)	39.45 (53.1)	6.0 (53.8)	12.0 (58.8)	21.0 (58.8)	36.0 (58.9)	43.16 (58.8)
Reducing sugars (as dextrose %)	4.7 (41.6)	9.44 (41.8)	16.35 (41.8)	25.9 (42.0)	31.29 (42.1)	4.6 (45.1)	9.3 (45.4)	16.3 (45.7)	28.1 (46.0)	33.96 (46.5)
Titrable acidity (as malic acid %)	1.54 (13.6)	3.08 (13.6)	5.30 (13.6)	8.34 (13.5)	10.10 (13.6)	1.41 (13.8)	2.82 (13.8)	4.93 (13.8)	8.4 (13.7)	10.01 (13.6)
Colour (OD at 440 nm)	0.15	0.15	0.17	0.18	0.20	0.15	0.15	0.19	0.21	0.27
Stormer viscosity (sec)	5	6	6	38	168	5	5	7	40	161

Figures in parenthesis indicate values on dry matter basis.

TABLE 6. PHYSICO-CHEMICAL CHANGES DURING CONCENTRATION OF SATSUMA PLUM JUICE

Parameter	Cold Pressed					Hot Pressed				
Total soluble solids (°Brix)	9	18	37	60	72	9	18	37	51	68
Total solids (%)	9.2	18.5	37.9	61.4	73.8	9.2	18.4	38.0	52.5	70.3
Total sugars (as dextrose %)	5.0 (53.3)	10.05 (54.3)	20.57 (54.3)	33.38 (54.4)	40.1 (54.3)	5.7 (62.0)	11.4 (62.0)	23.55 (62.0)	32.5 (61.6)	43.56 (62.0)
Reducing sugars (as dextrose %)	4.4 (47.8)	8.99 (48.6)	18.6 (49.0)	30.19 (49.2)	36.9 (50.0)	4.9 (53.3)	9.9 (53.8)	20.5 (54.0)	28.39 (54.0)	38.6 (54.9)
Titrable acidity (as malic acid %)	1.19 (12.9)	2.37 (12.8)	4.87 (12.8)	7.78 (12.7)	9.47 (12.8)	1.17 (12.7)	2.35 (12.8)	4.80 (12.6)	6.66 (12.7)	8.90 (12.7)
Colour (OD at 440 nm)	0.16	0.16	0.18	0.26	0.30	0.16	0.16	0.21	0.28	0.34
Stormer viscosity (sec)	4	4	7	51	165	5	5	6	37	147

Figures in parenthesis indicate values on dry matter basis.

concentrated on large scale in commercial apple concentrate plant. Thereby, the plant efficiency can be increased because the plum is available at that time when other temperate fruits are not available.

References

1. Siddappa G S and Girdhari Lal, Changes in ascorbic acid content of orange juice concentration and conversion into orange juice powder, *J Scient Ind Res* 1956, **15C**, 28.
2. Pruthi J S and Girdhari Lal, Studies on passion fruit juice concentrate and powder, *Fd Sci*, 1959, **8** 1.
3. Muralikrishna M, Nanjundaswamy A M and Siddappa G S, Physico-chemical changes during concentration of guava juice. *Indian Fd Pckr*, 1973, **27**, 10.
4. Setty G R, Nanjundaswamy A M, Moorthy N V N and Ramana K V R, Physico-chemical changes during concentration of orange juice using industrial plate evaporator, *Indian Fd Pckr*, 1973, **27**, 10.
5. Sandhu K S and Bhatia B S, Physico-chemical changes during preparation of fruit juice concentrates, *J Fd Sci Technol* 1985, 22 201.
6. Aulch W, Manufacture of concentrates of plum puree, *German Democratic Republic Patent* 138597, 1979.
7. *Official Methods of Analysis*, Association of Official Analytical Chem sts, Washington, 1975, 12th Edn.
8. Ranganna S, *Manual of Analysis of Fruits and Vegetable Products*, Tata McGraw Hill Publishing Company Ltd. New Delhi 1977.
9. Bauman I G, Changes in acid concentration in fruit juice concentrates, *Industrielle Obst.* Und Gemiisever-wentung, 1979 **68** 205.

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Spectrophotometric Method for Determination of Phosphine Residues in Coffee Seeds

J.R. RANGASWAMY
Infestation Control and Protectants Discipline,
Central Food Technological Research Institute, Mysore — 570 013, India.

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A method for determination of phosphine (PH_3) residues on/in coffee seeds has been developed based on the reaction of phosphine with silver nitrate in 2% aqueous methanol. The linear relationship between absorbance of egg yellow chromophore and concentration of PH_3 is obeyed between 0.025 and 0.2 μg PH_3 in 3 ml solvent. PH_3 residue in fumigated coffee seeds is determined by soaking 10g sample in 15 to 30 ml solvent for one hr and the absorbance at 410 nm of the filtrate is read against the crop control blank of the same sample size. The method is sensitive with lower detectable and estimatable limits of 0.01 and 0.025 μg PH_3 respectively. Recovery of added PH_3 from a closed system is 95%.

Phosphine (hydrogen phosphide) liberated from aluminium phosphide preparations is a common fumigant used all over the world for fumigation of almost all food commodities. The popularity of this fumigant is due to ease of fumigation, simple to handle and its high toxicity to insects. In most coffee growing areas[1-3], PH_3 fumigation has been in practice to prevent infestation of coffee beetle (*Araecerus fasciculatus*) in coffee seeds in storage. In India, pyrethreum was recommended for treatment of monsooned coffee[4]. The most common fumigant now employed in India for fumigation of coffee in storage is a mixture of ethylene-di-bromide and methyl bromide (EDB + MB), though not much is known about the free and bound residues of these two fumigants in coffee seeds.

For determination of PH_3 in air many gas chromatographic (GC) methods have been reported[5-7]. PH_3 residues in wheat[8,9] are determined by passing nitrogen through the commodity and collecting the desorbed PH_3 in a trap cooled with dry ice; residues are quantitated by GC. Kashi and Muthu[10] used a mixed indicator strip for PH_3 detection. But all these methods determine only the desorbed PH_3, but not total PH_3 residue found in commodity.

In an earlier communication, a spectrophotometric method based on the extraction of total free PH_3 residues with aqueous silver nitrate solution for determination of PH_3 residues in wheat was reported[11]. The method was later extended to determine PH_3 residues in rice types[12]; which was again modified for determination of PH_3 residues in cashew kernels.[13]

The present paper describes a simple spectrophotometric method for determination of PH_3 residues in coffee seeds. The method consists of extracting PH_3 residues by soaking 10g coffee seeds in a known volume of 2 per cent aqueous methanolic $AgNO_3$ solution for one hour. The absorbance of the extract at 410 nm is read after filtration against solvent blank. The absorbance of the crop control extract is similarly determined and subtracted from absorbance of the extract of the PH_3 fumigated coffee seeds. The calibration line between concentration of PH_3 and absorbance of the chromophore is obtained by passing a known volume of air-borne PH_3 through an aqueous methanolic solution containing 30 μg $AgNO_3$/ml. Reaction between PH_3 and $AgNO_3$ is allowed to proceed for 1 hr when an egg yellow chromophore is formed. The intensity of the chromophore bears a direct relationship to the concentration of PH_3.

Materials and Methods

(a) Spectrophotometer. Bausch and Lomb spectronic 21,

(b) *Tubes:* 5.5 cm × 11 mm id with gas tight rubber septum

(c) *Conical flasks:* 50, 100 and 500 ml flasks with side spout fitted with rubber septum to permit injection of PH_3 and gas sampling.

(d) *Methanol (Analytical grade)*: Make it absolute by distilling over a few pellets of KOH.

(e) *Silver nitrate solution:* Dissolve 150 mg $AgNO_3$ crystals (Analytical grade, British Drug House) in 50 ml glass-distilled water to prepare stock solution. Prepare working standards by diluting 1 ml stock solution and 1 ml distilled water to 100 ml with absolute methanol. Use 3 ml solution for each experiment.

(f) *Standard PH_3 :* Liberate PH_3 from Celphos or Phosfume tablets and store over water in gas burette. If phostoxin tablet is used, store PH_3 over 5% H_2SO_4 in gas burette.

(g) *Working standard of PH_3 :* Inject a known volume of PH_3 with a gas-tight microsyringe of 50 μ l capacity into a gas-tight syringe of 50 ml capacity whose nozzle is fixed with a gas-tight rubber septum. Dilute PH_3 with air to 50 ml by gently pulling the plunger after removing the septum. Again replace the septum. PH_3-borne air in the syringe must contain PH_3 in the range of 0.01 to 0.25 μg per ml aliquot.

Preparation of standard curve: Pipette 3 ml aliquots of working standard 2 per cent aqueous methanolic $AgNO_3$ solution into 6 ml tubes, and close with gas-tight rubber septum. Inject 0.05 - 0.20 ml working standard PH_3 borne air so as to contain PH_3 in the range of 0.01-0.5 μg with a 1 ml gas-tight microsyringe. Inject equal volume of air into 3 ml 2 per cent aqueous methanolic solution to serve as blank. Let the tubes stand for 1 hr for formation of chromophore. Measure the absorbance at 410 nm against blank (Fig. 1). Plot of absorbance *vs* concentration yields straight line over a range of 0.01-0.2 μ g PH_3 per 3 ml (Fig. 2).

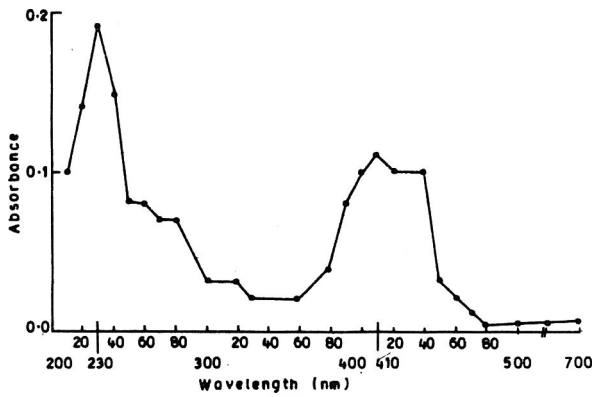

Fig.1. Absorption spectra of $AgNO_3$ - PH_3 chromophore in 2% aqueous methanol.

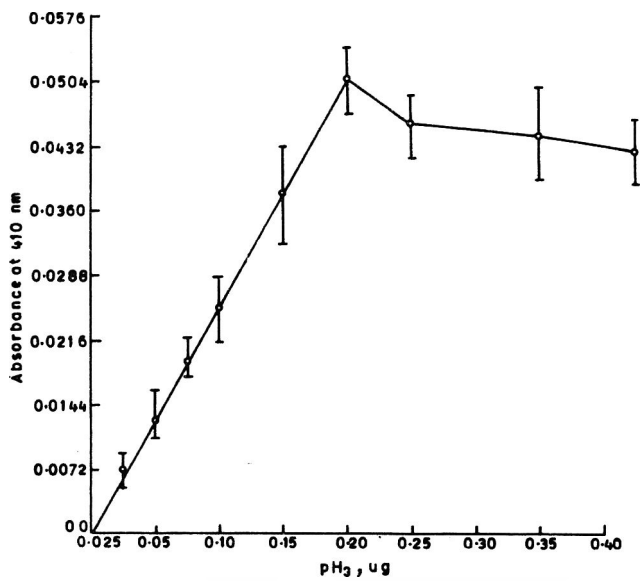

Fig.2. Linear relationship between absorbance at 410 nm and phosphine in μg.

Recovery study: Recovery of PH_3 from coffee seeds is worked out by second, third and fourth procedures described earlier.[11]

Second procedure: Fumigate 10 g coffee beans and peaberry seeds separately with different doses of PH_3 in 100 ml conical flask with side spout fitted with rubber septum to permit injection of PH_3. After 48 hrs fumigation, without disturbing either flask or its contents, remove glass stopper of the flask for 15 sec to drive out free PH_3 in head space. Quickly add 30 ml working standard aqueous methanolic $AgNO_3$ solution to the flask and replace the stopper tightly. Let the extraction of PH_3 and development of chromophore proceed for 1 hr with shaking at intervals. Filter the extract with Whatman filter circle of 9 cm dia. Read absorbance against solvent as blank. Similarly run crop control. Also run blank experiments without coffee seeds in pre-calibrated 100 ml conical flasks at every level of PH_3 used, to assess the extent of absorption of added PH_3 by glass, grease and rubber septum. After 48 hr, withdraw known aliquots of PH_3-borne air with micro-gas-tight syringe and analyse (Table 1).

Thira procedure: Repeat fumigation of 10 g coffee beans and peaberry with different doses of PH_3 as under second procedure. Also carry out crop control and blank experiments. After 48 hr fumigation, remove stopper of the flask, and quickly transfer the contents to a 50 ml tube containing 30 ml aqueous methanolic $AgNO_3$ solution. Tightly stopper tubes and let stand for 1 hr with shaking at intervals. Read absorbance of extracts (Table 2), as under second procedure.

The filtrate obtained in experiments dosed with 30 μg

TABLE 1. RECOVERY OF PHOSPHINE BY SECOND PROCEDURE

Dosed PH_3 (μg)	Coffee beans (μg)	Peaberry (μg)
30	1.64 ± 0.47	1.62 ± 0.19
60	4.11 ± 1.06	3.72 ± 0.74
120	7.35 ± 0.68	8.03 ± 0.94
180	8.58 ± 0.54	8.42 ± 0.48
240	18.13 ± 0.45	18.45 ± 2.19

Mean ± SD of 10 replicates

TABLE 2. RECOVERY OF PHOSPHINE BY THIRD PROCEDURE

Dosed PH_3 (μg)	Coffee beans (μg)	Peaberry (μg)
30	0.63 ± 0.09	0.78 ± 0.23
60	1.56 ± 0.43	1.18 ± 0.31
120	2.08 ± 0.66	3.71 ± 0.53
180	2.25 ± 0.58	5.38 ± 0.12
240	5.80 ± 0.88	5.83 ± 0.09

Mean ± SD of 10 replicates

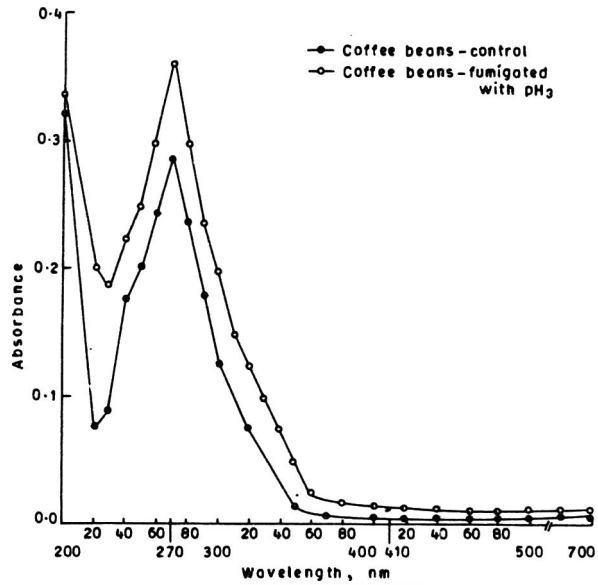

Fig.3. Absorption spectra of PH_3 residue - $AgNO_3$ chromophore from coffee beans (Robusta) in 2% aqueous methanol.

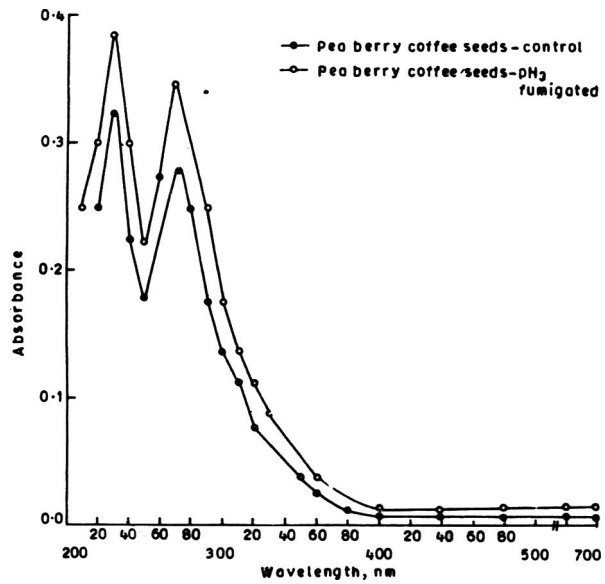

Fig.4. Absorption spectra of PH_3 residue - $AgNO_3$ chromophore from peaberry coffee seeds (Robusta) in 2% aqueous methanol.

PH_3 were employed to scan the absorption spectra to know the nature and number of bands formed due to interaction of PH_3 with constituents of coffee beans (Fig 3) and peaberry (Fig 4). For comparison, the spectra of the crop control coffee samples are also shown in these Figures.

Fourth procedure: Expose 500 g coffee beans and peaberry to 1.05 to 1.60 ml PH_3 for 2 weeks in 1L flask fitted with rubber septum that permits injection and gas sampling. Inject equal amount of PH_3 into another similar pre-calibrated empty flask to serve as control. At the end of 2 weeks, determine concentration of PH_3 in head space over coffee samples and in control flask by withdrawing known volumes of PH_3 — borne air. Compute amount of PH_3 taken up by 500 g coffee samples by subtracting head space concentration from concentration in control flask (Table 3).

Residue analysis: Results of PH_3 residue analyses from coffee beans and peaberry samples from model fumigation at two commercial doses of 3 and 6 tablets/ton are shown in Table 4. PH_3 residue employing this proposed method is determined in pre-aired and one day aired samples of these types of coffee seeds.

Results and Discussion

When aqueous $AgNO_3$ solution[11-13] is employed for extraction, the crop control coffee sample extracts (10 g in 30 ml), not only show high absorbance (0.70 to 0.77), but also the absorbance is increasing regularly on storing over 3 hr. When absolute methanolic $AgNO_3$ solution is employed, PH_3 is not found to react with $AgNO_3$, as the development of chromophore seems to depend on the presence of water, although crop control extracts show low absorbance of 0.029 to 0.033. Of various percentages of water in methanol tried, 2 per cent water in methanol when employed for extraction of crop control, the extract not only shows low absorbances (0.042 to 0.126) but also, allows full

TABLE 3. COMPUTED RECOVERY OF PH_3 BY FOURTH PROCEDURE

Dosed PH_3 (ml)	Found in control flask (μg)	Found in head space (μg)	Absorbed by commodity (μg)
		Coffee beans	
1.60	1824 (95%)	268.60 ± 5.13	1555.40 (85.3%)
1.25	1425 (95%)	231.64 ± 4.65	1211.35 (85.0%)
1.05	1197 (95%)	206.37 ± 4.78	990.63 (82.8%)
		Peaberry	
1.60	1824	390.59 ± 4.78	1433.41 (78.6%)
1.25	1425	276.17 ± 9.31	1148.83 (80.6%)
1.05	1197	278.80 ± 6.98	918.20 (76.7%)

Mean ± SD of 10 replicates. Values in parentheses in last column indicate % uptake of available phosphine by commodity.

TABLE 4. PHOSPHINE RESIDUE (PPM) IN FUMIGATED AND AIRED COFFEE SEEDS AT TWO COMMERCIAL DOSES

	3 tablets AIP/ton	6 tablets AIP/ton
	Coffee beans	
Pre-aired	0.106 ± 0.069	0.189 ± 0.033
One-day aired	0.052 ± 0.012	0.091 ± 0.004
	Peaberry	
Pre-aired	0.157 ± 0.016	0.204 ± 0.049
One-day aired	0.075 ± 0.038	0.097 ± 0.024

Mean ± SD of 6 replicates.

development of chromophore as a result of PH_3-$AgNO_3$ interaction. The changes in absorbances are marginal on standing the extracts over 3 hr. So, in the work with all types of coffee samples, 2 per cent aqueous methanol containing 30 μg $AgNO_3$ per ml solution is employed.

The minimum weight of PH_3 needed to produce a measurable chromophore is 0.025 μg, although detectable colour formation takes place at 0.01 μg PH_3. As seen in Fig 2, the linear relationship between the absorbance at 410 nm and the concentration of PH_3 is obeyed between 0.025 and 0.20 μg PH_3, thereafter the relationship ceases. The values plotted in Fig 2 are the mean of 10 replicate determinations and the limits of variance at each point are shown as vertical lines. As seen in Fig 1, the absorption spectra of $AgNO_3$—PH_3 chromophore in 2 per cent aqueous methanol has a well defined intense band at 230 nm and a hump between 360 and 480 nm. Absorbances from recovery experiments when read at 230 nm, the PH_3 recoveries are disproportionate to the dosed amounts, suggesting the band does not correspond to the absorption maximum of the chromophore. Although the hump between 360 and 480 nm is absent (Fig 3 and 4, later discussion) from the spectra of extracts of the fumigated coffee seeds, the absorbances when read at 410 nm afforded good recoveries within the dosed amounts at all doses (Tables 1, 2 and 3). Due to overlapping of chromophores, the band corresponding to absorption maximum at 410 nm might have spread between 300 and 480 nm (Fig.1), but its absence in the spectra of extracts with PH_3 fumigated coffee seed types is very difficult to reason out. In the work with all types of coffee seeds, the absorbance of the extract is read at 410 nm against the solvent as blank, and the absorbance of crop control is subtracted from those of fumigated samples.

Full development of chromophore takes over an hour. The chromophore formed is stable only for 2 hr, thereafter it starts decomposing as indicated by fall in absorbances. So, the absorbance of the extracts need be read within 2 hr.

As shown in the absorption spectra of coffee beans fumigated with PH_3 and that of crop control (Fig.3), the latter is having a well defined band at 270 nm, which increases in intensity on reaction with PH_3 during fumigation. On airing the fumigated coffee beans for a day, the increment shown in the intensity of the band vanishes due to decomposition of the reaction product. No other band is observed in the spectra. Absorption spectra of PH_3 fumigated peaberry and its crop control present a slightly different picture (Fig.4). The spectrum of crop control, in addition to a band at 270 nm, shows another more intense band at 230 nm. As observed in the spectrum of fumigated coffee beans, these bands also show an increase in intensity due to interaction with PH_3. Here also, on airing for a day, the interaction product decomposes as indicated by the loss of increment in intensity of bands. These observations suggest that at lower dosages of PH_3, bound residues of PH_3 are not encountered in coffee seeds indicating the suitability of PH_3 as fumigant for coffee seeds.

Recoveries by second procedure (Table 1) are the amounts of PH_3 determined in/on coffee seed types. Unlike with solid or liquid pesticides, complete recovery of fortified fumigant is very difficult to establish due to fugacity of gas. Some amount of fumigant escapes at every stage of sampling. It is likely that a small portion of PH_3 that had settled on coffee seeds might have also escaped when the stopper is removed for 15 sec to expel PH_3 in the head space. A different yardstick is employed to assess the recovery and hence the soundness of the method. As seen with two types of coffee seeds, the amount of PH_3 recovered increases as the dosed PH_3 increases from 30 to 240 μg. If this is taken as an index of the amount of PH_3 mixed indirectly with the types of coffee seeds, then these values indicate almost 100 per cent recovery of PH_3 fortified with these coffee seeds, indicating the soundness of the method. As for example at a dose of 240 μg, 18.13 μg PH_3 is held back by 10 g coffee beans, while 18.45 μg is held back by 10 g peaberry. At all these doses there is a 95-99 per cent recovery of added PH_3 from empty flasks.

The values in Table 2 also show the same trend of increase in recovered amount as the dosed PH_3 is increased from 30 to 240 μg. As for example at a dose of 240 μg both coffee beans and peaberry have retained about 5.8 μg. But at a dose of 180 μg due to excess loss of loosely held PH_3 from coffee beans during transfer, coffee bean has held back (2.25 μg) only half the amount of PH_3 that has been retained (5.38 μg) by peaberry. However, the trend of increased recoveries at higher doses is maintained. Comparison of recoveries in Tables 1 and 2 shows that values in Table 2 are on lower side at any given dose. This is due to loss of PH_3 loosely held by coffee seeds, as they are disturbed during transfer. Within the limits of PH_3 holding capacity of these two types of coffee seeds, 10 g sample of these types would hold only a definite amount of PH_3 under a given set of conditions, including the dosage as shown by second and third procedures, and this amount of PH_3 has been completely recovered by the new method.

The purpose of the fourth procedure (Table 3) is to completely determine PH_3 residue in/on coffee seeds by computing the headspace concentration and the amount of added PH_3 remaining after adsorption by glass, grease etc. Values in Table 3 show almost 100 per cent recovery of added PH_3. By this method also, the residue on coffee seeds decreases as the added PH_3 decreases. At a dose of 1.60 ml (1.92 mg) the residue on 500 g coffee beans is 1555 μg, while that on peaberry is 1433 μg. The residue levels on these two types of coffee seeds at a dose of 1.05 ml (1.26 mg) are 990.63 and 918 μg respectively. As seen by the values in parentheses in the last column, about 84 per cent of the available PH_3 is taken up by coffee beans, while the same by peaberry is about 79 per cent, indicating a slightly higher PH_3 holding

capacity of coffee beans.

The residue values shown in Table 4 indicate that the method can be applied for determination of PH_3 residues, as the crop material from coffee seeds has not interfered with the development of chromophore. Inspite of slightly higher capacity of coffee beans to hold PH_3, peaberry shows higher pre-aired residue of 0.157 and 0.204 p.p.m. at 3 and 6 tablets/ton than the corresponding levels of 0.106 and 0.187 p.p.m. in coffee beans. This is due to higher rate of desorption of PH_3 from coffee beans than that from peaberry. As for example, from the pre-aired samples dosed at 6 tablets/ton, 0.046 p.p.m. PH_3 is lost in the first hour of desorption from beans, while the corresponding loss from peaberry is 0.036 p.p.m. So larger amounts of PH_3 is lost from coffee beans on opening the fumigation cover. On airing for one day by spreading the samples as thin layer, about 52 per cent PH_3 residue is lost from all these samples.

Acknowledgement

The author thanks Mr. S.K. Majumder for his interest and Dr. B.L. Amla, Director for his encouragement.

References

1. Puzzi D and Orlando A, Studies on the dosage of fumigants for the control of *Araecerus fasciculatus* (DeGeer) under plastic covers, *Biologico Brazil*, 1963, **29**, 127.
2. Puzzi D and Orlando A, Preliminary studies on the dosage and time of exposure of phosphine in the control of pests of stored grains, *Biologico Brazil*, 1964, **30**, 5.
3. Countinho J M Puzzi D. and Orlando A The use of fumigant phosphine in the control of insects of stored seeds, *Biologico Brazil*, 1961, **27**, 271.
4. Subrahmanyan V Bhatia D S Natarajan C P and Majumder S K, Monsooned coffee, *Indian Coffee*, 1963, **27**, 11.
5. Dumas T Microdetermination of phosphine in air by gas chromatography, *J Agric Fd Chem*, 1969, **17** 1164.
6. Dumas T and Bond E J, Method of trapping low levels of phosphine at ambient temperature for gas chromatographic analysis, *J. Chromatogr*, 1981, **206**, 384.
7. Bond E J and Dumas T, A portable gas chromatograph for macro-and micro determination of fumigants in the field, *J agric Fd Chem*, 1982, **30**, 986.
8. Dumas T, Modified gas chromatographic determination of phosphine, *J. ass. off. Analyt Chem*, 1978, **61**, 5.
9. Dumas T Phosphine sorption and desorption by stored wheat and corn. *J Agric Fd Chem*, 1980, **28**, 337.
10. Kashi K P and Muthu M, A mixed indicator strip for phosphine detection, *Pestic Sci*, 1975, **6**, 511.
11. Rangaswamy J R, Simple spectrophotometric method for determination of phosphine residues in wheat, *J. ass. Off. Analyt Chem* 1984, **67**, 117.
12. Rangaswamy, J R and Muthu M, Spectrophotometric determination of phosphine residues in rice, *J ass Off analyt Chem.*, 1985, **68**, 205.
13. Rangaswamy J R, Spectrophotometric method for determination of phosphine residues in cashew kernels, *J ass Off analyt Chem*, 1988, **71**, 557.

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Suitability of Phosphine for Coffee Bean Fumigation and Its Residues During Storage

J.R. RANGASWAMY AND V.B. SASIKALA
Infestation Control and Protectants Discipline,
Central Food Technological Research Institute,
Mysore — 570 013, India.

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At a dose of 3 aluminium phosphide tablets/ton free phosphine (PH_3) residue in coffee beans is just half of that found in the round pea berry. Coffee beans (0.097 p.p.m.) is free of PH_3 residue at 20 days compared to that in the peaberry (0.176 p.p.m.) which takes 33 days. Decrease of computed residues from coffee beans is faster (14 days) than that from the peaberry (26 days). Coffee beans have higher PH_3 holding capacity than the peaberry variety. In its initial pattern of PH_3 desorption, coffee resembles wheat. Coffee beans and peaberry dosed at 6 AlP tablets/ton had desorbed larger amounts of PH_3 than those dosed at 3 tablets/ton. Even at this higher dose, both coffee beans and the peaberry varieties are free of PH_3 residue within 34 days. Absorption spectra of these fumigated coffee varieties reveal the absence of any bound residues of PH_3. Air oxidation of PH_3 to phosphorus compounds is predominant in coffee beans. Coffee beans can safely be fumigated at 3 AlP tablets/ton without having traces of either free or bound residues of PH_3.

In India, coffee seeds packed in jute bags and stored in warehouses during monsoon months are infested with the coffee bean weevil *Araecerus fasciculatus* Deg. To control this insect, fumigation with MB-EDB mixture is in practice, although little is known about the extent and mechanism of formation of the bound and free residues of these fumigants in coffee beans. In Brazil, fumigation of coffee using aluminium phosphide (AlP) ("Phostoxin") tablets at 1 tablet per 3 bags (3-4 tablets/ton) is being permitted. It has been found that the PH_3 gas evolved from these tablets was found to be effective against all stages of *A. fasciculatus* within 24 hr under plastic covers.[1] Countinho *et al.*[2] found that in Brazil, complete control of all stages of *A. fasciculatus* was achieved by fumigating coffee with a dose of 15 'Phostoxin' tablets/ton for 24 hr under plastic sheeting. Puzzi *et al.*[3] in their report found that fumigation of coffee beans with 0.6 g Phostoxin tablet for 4 bags (0.5 tablets/ton) afforded complete eradication of immature and adults of *A. fasciculatus*. Although acrylonitrile as a fumigant was found to be effective to all stages of *A. fasciculatus*, in India,[4] coffee in warehouses is being fumigated with a mixture of ethylene dibromide + methyl bromide (EDB + MB) (1:1) at a dose of 32 g/m^3. Puzzi *et al*[3] have found complete eradication of immature and adults of *A. fasciculatus* on fumigation with MB at 22 ml liquid/m^3 (38 mg/l). Brazilian workers have not reported the levels of PH_3 residues in coffee beans even when such high dose of 15 tablets/ton was employed.[2] However, Muthu *et al.*[5] have reported a residue of 0.99 ± 0.12 p.p.m. of PH_3 in coffee beans dosed at 6 tablets of aluminium phosphide per ton on exposure of 5 days. Dieterich *et al.*[6] quote residues varying from 0.003 to 0.036 p.p.m. in green coffee beans fumigated in paper bags with 45 to 450 tablets per 11000 cft (0.2 to 2 tablets/ton). In order to get a clear idea of the terminal residues formed, an attempt has been made to standardise the fumigation of coffee with phosphine with respect to dosage and residue levels. A method has also been developed for the determination of PH_3 residue in various types of coffee[7]. The present paper describes these systematic approaches to PH_3 fumigation of coffee with respect to residue and storage for the benefit of the Indian coffee industry.

Materials and Methods

Fumigation and aeration: Two kilograms each of coffee beans and peaberry (round) in three replicates were fumigated at two doses of 3 and 6 Celphos tablets per ton by placing 'Celphos' (Aluminium phosphide + binders + pelleting agents but no ammonium carbamate) pellets (9 and 18 mg/kg respectively) in a paper pack underneath the coffee seeds in a 3 l flask. Nearly 6 and 12 mg of PH_3 should have been produced by 18 and 36 mg of the AlP formulation respectively. The flasks were closed with gas-tight silicon-greased glass stoppers. At the end of one week exposure at room temperature (28-30°C), the initial PH_3 residue, the trend in PH_3 desorption, and the fall in PH_3 residue during storage from pre-aired samples were determined. The

remaining samples were aired by spreading them as a thin layer in the open for 24 hr and at the end of this period, PH_3 residue was again determined[7].

Initial PH_3 residue before airing: The initial PH_3 residue in the fumigated coffee bean types before airing was determined by extracting 10 g of coffee beans with 30 ml of 2 per cent aqueous methanolic $AgNO_3$ solution (30 μg/ml)[7]. Ten grams of the corresponding crop controls were similarly extracted, and the absorbance value was subtracted from that of the PH_3 treated samples. The same extracts were employed for scanning the absorption spectra of the chromophore in the range of 200-700 nm in a Spectronic-21 spectrophotometer against the corresponding crop control as blank.

Trends in PH_3 desorption: A glass test tube containing a detector strip[8] (1 cm wide × 6 cm long) was attached to the side spout of the flask containing 200 g pre-aired coffee seeds. At the end of every hour, the tube containing the detector strip was pulled out, the red band portion of the detector strip was cut out and a fresh strip placed in the tube if needed, and the tube was replaced to the side spout of the flask quickly. The area (cm^2) of the red band was used to find the micrograms of PH_3 desorbed by the linear relationship between area vs micrograms of PH_3. Comparison of the trends in PH_3 desorption from two types of coffee seeds fumigated at two doses is shown in Fig.1.

Desorption experiments and computed residues of PH_3: A 200 g portion of coffee sample pre-aired and one-day aired, was sealed (four replicates) in 250 ml conical flasks with side spouts. A glass test tube containing a detector strip[8] was fixed gas-tight to the side spout of the flask as above. The flasks were stored in the laboratory at room temperature avoiding direct sun on the flasks. Fresh detector strips were placed in the tubes as and when needed after cutting out the red band at the end of every 24 hr. In order to gauge the amount of PH_3 desorbed regularly, the storage was continued till the red band ceased to form. The phosphine residues on each day of storage from zero to 'no-band' day was computed by observing the amount of total PH_3 desorbed over the entire period. The amount of PH_3 (p.p.m) desorbed over the entire period was taken as the total PH_3 residue in the commodity. The amount of PH_3 desorbed over the next 24 hr was subtracted from the total value successively from the first day onwards to get the computed residue for the previous day.

The fall of the PH_3 residue in two types of coffee beans during the first 34 days of storage and the absorption spectra of $AgNO_3$-PH_3 chromophore, are shown in Fig 2 to 5. Each value in these plots is the mean of 4 replicates.

Phosphorus determination: Five gram samples of coffee beans were dried at 100°C and powdered well in an iron pestle and mortar. The powdered material was soaked in boiling water (200 ml) containing 1 ml 6N H_2SO_4 with intermittent stirring. The suspension was filtered and 1.0 ml filtrate was used for colour development[7]. At the end of the storage period, the dusty patches formed on the inner walls of test tubes due to oxidation of PH_3 were used to determine the amount of phosphorus compounds formed. The dust was dissolved in hot distilled water containing 1-2 drops of 6N H_2SO_4. Complete dissolution was effected by scratching the inner walls of the test tubes with a glass rod. The washing and scratching were repeated 3-4 times and then the combined washings were made upto 10 mL. A 5 ml aliquot of this solution was employed for determination of phosphorus[9]. The amount of phosphorus was calculated from the calibration line drawn by employing KH_2PO_4 as the standard by the same method. Phosphine and inorganic phosphorus residues in the samples dosed at 3 and 6 Celphos tablets/ton are shown in Tables 1 and 2 respectively.

Results and Discussion

Both coffee beans (0.047 p.p.m.) and the peaberry (0.035 p.p.m.) dosed at 6 tablets/ton have desorbed larger amounts of PH_3 than those (0.022 and 0.012 p.p.m. respectively) dosed at 3 tablets/ton, as a result of higher uptake of PH_3 at higher dose (Fig.1). Due to slightly higher PH_3 holding capacity of coffee beans than peaberry,[7] at both the doses coffee beans had taken up higher amounts of PH_3 than the peaberry, which has resulted in larger amounts of desorption from coffee beans during first 4 hr of storage. During the next 4 hr at both the doses, desorption from both types of coffee seeds was almost uniform. At 6 tablets/ton, although there was no difference in per cent decrease in PH_3 desorption from coffee beans (70 per cent) and peaberry (71 per cent) during first 4 hr the latter at 3 tablets/ton showed a decrease of only 33 per cent compared to 73 per cent by coffee beans. This difference was due to slightly faster rate of desorption from coffee beans than peaberry (later discussion). In their PH_3 desorption pattern, coffee seeds behaved similar to wheat.[10,11]

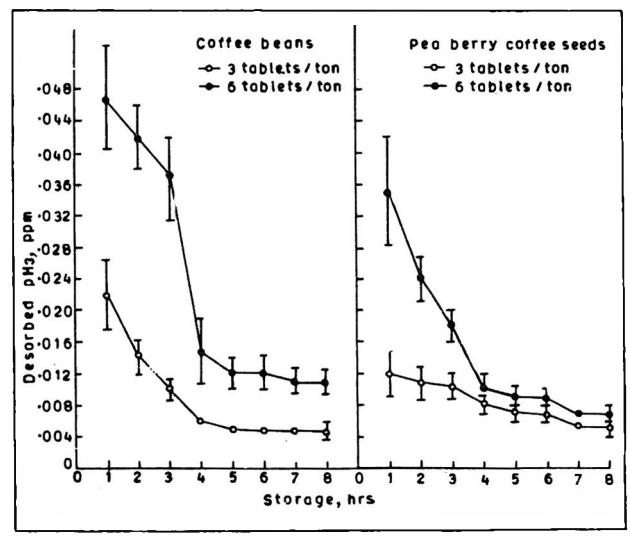

Fig.1. Initial trends in PH_3 desorption from coffee seeds fumigated with aluminium phosphide (Celphos)

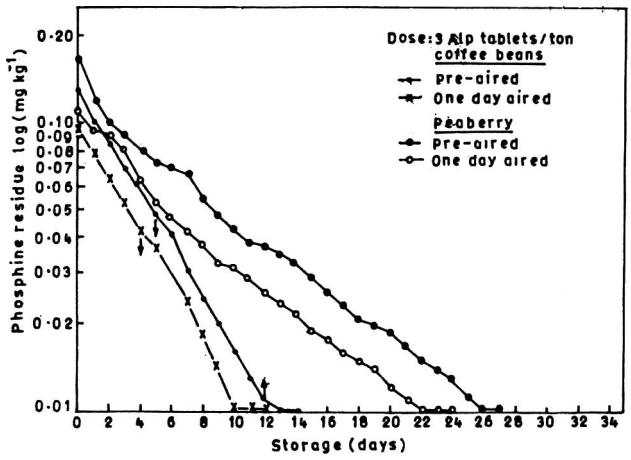

Fig.2. Fall in computed residues in coffee seeds fumigated with 3 AlP tablets/ton during storage.

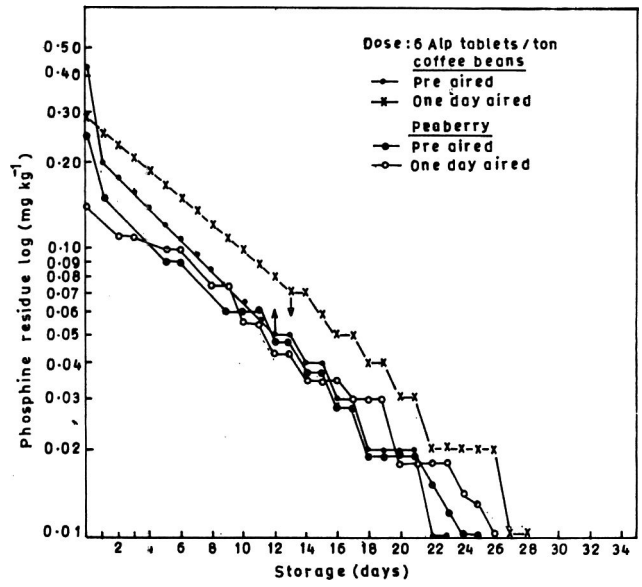

Fig.3. Fall in computed residues in coffee seeds fumigated with 6 AlP tablets/ton during storage.

At 3 tablets/ton dose, the computed residue had fallen to 0.01 ppm in coffee beans within 14 days compared to 22-26 days in peaberry (Fig.2). In coffee beans, the fall in PH_3 residue in pre-aired was linear between 0-5 and 7-12 days, while that in one day aired samples it was linear only between 0-4 days. The fall of residue in both pre and one day-aired samples of peaberry was irregular. At higher dose of 6 tablets/ton, the residue in both coffee beans and peaberry had taken almost the same duration of 22-27 days to reach 0.01 p.p.m. (Fig.3). At this dose also, the fall of residue in pre and one day-aired samples of coffee beans was linear between 1-12 and 0-13 days respectively, while the same in corresponding samples of peaberry was irregular. The shape of the seeds (peaberry is round, coffee beans are split) may be one of the factors responsible for such differences in the fall of the residue.

In all these trials, the time that elapsed between opening of the fumigation flask and pouring of the sample to the 6th replicate tube was kept between 40-45 sec. The pre-aired samples were quickly poured upto a mark precalibrated to

TABLE 1. PHOSPHINE AND INORGANIC PHOSPHORUS RESIDUES IN COFFEE SEEDS DOSED AT 3 AIP TABLETS/TON

Period of airing	PH_3 residue		Inorganic P in coffee seeds (mg/100g)	Inorganic P in side tube (μg)
	Determined (p.p.m.)	Computed (p.p.m.)		
		Coffee beans		
Pre-aired				
Immediately	0.0974 ± 0.0653	0.1260 ± 0.0127	0.189 ± 0.034	—
After 20 days storage	BEL	0.007 ± 0.0004	0.312 ± 0.075	7.73 ± 0.64
One day aired				
Immediately	0.0482 ± 0.0114	0.0953 ± 0.0084	0.225 ± 0.066	—
After 16 days storage	BEL	0.0014 ± 0.0005	0.534 ± 0.026	6.5 ± 0.66
		Pea berry		
Pre-aired				
Immediately	0.1760 ± 0.0376	0.1676 ± 0.0209	0.75 ± 0.015	—
After 33 days storage	BEL	0.0005 ± 0.0003	1.35 ± 0.316	8.34 ± 1.22
One day aired				
Immediately	0.0900 ± 0.0294	0.1118 ± 0.0193	0.736 ± 0.033	—
After 29 days storage	BEL	0.0030 ± 0.0015	0.803 ± 0.35	12.50 ± 0.97

Mean ± SD of 6 replicates
BEL, Below the estimatable limit of the method,

10 g on a 50 ml wide mouth B_{24} glass test tube. At a dose of 3 tablets/ton coffee beans showed a pre-aired determinable PH_3 residue almost half of that showed by the corresponding samples of peaberry (Table 1). Inspite of slightly higher capacity of coffee beans to hold PH_3 than peaberry,[7] the former had shown smaller residue due to faster rate of PH_3 desorption from it. Within 40-45 sec. of sampling time larger amounts of PH_3 had escaped from beans than from pea berry. When these samples were aired for one day, the decrease in determined residue was by 50 per cent in coffee beans, while the same in peaberry was by 47 per cent. The determinable free PH_3 residue in pre-aired coffee beans was below the limit of the method after 20 days storage, while the same was attained after 33 days of storage in peaberry. In case of one day aired samples, the determinable residue in coffee beans was below the limit of the method after 16 days, while the same in peaberry was after 29 days.

Because of slow desorption of PH_3 from peaberry both pre-aired (0.1676 p.p.m.) and one day aired (0.1118 p.p.m.) samples hold higher computed residues than the corresponding samples of coffee beans (0.1260 and 0.095? p.p.m. respectively). Computed residue in pre and one day aired samples of coffee beans decreased by 99 per cent over 20 and 16 days respectively, the corresponding decrease in the respective samples of peaberry being over 33 and 29 days respectively, indicating the faster rate of PH_3 desorption from coffee beans than from peaberry.

In pre-aired coffee beans, phosphorus compounds formed due to oxidatton of PH_3 was increased by 67 per cent over 20 days, while in the corresponding peaberry samples the increase was by 80 per cent. The trend was reversed in the case of one day aired samples. In case of one day aired coffee beans, the increase was as high as 137 per cent; in the orresponding peaberry samples, the increase was only 91 per cent. Unlike in cashew nuts[10] the PH_3 residues in both the types were accessible to air oxidation. Although the reported[12] phosphorus content in coffee was 130-165 mg/100g, many samples examined by the method described showed a mean value of 63.7 mg/100 g in coffee beans and 41.6 mg/100 g in peaberry. The insignificant amounts of phosphorus formed in the side tubes (6.5 to 12.5 μg) suggested that the interaction of PH_3 with the detector strip was quite fast and quantitative.

The same trends were observed in the levels of determinable free residues in coffee beans and peaberry even at the higher dose of 6 tablets/ton, and for the reasons stated above, peaberry showed a larger pre-aired residue than coffee beans (Table 2). The trends in their decrease on airing for one day had been altered, this may be due to large amount of free PH_3 residue. The decrease in the pre-aired residue in coffee beans was by 34 per cent during one day airing, while the corresponding decrease in peaberry was 38 per cent. Similar alterations were also noticed in the decrease of free determinable residues from pre-aired and one day aired samples of coffee beans and peaberry. Irrespective of the

TABLE 2. RESIDUE OF PHOSPHINE AND INORGANIC PHOSPHORUS IN COFFEE SEEDS DOSED AT 6 AIP TABLETS/TON

Period of airing	PH_3 residue		Inorganic p in coffee seeds (mg/100g)	Inorganic p in side tube (μg)
	Determined (p.p.m.)	Computed (p.p.m.)		
		Coffee beans		
Pre-aired				
Immediately	0.1505 ± 0.0445	0.2545 ± 0.0446	1.834 ± 0.359	—
After 34 days storage	BEL	0.0005 ± 0.0001	1.389 ± 0.075	19.87 ± 1.50
One day aired				
Immediately	0.0997 ± 0.0281	0.3181 ± 0.1440	0.742 ± 0.101	—
After 33 days storage	BEL	0.0007 ± 0.0007	2.070 ± 0.280	11.56 ± 0.76
		Peaberry		
Pre-aired				
Immediately	0.1951 ± 0.0310	0.4391 ± 0.0514	1.45 ± 0.299	—
After 33 days storage	BEL	0.0019 ± 0.0005	1.546 ± 0.159	10.97 ± 0.79
One day aired				
Immediately	0.1201 ± 0.0784	0.2888 ± 0.0383	1.485 ± 0.299	—
After 34 days storage	BEL	0.0020 ± 0.0003	1.582 ± 0.532	11.88 ± 0.72

Mean + SD of 6 replicates
BEL, Below the estimatable limit of the method,

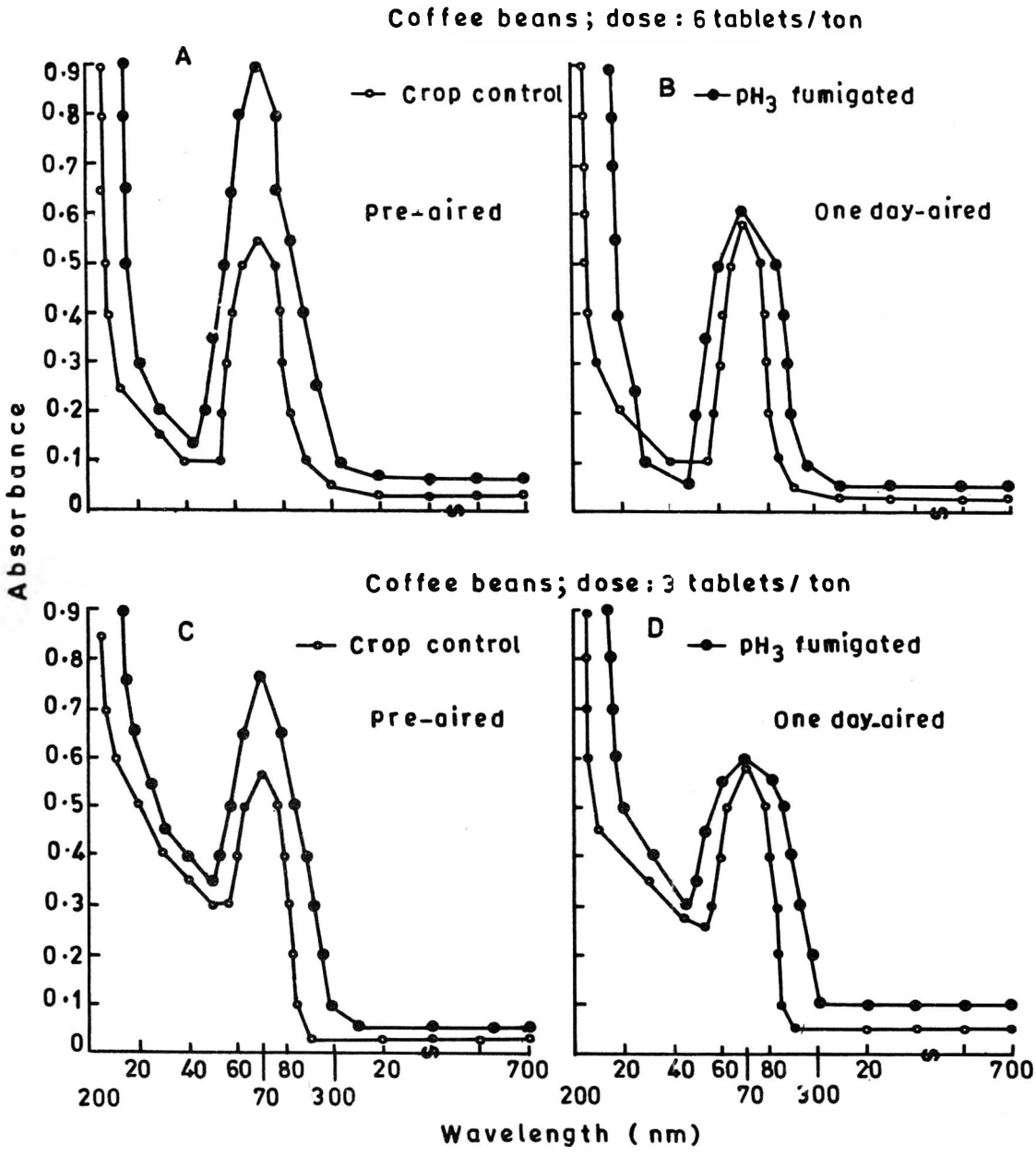

Fig.4. Absorption spectra of PH_3 residue - $AgNO_3$ chromophore from coffee beans fumigated with 6 and 3 AlP tablets/ton.

amount of determinable residues in these samples, these residues have reached a level which is below the estimatable limit of the method within 33-34 days in storage.

As observed at the lower dose, pre-aired (0.439 p.p.m.) and one day aired (0.288 p.p.m.) samples of peaberry hold a higher computed residue than the corresponding samples of coffee beans (0.255 and 0.138 p.p.m. respectively) during storage. In all these samples, the computed residues have decreased by 99.3 to 99.8 per cent within 33-34 days in storage. Larger amounts of phosphorus formation compared to that in 3 tablets/ton was noticed in both the types of coffee seeds due to excess of available PH_3 during fumigation. The changes in phosphorus content in these samples on airing and on storing were not marked except in the case of one day aired coffee beans, which had shown an increase of about 15 per cent on storing for 33 days. Only traces of phosphorus were formed in the side tubes during storage.

Absorption spectra of pre-aired and one day aired coffee beans and peaberry fumigated with 6 and 3 tablets/ton separately shown in Fig.4 and 5 respectively revealed some interesting points. Absorption spectra of the crop control of coffee beans showed a band at 270 nm due to a specific crop extractive (Fig.4). On fumigation with Celphos, PH_3 interaction with this crop extractive increases the intensity of the band in the absrption spectra of the pre-aired samples.

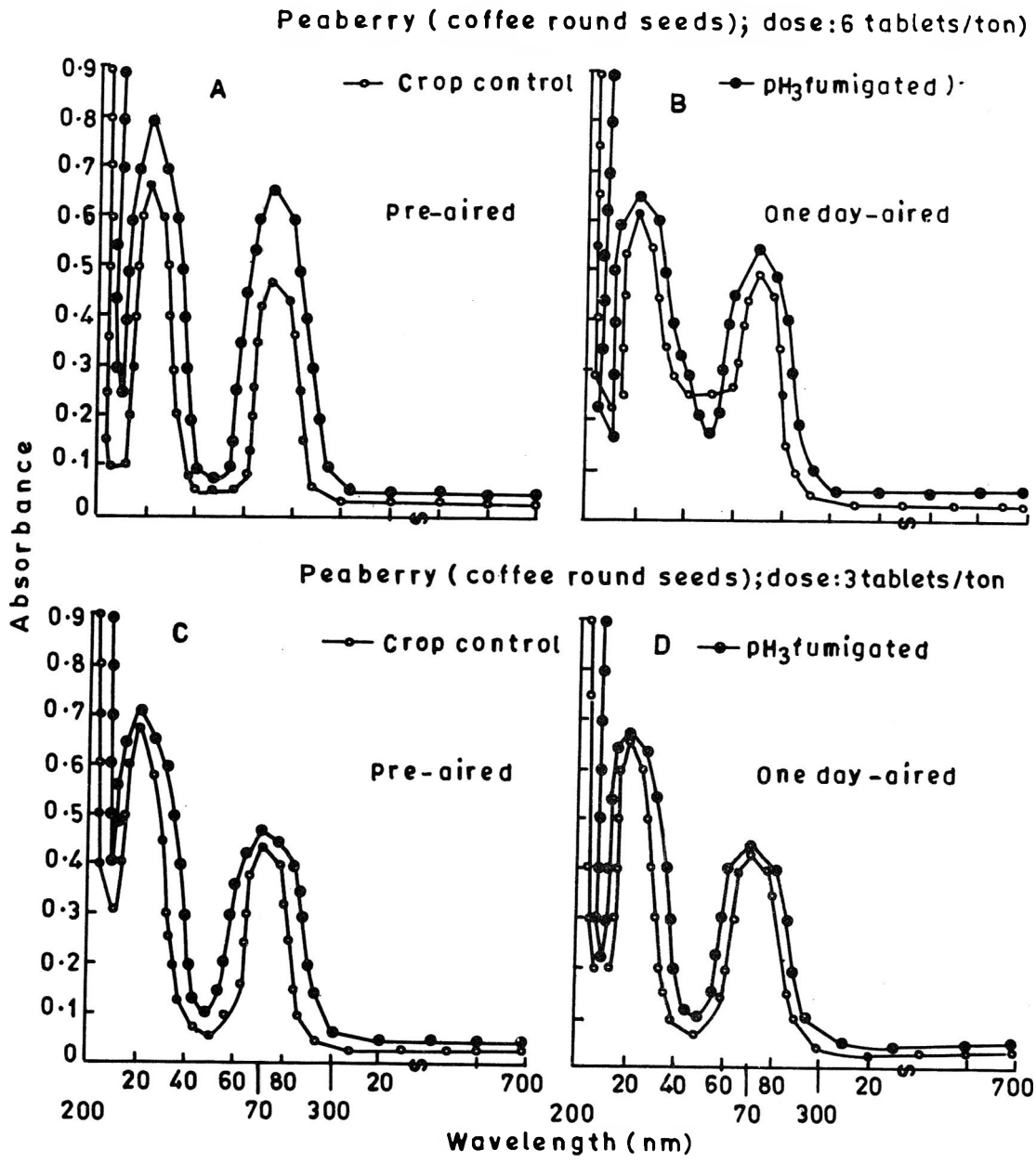

Fig.5. Absorption spectra of PH_3 residue - $AgNO_3$ chromophore from peaberry fumigated with 6 and 3 AlP tablets/ton.

The observed increment in the intensity of the band was more with a dose of 6 tablets/ton than with 3 tablets/ton. On airing for a day the increment in the intensity of the band in the absorption spectra of the samples fumigated with both these doses decreased almost completely due to decomposition of the interaction product, as revealed by the absorption spectra of one day aired coffee bean samples.

The corresponding absorption spectra of peaberry samples shown in Fig.5 are different from those of coffee beans, as the former show an additional band at 220 nm. The band at 220 nm is more intense than the one at 270 nm. Both these bands behave similar to that in coffee beans on fumigation with two doses and aeration for one day. The band at 270 nm appears to be common to these two types of coffee seeds. No other band is observed in the spectra between 200 and 700 nm except a hump between 380-480 nm (not shown in Fig) due to free PH_3 residue indicating the absence of bound residues of PH_3 in coffee beans and peaberry fumigated at these two commercial doses. Identification of the causative extractive of these two bands from coffee beans and peaberry (under progress) and their reaction with PH_3 will throw more light on the type of the linkage formed.

The extensive data provided above conclude that coffee beans and peaberry can safely be fumigated with aluminium phosphide at a dose of 3 tablets/ton. In both the coffee types the determinable free residues of PH_3 fall to a low level of

0.01 ppm within a week. The computed residues decrease to similar level in about 10-13 days in coffee beans and 24-26 days in peaberry.

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References

1. Puzzi D and Orlando A, Studies on the dosage of fumigants for control of *A. fasciculatus* under plastic covers, *Biologico*, 1963, **29**, 127.
2. Countinho J M Puzzi D and Orlando A, The use of the fumigant phosphine in control of insects of stored coffee seeds, *Biologico*, 1961, **27**, 271.
3. Puzzi D Pereira H F Bitran E A and Campos T B Studies on the influence of temperature on the effectiveness of methyl bromide and phosphine for fumigation of the coffee beetle, *A. fasciculatus*, *Biologico*, 1968, **34**, 51.
4. Majumder S K Muthu M Sreenivasan K S Natarajan C P, Bhatia D S and Subrahmanyan V, Studies on the storage of coffee beans — IV. Control of *A. fasciculatus* in monsooned coffee and related storage experiments, *Fd Sci*, 1961, **10**, 332.
5. Muthu M Kashi K P and Majumder S K, A simple method of determining the sorption affinity of foodstuff to phosphine, *Chem. Ind.* 1978, **18**, 129.
6. Dieterich W H, Gero Mayr, Karl Hild, Sullivan, J B and Murphy J, Hydrogen phosphide as a fumigant for foods, feeds and processed food procucts, *Residue Rev*, 1967, **19**, 135.
7. Rangaswamy J R, Spectrophotometric method for determination of phosphine residues in coffee seeds. *J Fd Sci Tech*. 1990, 27,33.
8. Kashi K P, and Muthu M, A mixed indicator strip for phosphine detection *Pesticide Sci.*, 1975, **6**, 511.
9. Bruce R B Robinson J R and Tuft T O, Phosphine residues from phostoxin-treated grain, *J Agric Fd Chem*, 1962, **10**, 18.
10. Rangaswamy J R and Sasikala V B, Comparison of dissipation, behaviour and levels of phospine residues in cashew kernel and wheat, *J Fd Sci Tech*, (Accepted).
11. Rangaswamy J R and Sasikala V B, Phosphine residues in cashew nuts and wheat at a commercial dose of 6 tablets/ton, *Lebensm,-wiss, u-Technol* 1988, **21**, 1.
12. Heath H B, *Source Book of Flavours*. The AVI Publishing Company Inc. West Port, Connecticut, 1981, 164.

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Sensitivity of Sterilization Effect from Process Parameters

S. AKTERIAN, K. VIDEV AND H. DAS*
Higher Institute of Food & Flavour Industries,
Department of Machines & Apparatuses for Food & Flavour Industries,
Lenin-26, Plovdiv, Bulgaria

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Sensitivity of sterilization effect (SSE) define changes in F-effect caused by relative changing of respective determining parameter. Analytical and correlative dependences of SSE which have a bearing on convectionally heated canned foods are presented. Generelized intervals of changing SSE, involved in definite multitude from sterilization regimes are given. SSE is calculated on the basis of worked out private programmed products. Way of defining of the complete relative changing of F-effect, caused by simultaneously relative changing of all defined parameters, is also shown.

With respect to thermophysical and micrrobiological conditions of sterilization, the bulk of canned foods: stewed fruit, gherkins, fresh beans, peas, fleshy peppers, bear, fruit juices, nectars, milk, etc., can be classified as convectionally heated foods (where during the time of whole process, temperature of the liquid in canned foods is uniform in whole volume).

It is established that the changes in the temperature of canned foods, with enough accuracy for practice, can be written using following equation[1,2]:

$$E\ \frac{dT_c}{dt} + T_c - T = O. \qquad (1)$$

Temperature change in heating medium in still, rotary, hydrostatic and open tunnel retorts can be approximated with linear change in n phases. Let temperature in i-phase change linearly from T_{bi} to T_{fi} for time t_i. Temperature of the liquid in canned food in the beginning of the phase is T_{ci} and the coefficient of thermoinertia is E_i (Fig.1). Temperature changes in canned food[3] can be defined from equation (1):

$$T_c = T - \frac{T_{fi}-T_{bi}}{t_i} E_i - (T_{bi} - T_{ci} - \frac{T_{fi}-T_{bi}}{t_i} E_i).\ \exp(-t/E_i) \qquad (2)$$

Sterilization effect of i-phase can be defined with the following formulae[3,4]:

$$F_i = \int_0^{t_i} 10^{\frac{T_c - T_e}{z}}\ dt, \qquad (3)$$

$$F_i = E_i \exp(-C_i) \int_0^{N_i} \exp[B_i x + A_i \exp(-x)]dx. \qquad (4)$$

The F-effect for whole sterilization process is defined by $F = F_1 + F_2 + \ldots + F_i + \ldots + F_n$.

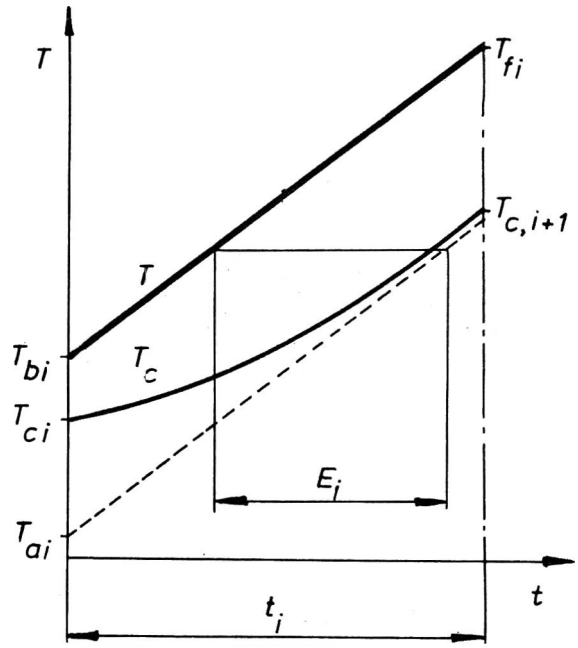

Fig. 1 Temperature curves of the heating medium and the canned food in i-th phase of the sterilization process.

The values of F-effect are compared for assessment of adequateness of described model with equations (2) and (3). The values of F-effect are calculated according to the model and on the basis of experimental data representing the change of the temperature of the liquid in canned foods during during the sterilization process. By means of these data, F-effect is calculated through numerical integration of equation (4). Average deviation among the two types of F-effect, which are determined, is 8 per cent, as for stewed fruit from morello with 76 times repetition of the experiment - (8+7)%, for stewed fruit from cherry with 60 experiments - (7+6)%; for

* To whom all correspondence should be addressed.

stewed fruit from plum with 83 experiments - (8+5)%; for stewed fruit from quince with 80 experiments - (9+6)% and for stewed fruit from pear with 40 experiments - (9+7)%[56]:

Sensitivity of sterilization effect (SSE) represents relative differential changing of F-effect of whole process caused relative differential changing of determining parameter P_i and is defined by the following formulae[3,7-12]:

$$\phi_{Tci} = \frac{T_{ci}.dF}{F.dT_{ci}} = \sum_{i=1}^{n} \varphi_{Tci} \frac{F_i}{F} \cdot \frac{T_{cl}}{T_{ci}} \cdot \frac{dT_{ci}}{dT_{cl}}, \text{ where: } \varphi_{Tci} = \frac{T_{ci}.dF_i}{F_i.dT_{ci}}, \quad (5)$$

$$\phi_z = \frac{z.dF}{F.dz} = \sum_{i=1}^{n} \varphi \frac{F_i}{F}, \text{ where: } \varphi_{zi} = \frac{z.dF_i}{F_i.dz}, \quad (6)$$

$$\phi_{ti} = \frac{t_i.dF}{F.dt_i} = \frac{F_i}{F} \varphi_{ti} + \sum_{j=i+1}^{n} \varphi_{Tcj} \frac{F_j}{F} \cdot \frac{t_i}{T_{cj}} \cdot \frac{dT_{cj}}{dt_i} \quad \phi_{tp} = \sum_{i=1}^{n} \phi_{ti}, \quad (7)$$

where: $\varphi_{ti} = \frac{t_i.dF_i}{F_i.dt_i}$,

$$\phi_{Ei} = \frac{E_i.dF}{F.dE_i} = \frac{F_i}{F} \varphi_{Ei} + \sum_{j=i+1}^{n} \varphi_{Tcj} \frac{F_j}{F} \cdot \frac{E_i}{T_{cj}} \cdot \frac{dT_{cj}}{dE_i} \quad \varphi_{Ei} = \frac{E_i.dF_i}{F_i.dE_i} \quad (8)$$

$$\phi_{Tbi} = \frac{T_{bi}.dF}{F.dt_{bi}} = \frac{F_i}{F} \varphi_{Tbi} + \sum_{j=i+1}^{n} \varphi_{Tcj} \frac{F_j}{F} \cdot \frac{T_{bi}}{T_{cj}} \cdot \frac{dT_{cj}}{dT_{bi}} \quad (9)$$

where: $\varphi_{Tbi} = \frac{T_{bi}.dF_i}{F_i.dT_{bi}}$,

$$\phi_{Tfi} = \frac{T_{fi}.dF}{F.dT_{fi}} = \frac{F_i}{F} \varphi_{Tbi} + \sum_{j=i+1}^{n} \varphi_{Tcj} \frac{F_j}{F} \cdot \frac{T_{fi}}{T_{cj}} \cdot \frac{dT_{cj}}{dT_{fi}} \quad (10)$$

where: $\varphi_{Tbi} = \frac{T_{bi}.dF_i}{F_i.dT_{bi}}$

Minimum, maximum and average values of changing of SSE, determined on the basis of analysis of about 300 sterilization regimes are given in Table 1. These regimes apply to:

—for retorts with continuous process (tunnel retorts) and with batch process (autoclaves);

—for convectionally heated preserved foods: stewed fruits (from cherry, quince, plum, pear, strawberry, peach, apricot, apple etc.), canned vegetables (from french bean, pea, gherkin, fleshy pepper etc.), beer and nectars;

—for packed preserved foods: glass jar - 0.8 dm^3 (ϕ105×130 mm), metal can - 1 dm^3 (D = 99 mm) and glass bottles - 0.5 dm^3.

These results are according to Akterian and Videv[13]. Regimes are in conformity with and coefficients of thermal inertia are according Tanchev[14] and Videv[15]. The values of F-effect and SSE are calculated in computer by means of complex programmed products of FORTRAN, FOCAL and BASIC[3,4,13].

On the basis of correlation analysis, correlation dependencies are constituted for determining SSE[16] from parameters which are most significant and contribute to changes in biggest particular change of F-effect:

$$\phi_z = 2.08 \times \log (E_2/F) + 1.64, \quad \delta = 0.24, \quad \delta = 0.71, \quad (11)$$

$$\phi_{Trd} = 246 \times z^{-0.573} - 6.80 \times \log(t_2/F) - 28.8, \quad \delta = 1.39, \quad \delta = -15.01, \quad (12)$$

$$\phi_{t2} = 0.183 \times \log (t_2/F) + 1.94, \quad \delta = 1.50, \quad \delta = -14.87, \quad (13)$$

$$\phi_{Tcl} = -0.0379 \times \log F + 0.185 - 0.00604 \times (t_1/E_1) \times (t_2/E_2), \quad (14)$$
$$\delta = 0.89, \quad \delta = 3.08$$

$$\phi_{E2} = -0.174 \times \log (E_2/F) - 0.387, \quad \delta = 2.32, \quad \delta = 19.71, \quad (15)$$

TABLE 1. LIMITS FOR CHANGING OF SENSITIVITY, PARTICULAR CHANGES OF STERILIZATION EFFECT AND VARIATIONS OF PROCESS PARAMETERS

P_i	Autoclave							Tunnel Retort						
	ϕ_{Pi}			$\frac{\Delta Pi}{Pi}$ (%)	$\left(\frac{\Delta F}{F}\right)_{Pi}$ (%)			ϕ_{Pi}			$\frac{\Delta Pi}{Pi}$ (%)	$\left(\frac{\Delta F}{F}\right)_{Pi}$ (%)		
	min	mid	max		min	mid	max	min	mid	max		min	mid	max
z	0	1.5	8.5	20	0	30	170	0	0.2	4	20	0	5	80
T_{cl}	0	0.4	1.5	20	0	8	30	0	0.4	1	20	0	8	20
T_{rw}	0.2	1	3	8	1.6	8	24	0	1.5	3	8	0	12	24
T_{rd}	15	25	50	2	30	50	100	15	20	25	2	30	40	50
T_{rc}	0	0.2	0.7	16	0	4	11	0	0	0	16	0	0	0
t_{id}	0.4	2	5.5	5	2	10	27	1.5	2	2.8	0	0	0	0
t_i	0	0.7	2.5	10	0	7	25	0	0	0	0	0	0	0
t_p	1.2	3	5	10	12	30	50	1.5	2.2	3.5	2	3	4.5	7
E_{wd}	0	1	2.3	6.5	0	6.5	15	0	0.7	2	5	0	3.5	10
E_c	0	0.1	0.6	9	0	1.3	5.4	0	.02	.15	5	0	0.1	0.7
					min	mid	max					min	mid	max
$\left(\frac{\Delta F}{F}\right)_{sq}$ (%)					30	60	200					30	50	110

These dependencies are received through analysis of 144 regimes from the above mentioned regimes, applied for sterilization of 12 types of convectionally heated canned foods in autoclaves, at $T_e = 121.1°C$.

Relative changing of F-effect, caused by simultaneous relative changing of all determining parameters P_i can be defined by[13]:

$$\frac{\Delta F}{F} = \phi_{Tcl}\frac{\Delta T_{cl}}{T_{cl}} + \phi_z\frac{\Delta z}{z} + \sum_{i=1}^{n}\phi_{t_i}\frac{\Delta t_i}{t_i} + \sum_{i=1}^{n}\phi_{E_i}\frac{\Delta E_i}{E_i} + \sum\phi_{T_{bi}}\frac{\Delta T_{bi}}{T_{bi}} + \sum_{i=1}^{n}\phi_{T_{fi}}\frac{\Delta T_{fi}}{T_{fi}} \quad (16)$$

The occasional variations of F-effect during the time of process can be evaluated by average quadratic variation of F-effect, caused by occasional variations of parameters[13]:

$$\left(\frac{\Delta F}{F}\right)_{sq} = \sqrt{\sum_{Pi}\sum_{i=1}^{n}\left(\frac{\Delta F}{F}\right)^2_{P_i}} \quad (17)$$

Minimum, maximum and average values of particular ($\Delta F/F$) and average quadratic variation of F-effect, applied for the above mentioned groups from 300 regimes are shown in Table 1. Average variations of parameters ($\Delta P_i/P_i$) are also given in the same Table. Evaluations of these variations are done on the basis of direct supervision in production conditions and analysis of methods for determination of z and E_i.

One of the possible ways for defining the recommended accuracy for regulation of T_{rd}, T_{rw}, T_{rc}, T_{cl}, t_i and the accuracy for identification of z and E_i the use of SSE:

$$(\Delta P_i/P_i) < \frac{1}{R = \phi_{Pi}\sqrt{m}}(\Delta F/F)_{sq}, \quad (18)$$

The values of recommended accuracy for regulation and for identification of parameters, defined on the basis of equation (18) and after techno-economic analysis, are given in Table 2.

TABLE 2. REQUIRED ACCURACY (%) FOR REGULATION AND DETERMINATION OF PROCESS PARAMETERS

A. **Autoclave**

	P_i	z	T_{cl}	T_i	E_i	T_{rw}	T_{rd}	T_{rc}
$\frac{\Delta Pi}{Pi}$ R	F<600 sec,	10	15					
	F<600 sec,	20	50	10	10	10	0.5	20

B. **Tunnel Retort**

P_i	z	T_{cl}	t_p	E_i			T_{rd}	
$\frac{\Delta Pi}{Pi}$ R	20	20	5	10	20	10	5	0.5
T_{rd},(°C)					< 60	60-92	92-95	>95

Example: In order to calculate the sensitivity of the F-effect in relation to the parameters of the process during sterilization of green peas in glass bottle with volume 0.8 dm^3 (ϕ105×130 mm) using autoclave. The sterilization was completed under the following regime T_{cl}=40°C; $T_{rw}=T_{b1}$=60°C; $T_{rd}=T_{f1}=T_{b2}=T_{f2}=T_{b3}$=121°C $T_{rc}=T_{f3}$=30°C; t_1=1500 sec; t_2=1800 sec; t_3=1500 sec (4). The coefficients of thermal inertia are $E_1 = E_2$ = 318 sec; E_3 = 210 sec[15].

The F-effect was calculated through equations (1) and (2) at z=10°C and T_e=121.1°C is F=1422 sec. The sensitivities of the sterilization effect calculated through equations (5) to (15) are given in Table 3. If it is accepted that SSE from the most influential parameter T_{rd} is 100 per cent, then SSE of the next parameters accruing importance are ϕ_{t2}:4.5 %; ϕ_{E2}:1.3 %; ϕ:1.1 % and SSE from all the remaining parameters are below 1%.

The partial changes of F-effect determined on the basis of the currently applied accuracy for regulation of the process parameters taken for Table 1 and on the basis of the recommended accuracy taken for Table 2 are given in Table 3. The occasional variations of F-effect calculated as per equation (17) decrease from 55 per cent at the applied accuracy for regulation to 20 per cent at the recommendable accuracy.

On the basis of equation (16), it is possible to constitute equation to compensate the changes of F-effect ($\Delta F/F$=O) caused by the unfavourable systematic variation of T_{rd}, through the change of t_2:

$$\frac{\Delta t_2}{t_2} = -\frac{\phi T_{rd}}{\phi t_2}\cdot\frac{\Delta T_{rd}}{T_{rd}} \text{ or } \frac{\Delta t_2}{1800} = -\frac{27.3}{1.23}\frac{\Delta T_{rd}}{121} \text{ or } \Delta t_2 = -330\times\Delta T_{rd}$$

TABLE 3. SENSITIVITY AND PARTICULAR CHANGES OF F-EFFECT DURING STERILIZATION OF GREEN BEANS

P_i	ϕ_{Pi}	ϕ_{Pi}	$\left(\frac{\Delta F}{F}\right)_{Pi}$	$\left(\frac{\Delta F}{F}\right)_{Pi}$
	1	2	3	4
z	0.32	0.29	6.4	6.4
T_{cl}	0.01	-0.03	0.2	0.5
t_1	0.22	-	2.2	2.2
t_2	1.23	1.95	6.1	12.3
t_3	0.07	-	0.7	0.7
E_1	-0.22	-	1.4	2.2
E_2	-0.36	-0.27	2.3	3.6
E_3	0.05	-	0.4	3.6
T_{rw}	0.21	-	1.7	2.1
T_{rd}	27.3	36.2	54.6	13.6
T_{rc}	0.02	-	0.3	0.4

1 and 2 - values of SSE calculated as per equations (5) to (10) and (11) to (15) respectively;
3 and 4 - particular changes of F-effect determined on the basis of applied and recommended accuracy of regulating the process parameters P_i.

According to above mentioned equation, the decreasing of temperature T_{rd} by 1°C, can be compensated by extending the continuity by 300 sec. The following equations for compensating the unfavourable systematic change of T_{cl} are worked out as per analogical way:

$$\Delta T_{rd} = -0.001 \times \Delta T_{cl}; \quad \Delta t_2 = -0.366 \times \Delta T_{cl}.$$

On the basis of the completed analysis of SSE and the changes of F-effect, the following inferences can be drawn:

—Thermomicrobiological model (characterized by thermal stability with coefficient z) and thermophysical model (characterized by thermoinertia with coefficient E_i) describe the process of thermal sterilizations with enough accuracy. The perfection of two models must be completed simultaneously. But priority should be given to the first.

—At the existing accuracy for regulation of process parameters, the occasional variations of F-effect are in the order of 50 to 60 per cent. At the recommended accuracy for regulation, these variations decrease to half (Table 2) and can be evaluated as completely satisfactory. The occasional variations in tunnel retorts are smaller than those in autoclaves. Therefore, from this point of view, preference should be given to the first.

Nomenclature:

$A_i = 2.303\ (T_{ci}-T_{ai})/z$ - temperature difference (dimensionless) between T_{ci} and T_{ai} at the beginning of i phase;

$B_i = 2.303\ (T_{fi}-T_{bi})\ /\ (Z.N_i)$ - speed (dimensionless) of changing the temperature of heating medium in i-phase;

$C_i = 2.303\ (T_e-T_a)/z$ - temperature difference (dimensionless) between T_e and T_{ai} at the beginning of i-phase;

E, E_i, E_w, E_d, E_c - coefficient of thermal inertia: running value, of i - phase, at heating, retention and cooling; sec;

F, F_i - adjusted sterilization effect for whole process and for i - phase, sec;

i=1...n - phase number in row;

m - number of parameters P_i for given sterilization process;

$N=t_i/E_i$ - duration (dimensionless) of i-phase;

n - number of phases;

P_i - process parameters: $T_{cl}, z, t_i, E_i, T_{bi}, T_{fi}$;

T, T_{bi}, T_{fi} - temperature of heating medium: running value, at the beginning and at the end of i-phase, °C;

$T_{ai} = T_{bi} - (T_{fi}-T_{bi})\ E_i/t_i$ - asymptotic temperature at the beginning of i-phase, °C;

T_c, T_{ci} - temperature of liquid in canned food: running value and at the beginning of i-phase, °C;

T_e - standard temperature, °C;

T_{rw}, T_{rd}, T_{rc} - regulated temperature of heating medium during heating, retention and cooling, °C;

t, t_i, t_{id}, t_p - duration of process: running value, of i-phase, of the retention phase and of whole process, sec;

z - coefficient of temperature stability of microorganisms, °C;

$(\Delta F/F)_{sq}$ - average quadratic change of F-effect;

$\left(\frac{\Delta F}{F}\right)_{Tcl}, \left(\frac{\Delta F}{F}\right)_{z}, \left(\frac{\Delta F}{F}\right)_{ti}, \left(\frac{\Delta F}{F}\right)_{Ei},$

$\left(\frac{\Delta F}{F}\right)_{Tbi}, \left(\frac{\Delta F}{F}\right)_{Tfi}$ — particular relative change of F-effect, caused by variation of parameters respectively P_i : T_{cl}, z, t_i, E_i, T_{bi} and T_{fi}; $(\Delta F/F)_{Pi} = (\Delta P_i/P_i)$, $(\Delta P_i/P_i)_R$ - applied and recommended relative variation of parameters P_i;

δ, δ - dispersion and highest value of relative variation of SSE calculated by using correlation dependences equations (11) to (15) and analytical dependences equations (5) to (10);

$\phi Pi, \phi fci, \phi z, \phi t, \phi Ei, \phi Tbi, \phi Tfi$ - sensitivity of F-effect from process parameters P_i : T_{ci}, z, t_i, E_i, T_{bi}, T_{fi};

$\varphi Pi, \varphi Tci, \varphi z, \varphi ti, \varphi Ei, \varphi Tbi, \varphi Tfi$ - function of sensitivity of F-effect from parameters P_i : T_{ci}, z, t_i, E_i, T_{bi}, T_{fi}.

References

1. Molodetzky E, *Theoretical and Experimental Study of Apparatus and Sterilization Process of Canned Foods with Aim to its Automation*. 1968. Ph D. Thesis, Odessa Technological Institute for Food Industries, USSR.
2. Postov P, Kolev D and Videv K, Study of heating process of preserved foods during the time of sterilization. *Scient. Works Scient, Res. Inst for Canning Ind*, 1963, 1, 7.
3. Videv K and Akterian S, Calculation of sterilization of convectionally heated preserved foods in batch retorts using computers, *Scient Works Higher Inst of Fd Flav. Ind.* 1982, 1, 285.
4. Akterian S, Videv K and Chaushev G, Packet programmes for calculation of effectiveness of sterilization processes and regimes, *Proceedings of 2nd conference on "Personal Computers"*, Varna, May 1987, 13.
5. Furlinski I, Videv K and Tanchev S, Time-inertia coefficients for stewed fruits from morello, cherry and plum, *Bulgarian Fruit, Veg Canned Fd* 1980, 10, 16.
6. Videv K, Furlinski I, Tanchev S, Ruskov P and Kash Kavaldziev P, Determination of time-inertia coefficients for stewed fruits from quince and pear, *Scient Works Higher Inst Fd Flav Ind*, 1978, 3, 10.
7. Videv K and Akterian S, Dependence of F-effect from accuracy of defining of thermal sensitivity of micro-organisms, *Proceedings of the Scientific and Technical Conference for Canning Industry*, Plovdiv, November 1982, 21.
8. Videv K and Akterian S, Sensitivity of F-effect from duration of sterilization process, *Proceedings of Scientific Session of Higher Institute of Food and Flavour Industries*, Plovdiv, May 1983, 24.
9. Videv K and Akterian S, Sensitivity of F-effect from process parameters, *Scient Works Higher Inst Fd Flavour Ind*, 1985, 2, 185.
10. Videv K and Akterian S, Sensitivity of F-effect from starting temperature of preserved foods, *Scient Works Higher Inst Fd Flavour Ind*, 1985, 2, 135.
11. Videv K and Akterian S, Sensitivity of F-effect from the variations of temperature of heat carrier. *Proceedings of the Scientific and Technical Conference for Canning Industry*, Plovdiv, November 1982, 20.

12. Videv K and Akterian S, Study of the influence of variations of coefficients of thermal inertia on sterilization process, *Proceedings of Scientific Session of Higher Institute of Food and Flavour Industries*, Plovdiv, May 1983, 25.
13. Akterian S and Videv K, Sensitivity of sterilization effect of convectionally heated canned foods, *Proceedings of the International Scientific and Technical Conference for Canning Industry*, Plovdiv, November 1987, 87.
14. Anon, *A Collection of Technological Instructions*, Scientific Research Institute for Canning Industry, Plovdiv, 1981, 4.
15. Tanchev S and Videv K, Correlation dependences for defining of sensitivity of sterilization effect, *Scient Works Higher Inst Fd Flav Ind.* 1986, 2, 325.
16. Akterian S and Videv K, Correlation dependences for defining of sensitivity of sterilization effect, *Scient Works Higher Inst Fd Flav Ind*, 1986, 2, 325.
17. Videv K, *Mathematical Model for Sterilization Process of Canned Foods During Convection Heating*, 1972, Ph.D. Thesis, Higher Institute of Food and Flavour Industries, Plovdiv, Bulgaria.

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EFFECT OF SPICES, ADDITIVES AND AUTOXIDISED OILS ON ANALYSIS OF SORBIC ACID IN FOODS

G.K. SHARMA AND S.S. ARYA
Defence Food Research Laboratory, Mysore — 570 011, India.

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Effect of spices, antioxidant and other additives on the recovery of sorbic acid by the use of spectrophotometric and colorimetric method has been compared. Except fumaric acid and its derivatives and cumene oleoresin, the other spices and additives and oxidised fats do not interfere in the analysis of sorbic acid.

Sorbic acid is widely used in bakery, confectionery and fruit products mainly for the prevention of fungal attack during storage[1]. For analysis, sorbic acid is isolated by steam distillation of a known quantity of food material and its concentration is determined either by measuring the absorbance[2] of distillate at 258 nm or alternatively sorbic acid in the distillate is oxidised with potassium dichromate and oxidation products are reacted with 2-thiobarbituric acid producing red chromogen. The intensity of the red chromogen at 532 nm is linearly related to sorbic acid concentration[3]. Since some of the spices, additives and food constituents as well as autoxidation products of polyunsaturated fatty acid are steam volatile and also have absorbance in UV region, these may interfere in the determination of sorbic acid in foods. The present communication describes the role of some of these ingredients in sorbic acid recovery by both spectrophotometric and colorimetric methods.

In order to study the effect of various additives and spices on sorbic acid recovery, 1 ml aliquots of aqueous sorbic acid solution (1 mg/ml) were treated with 50 ml 0.1 N sulphuric acid and 50 g magnesium sulphate both with and without spices (chilli, cinnamon, turmeric, clove, cardamom, garlic, mace, black pepper, ginger, coriander, cumene powder, 10 mg each), additives (calcium propionate, butylated hydroxytoluene, butylated hydroxyanisole, tertiary butylated hydroquinone (TBHQ), dimethyl fumarate, fumaric acid, propylgallate, hydroxymethylfurfural, sodium benzoate, 1 mg each) and stored oils (refined groundnut, safflower and sunflower oils, 250 mg each). The contents were steam distilled in an all glass apparatus and 350 ml of the distillate was collected. The volume of the distillate was made upto 500 ml and its absorbance was measured at 258 nm. The concentration of sorbic acid was calculated using $E^{1\%}_{258} = 2150$.

For colorimetric estimation, 2 ml aliquots of the distillate were treated with 1 ml of 0.3 N sulphuric acid and 1 ml of potassium dichromate solution (147 mg/100 ml) and kept in boiling water for 5 min in 20 ml test tubes. The tubes were cooled in ice bath and 2 ml of 0.5 per cent thiobarbituric acid solution was added and subsequently heated for 10 min in boiling water. After cooling, the absorbance was measured at 532 nm and the concentration of sorbic acid was calculated from the standard curve drawn between sorbic acid concentration and absorbance. All experiments were conducted in triplicate and the results presented are mean of atleast three values.

TABLE 1. RECOVERY (PER CENT) OF SORBIC ACID WITH VARIOUS ADDITIVES BY SPECTROPHOTOMETRIC AND COLORIMETRIC METHOD

Additives	Spectrophotometric method	Colorimetric method
Sorbic acid (SA)	99.7 ± 1.6	100.7 ± 1.6
SA + calcium propionate	102.9 ± 1.2	102.5 ± 3.5
SA + butylated hydroxy anisole	103.1 ± 1.3	105.6 ± 2.8
SA + butylated hydroxy toluene	101.8 ± 2.1	101.3 ± 1.8
SA + tertiary butylhydro quinone	104.6 ± 0.2	101.3 ± 1.2
SA + propylgallate	105.6 ± 1.3	103.8 ± 1.8
SA + sodium benzoate	99.6 ± 0.2	100.0 ± 1.3
SA + hydroxymethyl furfural	101.2 ± 1.6	100.0 ± 0.1
SA + dimethyl fumarate	108.9 ± 1.6	98.8 ± 1.7
SA + fumaric acid	109.1 ± 1.3	100.5 ± 0.2
SA + cumene oleoresin	117.8 ± 1.6	101.6 ± 1.8
SA + cumene powder	104.2 ± 0.9	99.0 ± 1.4
SA + chill	100.0 ± 0.3	102.5 ± 1.0
SA + cinnamon	102.8 ± 2.2	103.8 ± 1.8
SA + turmeric	104.4 ± 1.5	102.5 ± 1.0
SA + clove	101.2 ± 1.6	102.4 ± 0.2
SA + cardamom small	103.3 ± 1.2	101.3 ± 1.8
SA + mace	106.8 ± 0.4	101.3 ± 1.8
SA + thyme	101.7 ± 2.9	101.3 ± 1.3
SA + garlic	102.3 ± 0.4	105.7 ± 0.9
SA + black pepper	101.5 ± 1.3	101.3 ± 1.0
SA + dry ginger	101.4 ± 1.6	100.0 ± 0.4
SA + coriander	102.3 ± 0.2	99.3 ± 1.1
SA + groundnut oil	98.0 ± 0.3	99.0 ± 1.1
SA + safflower oil	97.8 ± 0.2	100.0 ± 0.9
SA + sunflower oil	99.8 ± 0.3	99.0 ± 0.6

The effect of various additives, spices and stored oils on the percentage recovery of sorbic acid determined both by spectrophotometric and colorimetric methods is given in Table 1. The mean recovery of sorbic acid without any additives were 99.7 ± 1.6 and 100.7 ± 1.6 per cent by spectrophotometric and colorimetric methods respectively. The reproducibility of results by both these methods was excellent and coefficient of variation in both cases ranged between 0.5 and 2.5 per cent. Accordingly, the recoveries above 104% and below 96 per cent were found significant at 99 per cent confidence limit.

Among the products tested, dimethyl fumarate, fumaric acid and cumene oleoresin were found to interfere considerably in the determination of sorbic acid by spectrophotometric method whereas no interference was observed in colorimetric procedure from the above ingredients. Very slight but significant interference (104-106 per cent recovery) was also observed due to propylgallate, tertiary butylated hydroquinone, turmeric, cumene and mace powders in the spectrophotometric method. On the other hand, slightly higher recoveries (105 per cent) were also observed in colorimetric method in the presence of garlic and butylated hydroxyanisole.

Stored vegetable oils (peroxide value 50-100 meq oxygen per kg fat) did not interfere in sorbic acid determination either by spectrophotometric or colorimetric procedure. Autoxidised fatty acids containing conjugated diene have considerable absorbance in 230-270 nm region but these do not seem to get steam distilled along with sorbic acid.

The results of this investigation suggest that except fumaric acid and its derivatives and cumene oleoresin, the other spices, additives and oxidised fats do not substantially interfere in the analysis of sorbic acid.

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References

1. Luck E, Sorbic acid as food preservative, *Int Flavours*, 1976, May/June, 122.
2. Vidyasagar K and Arya S S, Stability of sorbic acid in orange squash, *J agric, Fd Chem*, 1983, **31**, 1162.
3. Schmidt H, Colorimetrische Bestimmung der sorbinsaure in Wein, *Dtsch Lebensm — runds*, 1962, **58**, 1.

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QUALITY ASSESSMENT OF WHEAT IN FLOOD AFFECTED AREAS OF SAHARSA IN BIHAR

P.L. SINGH AND S.K. AHMAD
University Department of Botany,
Bhagalpur University, Bhagalpur — 812 007, India.

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Flood affected areas surveyed just after flood and samples of wheat grain and flour were collected from various fair price shops and general stores. They were analysed for aflatoxin contamination and also for nutritive components (Protein, sugar, starch). Out of 60 samples of grain and flour, almost 20% were contaminated with aflatoxin B_1; the level of contamination ranging from 70-339 μg/kg. Deterioration of food constituents like protein, sugar and starch was recorded in all the samples as they were infected with *Alternaria alternata, Aspergillus flavus. Aspergillus niger, Fusarium, Penicillium* spp. etc. Deterioration was, less in grains/flour having no aflatoxin contamination.

Wheat (*Triticum aestivum*) is a staple diet of the majority of population in India. During flood or just after that, the cereal available in the affected areas is mainly wheat grains and flour. This crop is harvested during March and April. These are the pre-monsoons months. Unseasonal rains during harvesting and stacking makes the grain highly receptive for growth of moulds that causes deterioration in essential constituents and mycotoxin elaboration[1]. In the present investigation, a survey was made to assess the nutritional quality of wheat grain/flour available in the affected areas of Saharsa (Bihar).

Samples collected from Saharsa and its suburb were kept in polythene bags. Prior to analysis, they were subjected to hot air treatment for 5-6 hr in an oven at 60°C. After complete drying, grains were powdered and 25 g of the powdered samples were extracted chemically for aflatoxin following the methods of Jones[2]. Same amounts of the flour samples were also subjected to extraction.

Qualitative estimation of aflatoxins was done on TLC plates using solvent system suggested by Reddy *et al.*[3]. Chemical confirmation of aflatoxin was done by spraying trifluoroacetic acid[4] while quantity was calculated spectrophotometrically[5].

Protein, total sugar and starch were estimated by the methods of Lowry *et al*,[6] Dubois *et al*[7] and Snell *et al*[8] respectively.

Aspergillus flavus, A. niger, A. versicolor, Penicillium and *Fusarium* spp. were the commonly associated moulds with wheat grains (Percentage occurrence 50 per cent or more). Other fungi showing higher percentage occurrence (more than 10 per cent) were *Alternaria* spp. *Cladosporium herbarum, Streptomyces, Rhizopus* spp. *Aspergillus ochraceus*, and *A. nidulens.*

Aflatoxin B_1 contamination was detected in 25 and 15 per cent grain samples of fair price shops and general stores respectively (Table 1). The level of contamination in wheat grain ranged from 160-339 μg/kg in samples of fair price shops and 140.8-205 μg/kg in those of general stores. The amount of aflatoxin B_1 in flour was 95-210 μ g/kg (samples of fair price shops) and 70—250 μ g/kg (samples of general stores). The percentage of contamination was, however, less in packet flours of fair price shops (20 per cent) as compared to that of general stores (40 per cent).

A considerable loss in protein, total sugar and starch was recorded in all the samples (Table 2). The level of deterioration was, however, high in aflatoxin contaminated samples, obviously due to higher fungal infection. Protein, total sugar and starch contents were 4-6.9, 1.5-2.1 and 16.2-28.4 per cent respectively in aflatoxin contaminated grains. Grain samples with no detectable aflatoxins were comparatively more nutritious having 5.3-10.0, 2.5-3.8 and 23.5-50.0 per cent protein, total sugar and starch respectively. The contaminated wheat flour showed 5-6 per cent protein, 1.7-2.0 per cent total sugars and 18-29.5 per cent starch where

TABLE 1. OCCURRENCE OF AFLATOXIN B_1 IN WHEAT GRAIN AND FLOUR FROM FLOOD AFFECTED AREAS

Samples tested (No.)	Types of sample	No. of contaminated samples	Aflatoxin B_1 (μg/kg)
	Wheat grain		
20	Fair price shops	5	160.0 — 339.0
20	General stores	3	140.8 — 205.0
	Wheat flour		
10	Packet flour	2	95.0 — 210.0
10	General stores	4	70.0 — 250.0

TABLE 2. NUTRITIVE QUALITY OF NON-CONTAMINATED AND AFLATOXIN CONTAMINATED SAMPLES

Food samples	Types of sample	Protein (%)	Sugar (%)	Starch (%)
	Wheat grain			
Fair price shops	Non-contaminated	5.3—10.0	2.9—3.8	23.5—50.0
	Contaminated	4.0—5.7	1.5—1.8	16.2—27.5
General stores	Non-contaminated	6.3—8.5	2.5—3.3	27.0—42.0
	Contaminated	5.5—6.9	1.7—2.1	26.1—28.4
	Wheat flour			
Packet	Non-contaminated	6.7—7.4	1.5—3.0	28.0—46.0
	Contaminated	5.0—6.0	1.7—2.0	18.0—29.5
General stores	Non-contaminated	5.7—6.5	1.5—2.4	22.6—30.0
	Contaminated	5.0—5.9	1.7—1.9	24.0—28.6

as these constituents were 5.7-7.4, 1.5-3.0 and 22.6-46.0 per cent respectively in flour samples with no aflatoxin contamination.

The level of aflatoxin B_1 contamination either in grain or flour, was high as compared to its tolerance level fixed by WHO[9]. Bhat *et al*[10] have also suggested a maximum tolerance level of 40 μ g/kg for foodstuffs. Consumption of this contaminated wheat is not safe as it creates several health problems including liver cancer.

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References

1. Sinha K K and Sinha A K, Occurrence of Aflatoxin and *Aspergillus flavus* in marketed wheat, *Indian Phytopath*, 1988, **41** 44.
2. Jones B D, *Methods of Aflatoxins Analysis*, Tropical Products Institute. London, 1972, G 70 58.
3. Reddy R V Viswanathan L and Venkitasubramanian T A, Thin Layer Chromatography of aflatoxins, *Analyt Biochem*, 1970, **38**, 568.
4. Stack M E and Pohland A E, Collaborative study of a method for chemical confirmation of the identity of aflatoxins, *J Assoc Off Anal Chem*, 1975, **58**, 110.
5. Nabney J and Nesbitt B F, A spectrophotometric method for determining the aflatoxins, *Analyst*. London, 1965, **0**, 155.
6. Lowry O H Rosenbrough M J Farr A L and Randall R J. Protein measurement with the Folin-phenol reagent. *J Biol Chem*, 1951, **193**, 265.
7. Dubois M Gill K A Hamilton J K Robers P A and Smith F, *Methods in Microbiology*, Academic Pres, London, 1956, 272.
8. Snell F D Snell C T and Snell C A, *Colorimetric methods of analysis*, Van Nostrand Princeton, New Jersey, 1961, 23.
9. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, International Agency of Research on Cancer, Lyon WHO 1976 Vol. 10, 51.
10. Bhat R V, Nagarajan V and Tulpule P G, *Health Hazard of Mycotoxin in India*, ICMR Publication, New Delhi, 1978.

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RELATIONSHIP BETWEEN FAECAL CLOSTRIDIAL COUNTS AND AGE IN FLATULENT INDIVIDUALS

S.K. GARG AND J. VERMA
Department of Microbiology, College of Basic Sciences & Humanities
G.B. Pant University of Agriculture & Technology,
Pantnagar — 263 145. India.

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A significant positive correlation between the age of flatulent human individuals and clostridial counts in their faecal samples was observed. Using age as an independent variable to predict the clostridial counts in flatulent individuals, the computed simple and cubic linear regression equations could account 81 and 97.8% variation in the clostridial counts, respectively. Chi-square test of independence showed that clostridial counts in any group was independent of age.

The foods rich in galacto-oligosaccharides have been reported to be responsible for flatulence[1], owing to the absence of α 1, 6-galactosidase enzyme in the intestinal mucosa of humans. Consequently these oligosaccharides (flatulence factors) identified as melibiose, raffinose, stachyose and verbascose[2,3] are metabolized by intestinal microflora resulting in acid and gas production which actually cause discomfort[4]. The bean substrates and some other vegetables have been reported to have direct relationship with *in vitro* and *in vivo* gas production by intestinal clostridia[5,6]. The present study was, therefore undertaken to examine the relationship between age and intestinal clostridial counts in the gastric cases in human male volunteers.

Sample selection: Survey of gastro-intestinal gas production was conducted on human male volunteers in five age groups viz., 16-20, 21-25, 26-30, 31-35 and 36-40 years. Ten random samples were drawn from each group having otherwise normal health for the past two months. Special care was taken to select those persons only who had not taken any antibiotic for considerable preceding period.

Faecal sample preparation: Sterilized sodium thioglycolate broth containing the following ingredients (per litre) was used for the preparation of faecal samples[7]: bacto peptone (5 g), beef extract (1 g), glucose (5 g), sodium chloride (5 g), yeast extract (2 g), sodium thioglycolate (1 g) and distilled water (1000 ml). One gram fresh sample of stool was aseptically transferred to each screwcapped tube containing 9 ml of carbon dioxide flushed sterilized broth adjusted at neutral pH. After mixing thoroughly, the contents were given heat shock at 80 ± 1°C for 30 min in constant temperature water bath. The tubes were then allowed to stay overnight at 37°C to trigger the germination of clostridial endospores and to kill the vegetative cells of non-spore forming bacteria.

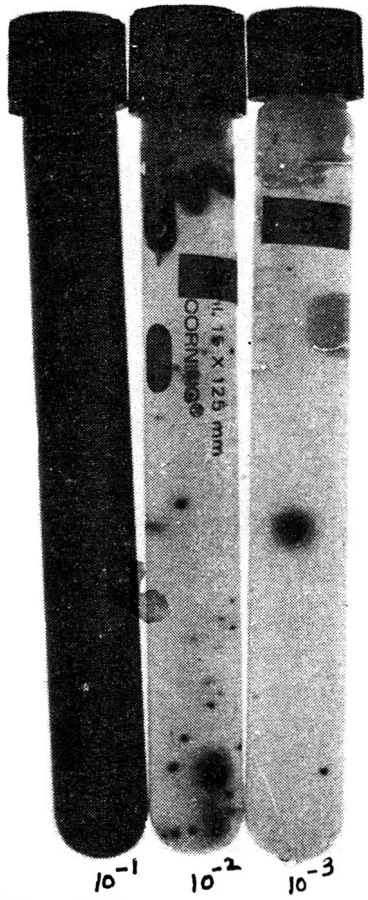

Fig.1. Specific black colonies of clostridia.

The black colony method of Mossel *et al.*[8] was followed for the enumeration of clostridia.

Fig.1. depicts the specific black colonies of clostridia observed in our experiments. General survey revealed that 4, 6, 7, 7 and 8 persons out of 10 had significant number of clostridial colonies in ages from 16-20, 21-25, 26-30, 31-35 and 36-40 years, respectively. The correlation coefficient (r) obtained between age and clostridial counts were 0.848, 0.971, 0.88, 0.908 and 0.828 in 16-20, 21-25, 26-30, 31-35 and 36-40 years age groups, respectively. Chi-square test for homogeneity of correlation coefficients was performed and the calculated Chi-square value was 8.30 at 4 degrees of freedom. Since the calculated Chi-square value was less than the table value (9.49 at p=0.05), all the "r" values are of the same order at 5 per cent probability level. The correlation coefficient for pooled data was significant at 1 per cent probability level (r=0.90 at 30 degrees of freedom which indicated high correlation between age and number of intestinal clostridia or gas production. So, as the age of the individual increased, the gas production tendency also

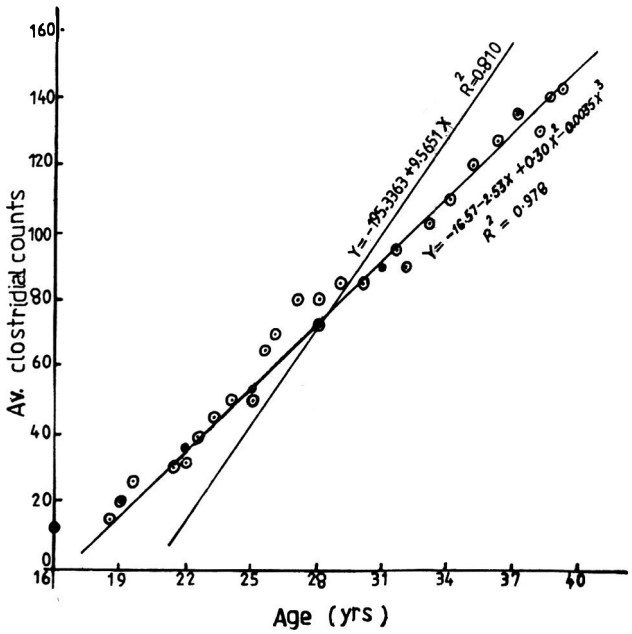

Fig.2. Scatter diagram between clostridial counts and age of the individual.

increased in the persons who were already flatulent. Drasser and Hill[9] reported increased number of clostridia present in faeces of older people.

A scatter diagram between age and the average number of clostridial colonies is shown in Fig.2. In order to predict the number of colonies of clostridia at various ages, the simple linear regression equation was computed. The value of the coefficient of determination for linear regression showed that 81 per cent of the variation in the average number of colonies (Y) could be explained by its linear regression on the age (X) of the flatulent patient. However, the scatter of the points around the fitted regression line had no meaningful values of Y for X less than 21.5 per cent revealed the limitations of the simple linear regression equation for the prediction purpose. Therefore, a cubic regression equation of Y on X was tried, which yielded a far better fit than the simple linear equation, as the value of coefficient of determination increased to 0.9785, leaving only 2.2 per cent of the unaccounted variations in Y due to the factors other than the age of the flatulent individuals.

Therefore, the cubic linear regression was much better for the prediction purpose. In order to see if with the increase in age, was there any change in the number of gas-producing individuals, Chi-square test of independence was performed. The calculated Chi-square value (17.49 at 4 degrees of freedom) was greater than the table value (14.86 at p=0.005) which indicated that the number of gas producing individuals in each age group was independent of the age. Thus, age is not a factor for gastric troubles. However, if one has this trouble, its severity is likely to increase with age.

References

1. Jha K and Verma J, Flatulence producing abilities of different Indian foods and effect of certain spices on flatulence, *Indian J Pathol Microbiol*, 1980, **23**, 279.
2. Rossi M, Germondari J and Casin P, Composition of chick-pea cultivars. Chemical composition, nutritional evaluation and oligosaccharide content, *J agric Fd Chem*, 1984, **32**, 811.
3. Savitri A and Desikachar H S R, A comparative study of flatus production in relation to the oligosaccharide content of some legumes, *Nutr Rep Intl*, 1985, **31**, 337.
4. Rao V S and Vakil U K, Effect of gamma-irradiation on flatulence causing oligosaccharides in green gram (*Phaseolus aureus*), *J Fd Sci*, 1983, **48**, 1795.
5. Richard E A, Steggerda F R and Murata M D, Relationship of bean substrates and certain intestinal clostridia to gas production in dog, *Gastroenterol*, 1968, **55**, 502.
6. Garg S K, Banerjea A C, Verma J and Abraham M J, Effect of various treatments of pulses on *in vitro* gas production by selected intestinal clostridia, *J Fd Sci*, 1980, **45**, 1601.
7. Shapton D A and Board R G, *Isolation of Anaerobes*, Academic Press, London, 1971.
8. Mossel D A A, de Bruin A S, Van Diepen H M J, Vendrig C M A and Zoutewelle G, The enumeration of anaerobic bacteria of *Clostridium* sp in particular foods, *J appl Bacteriol*, 1956, **19**, 142.
9. Drasser B S and Hill M J, *Human Intestinal Flora*, Academic Press, London, 1974.

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FACTORS AFFECTING PELSHENKE TEST AS UNIFORM WHEAT QUALITY SCREENING TECHNIQUE

A.K. SAXENA, K.S. SEKHON, A.K. BAKHSHI AND B. SINGH BAWA
Department of Plant Breeding, Punjab Agricultural University, Ludhiana, India

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The wholemeal disintegration time test (Pelshenke) showed that grinding method, yeast concentration and kneading affected the results considerably. More uniformity was observed in samples ground using quadrumat junior mill. Reduced granulation increased Pelshenke values. The development of gluten through kneading increased the disintegration time as also induced reproducibility into results. Higher yeast concentration of 3.2% was found to reduce disintegration times which were in accordance with the results of other workers conducted on the similar test samples.

Pelshenke test is used the world over as a screening test for assessing the gluten quality in early generation segregating wheat materials. As at this stage of testing the sample number is large and the sample quantity is limited, the test comes to be very handy. The test was first developed by Saunders and Humpheries[1], and was later modified by Pelshenke[2] and Cutler and Worzella[3]. The test was primarily developed to evaluate gluten quality in small samples. The Pelshenke test has been adapted by several laboratories in India. It is observed that Pelshenke values obtained in these laboratories are not comparable. The possible causes of this variation could be the mode of grinding, concentration of yeast, time of dough development, etc. The effect of these variables was studied and the results are presented in this paper.

Grains of four varieties of wheat viz. 'K. Sona', WG 377', 'WL 711' and 'WG 357' and a triticale variety 'TL 319' grown at PAU farm under recommended package of practices were tested for fermentation time test.

The yeast solution of concentration 2.5 and 3.2 per cent (W/V) were made by suspending Bakers dried yeast in distilled water at 30°C for half an hour. All the test varieties were ground separately in different mills viz. Hand grinder (HG), Wiley mill (WM), Junior quadrumat (QM) and Cyclotec tecator (TM) mills. Four sets of trials were conducted with two yeast concentrations and gluten development. Gluten was developed by pressing and subsequent folding of dough ball, before it was dropped in the beaker. The experiments were conducted in triplicate and results presented as the means. The results are as follows:

Effects of method of grinding: In general, the wholemeals obtained from Cyclotec mill recorded higher disintegration time. The hand ground and Wiley mill ground samples showed a very high variability among different runs. The 'WG 357' recorded the highest disintegration time against 'TL 319' recording the least disintegration time with the above modes of grinding (Table 1). The recorded variation in different runs of samples obtained from quadrumat junior mill was minimal indicating its higher reliability. The disintegration times of samples ground with quadrumat junior mill were in accordance with the results obtained by other workers[4].

Effect of yeast concentration: The increase in yeast concentration decreased the disintegration time. The time of disintegration at two concentrations differed significantly from each other (Table 2). As at lower yeast concentration the test is more time consuming, the use of higher concentration is advisable.

Effect of gluten development: The results showed that development of gluten (Table 2) had great bearing on disintegration time. The disintegration time significantly increased with proper kneading of the dough ball. The repeat

TABLE 1. EFFECT OF MODE OF GRINDING, GLUTEN DEVELOPMENT AND YEAST CONCENTRATION (3.2%) ON WHOLEMEAL DISINTEGRATION TIME

Varieties	Without gluten development					After gluten development				
	Hand grinder	Jr Quadrumat	Wiley mill	Cyclotec tecator	Mean	Hand grinder	Jr Quadrumat	Wiley mill	Cyclotec tecator	Mean
K. Sona	46	48	62	92	62	53	64	113	77	77
WL 711	47	54	47	88	59	54	68	61	102	74
WG 357	73	80	96	124	93	93	159	93	138	121
WG 377	52	66	46	67	58	52	75	74	83	71
TL 319	52	61	59	77	62	51	72	78	82	71
Overall mean	74.45									

CD 5% For gluten development = 0.4365
For mode of grinding = 0.6172
For yeast concent. = 0.3913
For varieties = 0.6899

TABLE 2. EFFECT OF MODE OF GRINDING, GLUTEN DEVELOPMENT AND YEAST CONCENTRATION (2.5%) ON PELSHENKE VALUES

Varieties	Without gluten development					After gluten development				
	Hand grinder	Jr Quadrumat	Wiley mill	Cyclotec tecator	Mean	Hand grinder	Jr Quadrumat	Wiley mill	Cyclotec tecator	Mean
K. Sona	89	50	63	105	76	71	59	66	124	55
WL 711	49	51	64	176	60	61	62	59	126	77
WG 357	82	85	116	183	91	104	136	124	143	127
WG 377	48	55	54	85	61	64	67*	61	71	66
TL 319	66	58	60	68	63	61	59	66	69	64
Overall mean	81.39									

CD 5% For gluten development = 0.6676
For mode of grinding = 0.9439
For yeast concentration = 0.3913
For varieties = 1.055

runs were reproducible in kneaded doughs as compared to unkneaded dough balls. Pushman and Bingham[5] observed that the time of kneading did not affect the test significantly.

Various correlations studied were non-significant. However very high values of correlations were observed between Pelshenke value and loaf volume and protein and loaf volume (Table 3). Pelshenke[6] and Winter and Gustafson[7] found a linear relationship between loaf volume and disintegration time in wider range of quality types. A direct correlation between Pelshenke value and other quality characteristics was observed by Cutler and Worzella[8], Walsh and Norman[9] and Dischreider[10].

Since different grinding techniques, yeast concentration and kneading affect the results considerably (as indicated by significance test), utmost care has to be observed to conduct the experiment under uniform conditions. If the uniform conditions are not prevalent in different laboratories conducting the test, then it should be substituted by some other more reliable and simple test like SDS-sedimentation test which is also conducted on the wholemeals.

TABLE 3. QUALITY CHARACTERISTICS OF DIFFERENT TEST VARIETIES

Varieties	Pelshenke value[1] (min)	Protein (%)	Cookie spread (W/T)	Chapati score[2]	Loaf volume (cc)
K. Sona	68	12.2	6.85	29	500
WL 711	68	12.8	5.80	29	500
WG 357	108	11.8	5.40	29	610
WG 377	64	11.9	5.24	26	550
TL 319	65	12.9	6.40	16	430

[1]Mean of 16 samples.
[2]Out of a total of 30 units

References

1. Saunders H A, and Humphries S, The Saunders test (Appendix 1), *J Nat Inst Agric Bot*, 1928, **2**, 34.
2. Pelshenke P, Baking Qualities, *Milling* 1931, 77, 208.
3. Cutler G H, and Worzella W W, A modification of the Saunders test for measuring quality of wheats for different purposes. *J Am Soc Agron*, 1931, **23**, 1000.
4. Bakhshi A K, Sekhon K S, Sehghal K L, and Gill K S, The Chapati making performance of some promising wheat strains, *J Res PAU*, 1979, **16**, 267.
5. Pushman F M, and Bingham J, A reapprisal of the Pelshenke test as a screening technique in the breeding of breadmaking wheats for the United Kingdom, *J Agric Sci Camb*, 1975, **85**, 221.
6. Pelshenke P, A short note for the determination of gluten quality of wheat, *Cereal Chem*, 1933, **10**, 90.
7. Winter O B, and Gustafson A G, Some experience with wheat meal fermentation time test for evaluating soft wheats, *Cereal Chem*, 1934, **11**, 49.
8. Cutler G H, and Worzella W W, The wheat meal fermentation time test of quality wheat as adapted for small plant breeding samples, *Cereal Chem*, 1933, **10**, 250.
9. Walsh J R, and Norman R M, Use of modified Pelshenke test for early generation of wheat quality screening, *Crop Sci*, 1972, **12**, 80.
10. Dischreider A R, Relations entire indica pelshenke les centres er la graulosite des farine, *Bull Mennerie Belge*, 1948, **10**, 113.

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EFFECT OF VEGETABLE OILS ON THE STABILITY OF CAROTENOIDS IN FRIED BENGALGRAM DHAL

A.D. SEMWAL, AND S.S. ARYA
Defence Food Research Laboratory, Mysore — 570 011, India.

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Effect of oils on the stability of carotenoids in fried Bengalgram dhal during storage has been studied. Relatively carotenoids in Bengalgram dhal were more stable when fried in vanaspati than in refined groundnut, sunflower and safflower oils.

In foods, carotenoids are known to undergo co-oxidation with lipids which also affect their storage stability. Based on studies in isolated systems Arya *et al.*[1] reported that during initial stages of storage, both saturated and unsaturated fatty acid esters stabilize carotenoids. After the induction period, polyunsaturated fatty acid esters exert a strong pro-oxidant effect while saturated fatty acid esters continue to exhibit anti-oxygenic effect in the degradation of carotenoids. Bodowski and Bondi[2] also reported that unsaturated vegetable oils accelerate carotenoid oxidation. But Koloman and Gerhard[3] and Ramakrishna and Francis[4] have concluded that in the presence of polyunsaturated fatty acids, carotenoids are more stable because lipid itself accepts free radicals more readily than carotenoids. Saturated and mono-saturated fatty acids being relatively less reactive are unable to accept free radicals and therefore rate of carotenoid degradation is faster in the presence of these fatty acids.

Most of these studies have been conducted in isolated systems and very little information is available on the role of different vegetable oils on the stability of carotenoids in actual food systems. The present paper describes the role of vegetable oils on the stability of carotenoids in fried Bengalgram dhal, a popular Indian snack food.

Good quality Bengalgram dhal was procured from local market and cleaned before use. Vanaspati (Dalda), refined sunflower oil (Flora), refined groundnut oil (Postman) and refined safflower oil (Saffola) were procured in sealed 5-litre polyethylene containers.

Bengalgram dhal (1 kg) was soaked in water (4 l) containing sodium bicarbonate (60 g) for 4 hr. The soaked dhal was filtered through a muslin cloth and tightly squeezed to remove excess water. Soaked dhal was placed in a stainless steel wiremesh basket and fried in vegetable oil (2 kg) heated at 180°C in an aluminium pan. Immediately after placing the soaked dhal in oil, its temperature fell to 100°C and began to increase during frying. When the temperature increased to 130°C, dhal was removed from the oil and allowed to cool at room temperature. One kg lots of dhal were fried in each of the above oils.

One hundred grams of dhal were packed in polypropylene (60 μ) pouches (10 × 10 cm), heat sealed and stored in an incubator maintained at 37 ± 1°C. Initially and after every 15 days, duplicate samples were analysed for total carotenoids, peroxide value, TBA value, free fatty acids and moisture content by the methods described earlier[1].

Moisture content in fried dhals immediately after processing ranged between 2.5 and 3.0 per cent and it remained constant throughout the storage. The total carotenoid contents in dhals processed in different vegetable oils immediately after frying ranged between 23.43 and 23.93 μg/g and differences among samples fried in different oils were not significant. On storage, the carotenoid content in fried dhals decreased considerably; but upto 30 days storage differences in carotenoid levels in dhals fried in groundnut, safflower and sunflower oils were not significantly different (Table 1). Samples fried in vanaspati, however, contained significantly higher level of carotenoids. After 90 days of storage, the carotenoid contents of dhals fried in vanaspati, groundnut oil, sunflower oil and safflower oil were 11.96, 8.48, 7.45 and 6.87 μg/g respectively and the differences among samples fried in four oils were statistically significant at 99 per cent confidence (Table 1). The rate of decrease was highest in those fried in safflower and sunflower oils and lowest in vanaspati clearly indicating the pro-oxidant effect of polyunsaturated glycerides in carotenoid degradation in foods. The relationship between pro-oxidant activity and polyunsaturated fatty acid level in different vegetable oils was however, not linear. Naturally occurring antioxidants in refined vegetable oils may be responsible for this unexpected non-linear relationship. The effect of various vegetable oils on carotenoid degradation seems to be related to their susceptibility to autoxidation during storage. The safflower and sunflower oils which are relatively more susceptible to autoxidation exhibited highest pro-oxidant activity in carotenoid degradation while samples fried in vanaspati, which is relatively more stable to autoxidation, retained largest concentration of carotenoid during storage. Peroxide

TABLE 1. CAROTENOID CONTENT (μg/g) OF BENGALGRAM DHAL FRIED IN VARIOUS OILS ON STORAGE AT 37°C

Storage period (days)	Vanaspati	Sunflower oil	Groundnut oil	Safflower oil
0	23.92 ± 0.04	23.83 ± 0.11	23.93 ± 0.02	23.43 ± 0.06
15	17.95 ± 0.02	15.95 ± 0.02	15.94 ± 0.01	14.94 ± 0.02
30	17.48 ± 0.05	14.48 ± 0.05	14.98 ± 0.00	14.48 ± 0.05
45	15.48 ± 0.05	11.74 ± 0.06	12.99 ± 0.05	10.98 ± 0.05
60	14.48 ± 0.05	9.98 ± 0.04	8.98 ± 0.05	9.48 ± 0.05
90	11.96 ± 0.02	7.45 ± 0.03	8.48 ± 0.05	6.87 ± 0.01

TABLE 2. PEROXIDE VALUE (meq of O_2/kg oil) OF BENGALGRAM DHAL FRIED IN DIFFERENT OILS ON STORAGE AT 37°C

Storage period (days)	Vanaspati	Sunflower oil	Groundnut oil	Safflower oil
0	16.6±0.1	17.6±0.6	18.7±0.1	17.0±0.4
15	16.8±0.2	25.3±0.1	26.8±0.7	30.2±0.7
30	17.2±0.6	44.7±1.2	31.0±0.4	41.6±0.4
45	28.1±0.5	82.7±0.6	45.8±0.1	84.6±1.1
60	28.8±0.2	112.2±1.1	48.5±0.9	104.1±1.7
90	39.7±0.6	241.9±1.6	62.6±0.8	231.8±0.9

TABLE 3. TBA VALUE (mg malonaldehyde/kg) OF BENGALGRAM DHAL FRIED IN DIFFERENT OILS ON STORAGE AT 37°C

Storage period (days)	Vanaspati	Sunflower oil	Groundnut oil	Safflower oil
0	0.09±0.01	0.11±0.01	0.11±0.01	0.11±0.01
15	0.17±0.01	0.24±0.01	0.24±0.01	0.32±0.01
30	0.19±0.01	0.25±0.01	0.25±0.01	0.34±0.01
45	0.20±0.01	0.28±0.01	0.26±0.01	0.39±0.01
60	0.21±0.01	0.34±0.01	0.31±0.01	0.40±0.01
90	0.25±0.01	0.74±0.01	0.39±0.01	0.89±0.01

value and TBA (Tables 2 and 3) were also highest in dhals fried in sunflower and safflower oils and lowest in those fried in vanaspati. Relatively higher peroxide and TBA values in sunflower and safflower oils suggest that free radicals are definitely accepted by polyunsaturated fatty acids in these oils but in the presence of oxygen, this leads to rapid accumulation of hydroperoxides which catalyse carotenoid destruction. In case of oils containing mainly saturated and mono-unsaturated fatty acids due to low reactivity of these fatty acids and concomitantly lesser acceptance of free radicals by oils, the free radicals tend to react among themselves forming stable molecules. This leads to lesser accumulation of hydroperoxides and therefore lesser degradation of carotenoids in carotenoid-oil mixtures. This has been found to be the case in the present study.

The free fatty acid contents of dhals immediately after frying ranged between 1.05 and 1.13 per cent. After 90 days storage, free fatty acid contents were 1.87±0.02, 1.83±0.05, 1.87±0.03 and 1.83±0.05 in dhal samples fried in vanaspati, groundnut, safflower and sunflower oils respectively. Increase in free fatty acids, however, were not related to carotenoid degradation in stored fried dhals.

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References

1. Arya S S, Natesan V, Parihar D B and Vijayaraghavan P K, Stability of ß-carotene in isolated systems, *J Fd Technol*, 1979, **14**, 571.
2. Bodowski P and Bondi A, Autoxidation of carotene and vitamin A. Influence of fat and antioxidant, *Arch Biochem Biophys*, 1960, **89**, 66.
3. Koloman B and Gerhard W, Influence of insecticides in autoxidation process. I. Oxidation of ß-carotene in the presence of lipids and chlorinated hydrocarbon insecticides, *Z Lebensm — Unters Forsch*, 194, **154**, 252.
4. Ramakrishna T V and Francis F J, Coupled oxidation of carotenoids in fatty acid esters of varying unsaturations, *J Fd Quality*, 1979, **2**, 277.

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AFLATOXIN ELABORATION AND NUTRITIONAL DETERIORATION IN SOME PULSE CULTIVARS DURING INFESTATION WITH *A. FLAVUS*

PREMLATA SINGH, SITA BHAGAT AND SYED KHALID AHMAD
University Department of Botany, Bhagalpur University,
Bhagalpur — 812 007, India.

Received 24 November 1988; revised 23 February 1989.

Thirty eight different cultivars of pulses were screened to determine the varietal responses in respect of aflatoxin production. Two of them (Gram var. 'H-208', and Soybean var. 'PK-746') were highly resistant against aflatoxin elaboration while others exhibit different degrees of resistance and susceptibility. Biochemical analyses of healthy seeds showed that the amounts of total phenol and protein were greater in resistant variety while the amount of total sugar was more in susceptible varieties of the same pulses. Deterioration in protein, total sugar and phenols has been noted during infection in all samples, however, in general except soybean, the percentage of deterioration was more in susceptible varieties than resistant varieties.

Pulses contain more protein than any other vegetable products and constitute an important food commodity in India. The investigation in recent past has revealed that pulses are vulnerable to growth of *Aspergillus flavus* and aflatoxin elaboration.[1-4] Aflatoxin is a secondary metabolite[5] of *A. flavus* and is reported to be carcinogenic[6] as well as mutagenic[7] for various warm blooded animals including man. The best method for controlling toxigenic fungi and mycotoxin production is to find out resistant varieties. In the present study, eight varieties each of black gram (*Phaseolus mungo*), Pigeonpea (*Cajanus cajan*), soybean (*Glycine max*), gram (*Cicer arietinum*) and six varieties of green gram (*Phaseolus aureus*) were screened in order to determine the varietal response in respect of aflatoxin production. Furthermore, highly resistant and highly susceptible varieties of each pulse were analysed for deterioration in protein, total sugar and total phenol. An attempt has been made to find out the biochemical basis of resistance of various pulses.

Thirty grams of healthy seeds of each variety were treated with aqueous solution of sodium hypochlorite (sodium hypochlorite : Water, 2:98, v/v) for 10 min in a 250 ml conical flask. The sterilised seeds were thoroughly washed with sterilised distilled water and given deep freeze treatment to check the germination of seeds. The treated seeds were subsequently inoculated with 1 ml spore suspension (approximately 0.5×10^6 spores/ml) of *Aspergillus flavus* which was a potent aflatoxin B_1 producing strain. The flasks were incubated for 15 days at 28 ± 2°C and shaken regularly each alternate day. Aflatoxins from infested seeds were extracted[8] and estimated[9-10] after fifteen days of mycelial growth. Identity of aflatoxin was confirmed chemically by trifluoroacetic acid.[11]

For biochemical analysis, 20 g seeds of the most resistant and susceptible varieties of each pulse were inoculated with the same strain of *A. flavus* following pretreatments mentioned above. The flasks containing inoculated seeds were incubated for 30 days at 28 ± 2°C. After one month of mycelial growth the infested seeds were analysed for protein,[12] total sugar[13] and total phenol.[14]

Two varieties one each of Gram (H_{208}) and Soybean (PK_{746}) were found to be highly resistant against the mould

TABLE 1. AFLATOXIN PRODUCTION IN SEEDS OF DIFFERENT CULTIVARS OF PULSES

Degree of resistance	Amount of aflatoxin B_1 (μg/kg)	Gram	Green gram	Soybean	Black gram	Pigeon pea
Highly resistant	0-20	H 208 BR—78		PK 746		
Moderately resistant	21-100	C 235 SG-2	RAU-6 Pusa baisakhi			H 77-216
Moderately susceptible	101-250	C3-30 BR-77	K 851	PK 595 H 3-19	4H 82-44 4H 82-50 4H 80-9 4H 80-4	H 77-208 UPAS-120
Highly susceptible	>251	ST-4 Radhe	Pusa-119 Garma 12/333 T 44	Sh-2 Bragg I-WH PK-558 Sh-96	4H 28 T-9 4H 80-7 4H 82-41	T 2 1 H 82-1 H 82-12 Prabhat H 83-31

TABLE 2. NUTRITIONAL DETERIORATION IN HIGHLY RESISTANT AND SUSCEPTIBLE PULSE CULTIVARS DURING INFESTATION WITH *A. FLAVUS*.

Pulse cultivars	Protein			Sugar			Phenol		
	Control (mg/100 mg)	Treated (mg/100 mg)	%Deterioration	Control (mg/100 mg)	Treated (mg/100 mg)	%Deterioration	Control (mg/100 mg)	Treated (mg/100 mg)	%Deterioration
Gram (Radha)$^{+}$	15.80	10.25	35.12	3.88	3.33	14.17	0.016	0.013	18.75
Gram (H 208)$^{-}$	20.00	14.43	27.85	2.22	1.96	11.79	0.028	0.025	10.71
Green gram (T 44)$^{+}$	20.54	12.32	40.01	3.88	1.66	57.21	0.011	0.009	18.18
Green gram (RAU 6)$^{-}$	21.60	15.00	30.55	3.05	1.66	45.66	0.019	1.017	10.52
Soybean (SH-96)$^{+}$	27.64	13.80	50.07	1.94	1.66	14.43	0.013	0.011	15.38
Soybean (PK-746)$^{-}$	34.40	21 07	38.75	1.66	1.25	24.69	0.022	0.017	22.92
Black gram (4H 82-41)$^{+}$	17.64	10.80	38.77	2.77	1.13	59.09	0.022	0.012	46.45
Black gram (4H 82-44)$^{-}$	23.60	15.34	35.00	2.49	1.94	22.00	0.024	0.017	29.16
Pigeon pea (H 83-31)$^{+}$	16.79	12.50	25.55	3.88	2.49	35.82	0.014	0.008	42.85
Pigeon pea (H 77-216)$^{-}$	22.54	18.30	18.81	3.60	3.11	13.90	0.020	0.015	25.00

+ Susceptible
− Resistant

growth and aflatoxin production (Table 1). On the basis of the amount of aflatoxin B_1 produced, the varieties were placed in four different categories, highly resistant (0-20 μg/kg), moderately resistant (21-100 μg/kg), moderately susceptible (101-250 μg/kg) and highly susceptible (more than 250 μg/kg).

Biochemical analysis of healthy seeds of different varieties showed that the amounts of total phenol and protein were greater in resistant varieties than susceptible varieties (Table 2). The amount of total sugar was, however, recorded more in susceptible varieties.

Deterioration in protein, phenol and sugar content was recorded during infection in all the samples. In general however, the percentage of deterioration was recorded more in susceptible varieties than resistant ones (Table 2).

The maximum reduction in protein content was recorded in Soybean var. 'Sh-96' (50.07 per cent) while comparatively much lesser amount was deteriorated in Pigeon pea var. '$H_{77\text{-}216}$' (18.8%). Sugar and phenols deteriorated in the range of 11.79 per cent (gram var 'H_{208}') to 57.21 per cent (green gram var. 'T_{44}') and 10.52 per cent (green gram var. 'RAU-6') to 42.85 per cent (Pigeon pea var. '$H_{83\text{-}31}$') respectively in various pulses.

The variation in the amount of aflatoxin B_1 produced by the same fungal strain in different varieties of pulses indicate that the toxin production is germplasm dependent. This may also be due to certain inhibitor present inside the seeds as suggested by Nagarajan and Bhat[15] in case of maize. They identified the inhibitory factor as protein of low molecular weight, which was present in susceptible as well as resistant varieties, but the concentration of this inhibitory protein was significantly high in resistant varieties. Besides, the present study also elucidates the possible inhibitory role of phenolics present in the seeds. These compounds cause retardation in microbial colonization, thus making cultivated plants resistant to infection.[16] Seeds may also react to a potential microbial colonist by producing phytoalexins. These are generally phenolic compounds and are inhibitory to fungus and other microorganisms.[16]

The decrease in protein, sugar and phenolic content of seeds might be due to their enzymatic degradation into simpler compounds which are subsequently utilized by fungi.[17-19] The constituents of the resistant varieties were not easily accessible to deterioration by *A. flavus*, however, a co-relation between mycelial growth and aflatoxin production has not been found. It is much evident in soybean where resistant cultivar was comparatively more vulnerable to mycelial growth, hence its nutritive components were subjected to more deterioration.

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References

1. Kumari P and Sinha K K, Aflatoxin elaboration in mungbean seeds, *Indian Phytopath*, 1987, **40**, 201.
2. Nagarajan V, Bhat R V and Tulpule P G, Aflatoxin production in some varieties of soybean (*Glycine max* L.), *Experientia*, 1973, **29**, 1302.
3. Reddy B N and Nusrath M, Aflatoxin contamination in normal and insect damaged Khesari (*Lathyrus sativus* L.) in Andhra Pradesh, *Curr Sci*, 1983, **52**, 370.
4. Reddy B N and Nusrath M, Aflatoxin contamination in pulses from tribal areas of Medak district, Andhra Pradesh, *Curr Sci*, 1983, **52**, 1024.
5. Detroy R W, Lillehoj E B and Ciegler A, in *Microbial Toxins* (Fungal toxins), by Cigler A, Kadis A and Ajil S J, Academic Press, Inc, New York, 1971, Vol.-VI, 4.
6. Gupta R K, Paul and Bhatia K C, Effect of mycotoxins on animal system, *Proc of the Symp on Mycotoxin in Food and Feed*, Bhagalpur, 1983, 79.

7. Sinha S P, Mycotoxins as mutagens — A need for confirmation using mammalian test system, *Proc of the Symp on Mycotoxin in Food and Feed*, Bhagalpur, 1983, 123.
8. Thomas F, Eppley R M and Trucksess, Rapid screening method for aflatoxins and zearalenone in Corn, *J Assoc Off Analyt Chem*, 1975, **58**, 114.
9. Reddy T V, Viswanathan L and Venkitasubramanian T A, Thin layer chromatography of aflatoxins, *Analyst*, Lond., 1971, **90**, 155.
10. Nebney J and Nsbitt B E, A spectrophotometric method for determining the aflatoxins, *Analyst*, Lond., 1965, **90**, 155.
11. Stack M E and Pohland A E, Colaborative study of a method for chemical confirmation of the identity of aflatoxins, *J Assoc Off Analyst Chem*, 1975, **58**, 110.
12. Lowry D H, Rosebrough N J, Fam A L and Ronald R J, Protein measurement with the Folin phenol reagent, *J Biol Chem*, 1951, **193**, 265.
13. Dubois M, Gilles K, Hamilton J K, Roberts D A and Smith F, A colorimetric method for the determination of sugars, *Nature*, Lond., 1951, 168, 167.
14. Singh M Singh S S and Sanwal G G, A new calorimetric method for the determination of phenols, *Indian J Exptl Biology*, 1978, **16**, 712.
15. Nagarajan V and Bhat R V, Factors responsible for varietal differences in aflatoxin production in maize, *J Agric Fd Chem*, 1972, **20**, 911.
16. Alexander M, *Microbial Ecology*, John Wiley & Sons, Inc, New York, 1970, 64.
17. Houston D F, Straka R P, Hunter I R, Roberts R L and Kester E G, Changes in rough rice of different moisture content during storage of controlled temperature, *Cereal Chem*, 1957, **31**, 143.
18. Cherry J P, Young C Y and Bouchat L R, Changes in protein and free and total amino acids of peanuts (*Arachis hypogeae*) infested with *Aspergillus parasiticus*, *Can J Bot*, 1975, **53**, 2639.
19. Mahadevan A, Sambandam J and Sivaswamy N, Microbial degradation of phenolic substances, *Indian Rev Life Sci*, 1982, **2**, 1.

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RELATIONSHIP BETWEEN POTATO TUBER SIZE AND CHEMICAL COMPOSITION*

J.B. MISRA** AND PREM CHAND

Central Potato Research Station, Modipuram, Meerut — 250 110, India.

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Dry matter, reducing sugars, sucrose, total phenolics and ascorbic acid contents of potato tubers of varying sizes were determined for cvs 'Kufri Chandramukhi' and Kufri Sindhuri'. The values obtained showed linear negative relationship between tuber size and total phenolics and between tuber size and reducing sugars. Between the two cultivars, the reducing sugars and total phenolics, irrespective of size, were more in the cv 'Kufri Sindhuri'.

The chemical composition of potato tubers varies with varieties, area of cultivation, cultural practices, maturity at harvest and storage conditions.[1] The variation in chemical composition within the tuber has also been reported[2,3]. The specific gravity of potato tubers was shown to be directly related with its dry matter and starch content[4-8]. The bulk crop produce normally comprises tubers of varying sizes in various proportions. Therefore, an attempt has been made to study the relationship between tuber size and content of its chemical constituents.

Tubers of variety 'Kufri Chandramukhi' and 'Kufri Sindhuri' were obtained from a demonstration crop raised by following standard package of practices at the Central Potato Research Station, Modipuram, Distt. Meerut. For obtaining sufficient number of tubers of various sizes, tuber weight was used as the index of tuber size. For this, first of all one tuber each with 1.5, 2.0, 3.0, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 cm diameter for 'Kufri Chandramukhi' and 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.5, 6.0, 6.5, 7.0 and 7.5 cm

TABLE 1. CHEMICAL COMPOSITION OF POTATO TUBERS IN RELATION TO TUBER SIZE

Tuber size (cm)	Dry matter (%)	Reducing sugars (mg/100 g)	Sucrose (mg/100 g)	Total phenolics (mg/100 g)	Ascorbic acid (mg/100 g)
		Kufri Chandramukhi			
1.5	18.6	150.0	250.0	44.0	9.6
2.0	19.8	241.6	124.0	40.3	9.0
3.0	20.2	86.0	266.6	34.0	11.1
4.0	19.9	64.0	206.6	27.3	11.8
4.5	20.1	48.6	251.6	28.0	12.5
5.0	20.5	58.3	210.0	27.3	11.6
5.5	20.7	39.0	233.0	25.3	13.1
6.0	20.4	41.6	221.6	22.0	13.3
6.5	20.1	30.6	240.0	22.3	10.8
7.0	19.5	35.0	210.0	20.0	9.4
7.5	19.7	41.3	73.3	22.3	9.4
8.0	18.8	47.0	180.0	26.6	9.9
CD (0.05)	0.3	34.0	37.0	4.8	1.8
r	NS	—0.7789**	NS	—0.8872**	NS
		Kufri Sindhuri			
2.0	18.7	733.0	45.9	100.0	
2.5	19.9	440.3	144.4	88.0	
3.0	19.5	358.0	144.4	85.6	
3.5	20.0	332.3	125.4	88.0	
4.0	21.4	427.0	71.8	74.0	
4.5	20.3	321.0	155.7	67.3	
5.5	20.8	64.6	277.4	63.0	
6.0	21.6	36.3	256.5	69.0	
6.5	21.3	178.0	175.4	69.0	
7.0	20.4	293.0	162.1	67.3	
7.5	19.4	294.0	154.9	62.0	
CD (0.05)	1.6	176.1	93.2	13.7	
r	NS	—0.6912*	NS	—0.8848**	

*Publication No. 1050, CPRI, Shimla (India)

**Present address: National Research Centre for Groundnut, PO : Timbawadi, Junagadh — 362 015, Gujarat

diameter for 'Kufri Sindhuri' were selected. The average of tuber length (heel to rose end) and breadth was taken as the tuber diameter. All the tubers obtained were weighed individually and subsequent selection of additional tubers was done on the basis of tuber weight (± 2.5 per cent) corresponding to the required size. For every size group, three samples of at least 7 tubers each were analysed. Tubers in each sample were cut into halves from heel to rose end. One set of halves was utilised for determination of dry matter and the other for determination of phenolics, sucrose, reducing sugars and ascorbic acid after dicing. The ascorbic acid was extracted from 25 g tissue in 5 per cent m-phosphoric acid and then estimated by the method of Roe[9]. Phenolics and sugars were extracted twice from 10 g diced tissue by refluxing for 1 hr with isopropanol and then making the volume to 100 ml. The phenolics in the extract were estimated by Folin-Denis reagent[10]. The reducing sugars and sucrose were first obtained in aqueous solution after evaporating isopropanol and then etimated by arsenomolybdate[11] and resorcinol[12] reagents, respectively. Dry matter was determined by oven drying 100 g diced tissue at 105°C for 18 hr. Ascorbic acid was analysed only in the tubers of 'Kufri Chandramukhi'. Standard statistical procedures were used for analysing the results.

A negative linear relationship was observed between tuber size and total phenolic contents and also between tuber size and reducing sugar contents (Table 1). The correlation coefficients are given in Table 1. No relationship of dry matter, sucrose and ascorbic acid contents was, however, observed with tuber size in any of the two varieties. Reducing sugar content of potato tubers is used to assess the chipping characteristics of potato tubers[13]. Phenolics of potato tubers are implicated in enzymatic browning reaction which occurs when raw potato tissue is cut or homogenised[14].

The results therefore indicate that for a given variety, the bigger the tuber the better the processing characteristics so far as reducing sugars and phenolics contents are concerned.

References

1. Van Es A and Hartmans K J, in *Storage of Potatoes* by Rastovski A and Van Es A, PUDOC, Wageningen, 1981. 71.
2. Neuberger A and Sanger F, The nitrogen of potato, *Biochem J*, 1942, **36**, 662.
3. Burton G W, *The Potato*, H Veenman and Zonen, Wageningen, 1966.
4. Scheele C Von, Svensson G and Rasmussen J, Die Bestimmung des stevikegehltes und der Trockensubstanz der Kartoffel mit Hilfe des Spezifischen Gewichts, *Landw Versstn*, 1937, **127**, 67.
5. Carlsson H, The relationship between specific gravity, dry matter and starch in potatoes, *Lantbr Hogsk*, 1967, **33**, 695.
6. Verma S C, Sharma T R and Joshi K C, Relation between specific gravity, starch and nitrogen content of potato tubers, *Potato Res*, 1975, **18**, 120.
7. Talley E A, Fitzpatrick T J, Porter W L and Murphy H J, Chemical composition of potatoes, I. Preliminary studies on the relationship between specific gravity and nitrogenous constituents, *J Fd Sci*, 1961, **26**, 351.
8. Killick R J, The analysis of penetro-meter data from a potato breeding programme. *Potato Res*, 1972, **15**, 91.
9. Roe E J, in *Methods of Biochemical Analysis*, Glick D, (Ed) Interscience Pub Inc, New York, 1954, Vol 1, 115.
10. Johnson G and Schaal L A, Accumulation of phenolic substances and ascorbic acid in potato tuber tissue upon injury and their possible role in disease resistance. *Am Pot J*, 1957, **34**, 200.
11. Somogyi M, A new reagent for determination of sugar, *J. biol Chem*, 1945, **160**, 61.
12. Ashwell G, in *Methods in Enzymology* Vol VII, Eds Colowick S P and Kaplan N O, (Eds) Academic Press, New York, 1957, Vol. VII, 73.
13. Talburt W F, Schwimmer S and Burr H K, in *Potato Processing*, by Talburt W F and Smith O, (Eds) The AVI Publishing Co, West Port, Connecticut, 1975, 11.
14. Adler G, Kartoffeln und Kartoffeln und KArtoffelerzengnisse, Grundlagen und Fortschritte der Lebensmittolunter-Suchung 13, Parey, Berlin Hamburg, 1971.

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STUDIES ON FORCE-DEFORMATION PROPERTIES OF FRESH CASSAVA (*MANIHOT ESCULENTA* CRANTZ)

K.C.M. RAJA, K. SUKUMARAN AND T.R. RAM MOHAN
Regional Research Laboratory, (CSIR), Trivandrum — 695 019, India.

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Studies were carried out to understand the mechanical failure of fresh cassava tubers of 'Malayan-4 (M-4)' variety. Compressive load testing was done using Instron Universal Testing Machine (Model 1195). Studies showed that tubers develop visible crack when compressed upto 25% deformation. The energy dissipated at this level of compression was 9 and 11.5J in the case of whole and deskinned tubers respectively.

In the case of plant organs such as roots, tubers, fruits and vegetables, an understanding of their mechanical properties is highly desirable in evolving methods for different post-harvest operations viz. storage, transportation and processing. Studies regarding the fracture characteristics of tissues such as potato, cabbage, apple, etc. have been carried out in detail and reported by different workers[1-4]. Cassava is an important food crop of tropical regions. Fresh cassava tubers are prone to spoilage, considerably faster than tubers such as potato, yam, sweet potato, etc. As no study appears to have been reported regarding the deformation properties of fresh cassava, preliminary investigations were carried out in order to understand the response of fresh tubers to static compression.

Tubers of 'Malayan-4' variety procured locally, were of 7-8 months maturity. They had moisture content between 58 and 60 per cent. Experiments were conducted using Instron Universal Testing Machine (Model 1195) adopting a cross-head speed of 5 mm/min under identical conditions of temperature (26±2°C) and RH (55-57 per cent).

Each tuber was subjected to compression by placing it between two rectangular plates having dimensions of 35×5×1.2 cm (length × breadth × thickness) as shown in Fig. 1. In one set of experiments, samples were compressed till they developed the first crack, while in the second, samples were compressed till they attained 25 per cent deformation measured in terms of reduction in the original diameter of the tuber at the point of compression. All the experiments were carried out using the whole (peels on) and deskinned tubers.

Results of the studies (Table 1) showed a direct correlation between breaking load and weight of the tuber. This was found to be true in the case of whole and deskinned samples. The average values of breaking load (L_{avg}), weight (W_{avg}) and diameter (D_{avg}) of the samples could be correlated with the

TABLE 1. BREAKING LOAD OF FRESH CASSAVA

Group	No. of tubers	Weight (kg) Mean±S.D.	Diameter (mm) Mean±S.D	Breaking load (KN) Mean±S.D
		Whole tubers		
I	17	0.13 ± 0.022 (0.110 — 0.185)	42.37 ± 13.37 (34.0 — 48.4)	0.733 ± 0.086 (0.60 — 0.85)
II	19	0.235 ± 0.024 (0.200 — 0.285)	46.52 ± 5.283 (36.8 — 53.0)	1.11 ± 0.090 (1.00 — 1.38)
III	10	0.347 ± 0.002 (0.315 — 0.435	51.5 ± 4.460 (41.5 — 61.0)	1.17 ± 0.130 (1.05 — 1.85)
		Deskinned tubers		
I	21	0.144 ± 0.020 (0.100 — 0.190)	40.12 ± 4.480 (30.0 — 48.4)	0.77 ± 0.086 (0.60 — 0.98)
II	21	0.238 ± 0.024 (0.200 — 0.290)	44.7 ± 5.944 (31.0 — 55.5)	1.131 ± 0.160 (0.95 — 1.38)
III	19	0.337 ± 0.034 (0.300 — 0.420)	46.06 ± 2.59 (40.0 — 53.0)	1.120 ± 0.210 (0.94 — 1.45)

Figures in parentheses indicate range

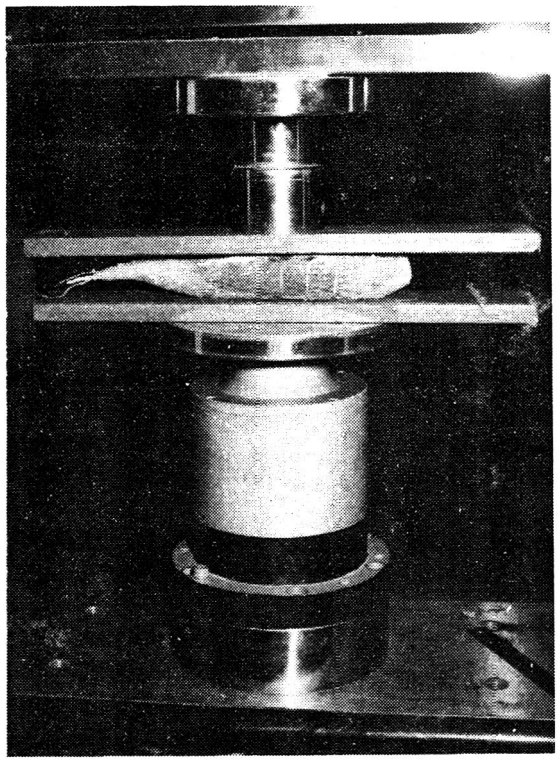

Fig.1: Compressive load testing of whole cassava (M-4 variety) using Instron UTM Model 1195

corresponding calculated values for load (L). Weight (W) and diameter (D) by means of the following equations (a) and (b) in each group for whole and deskinned samples respectively.

Group I (a) $\left[\frac{L}{L_{avg}}\right] = \left[\frac{D}{D_{avg}}\right]^{0.4} \left[\frac{W}{W_{avg}}\right]^{0.2}$ Mean standard error 11.4 per cent

(b) $\left[\frac{L}{L_{avg}}\right] = 0.95\left[\frac{D}{D_{avg}}\right]^{-0.7} \left[\frac{W}{W_{avg}}\right]^{0.8}$ Mean standard error 9.9 per cent

GroupII (a) $\left[\frac{L}{L_{avg}}\right] = 1.05\left[\frac{D}{D_{avg}}\right]^{-0.3} \left[\frac{W}{W_{avg}}\right]^{0.15}$ Mean standard error 11.6 per cent

(b) $\left[\frac{L}{L_{avg}}\right] = \left[\frac{D}{D_{avg}}\right]^{0.3} \left[\frac{W}{W_{avg}}\right]^{0.7}$ Mean standard error 11.3 per cent

Group III(a) $\left[\frac{L}{L_{avg}}\right] = 0.95\left[\frac{D}{D_{avg}}\right]^{-1.6} \left[\frac{W}{W_{avg}}\right]^{1.35}$ Mean standard error 9.1 per cent

(b) $\left[\frac{L}{L_{avg}}\right] = 0.95\left[\frac{D}{D_{avg}}\right]^{0.65} \left[\frac{W}{W_{avg}}\right]^{0.75}$ Mean standard error 13.8 per cent

Results of the second set of experiments (Table 2) could fit into the following mathematical expressions.

(a) $\left[\frac{L}{L_{avg}}\right] = 0.95\left[\frac{D}{D_{avg}}\right]^{0.5} \left[\frac{W}{W_{avg}}\right]^{0.85}$ Mean standard error 9.06 per cent

(b) $\left[\frac{L}{L_{avg}}\right] = 0.95\left[\frac{D}{D_{avg}}\right]^{0.45} \left[\frac{W}{W_{avg}}\right]^{1.05}$ Mean standard error 9.01 per cent

In all the above equations, the possitive coefficient of W suggests a direct relationship between the weight of the tuber and breaking load. The influence of the tuber diameter (D) to breaking load was not very consistant. In certain groups i.e. Group I(b), Group II(a), and Group III(a) tuber diameter showed an inverse relationship, as reflected from their negative coefficient values.

At 25 per cent deformation, fresh tubers showed visible crack and released watery exudate, confirming damage of the intact tissue. The values of the energy dissipated, as calculated from the area of load deflection curve was 9.0 and 11.5J for whole and deskinned tubers respectively. In the case of vegetable tissues such as potatoes, it has been mentioned that they undergo cracking when the energy value reached beyond a critical point. In the case of two varieties of potatoes, the above critical values have been reported to be 0.9 and 1.6J[5]. From the relatively higher values dissipated during compression upto 25 per cent deformation, it could be presumed that fresh cassava offers relatively higher resistance to static compression than tissues such as potatoes,

Authors express their gratitude to Dr. A.D. Damodaran, Director, Regional Research Laboratory for his encouragement and support.

TABLE 2. COMPRESSIVE LOAD AT 25 PER CENT DEFORMATION LEVEL OF FRESH CASSAVA

Batch	No. of tubers	Wt of the tuber (kg) Mean±S.D.	Diameter (mm) Mean±S.D	Compressive load (KN) Mean±S.D
Whole tubers	15	0.346 ± 0.03 (0.315 — 0.370)	48.6 ± 4.6 (46.0 — 56.6)	2.32 ± 0.62 (2.00 — 2.85)
Deskinned tubers	15	0.337 ± 0.031 (0.290 — 0.426)	46.13 ± 5.7 (40.0 — 49.0)	2.16 ± 0.65 (1.75 — 2.70)

References

1. Holt J E and Schoorl D, Cracking and energy dissipation in cabbages, *J Texture Studies*, 1983, **14,** 99.
2. Schoorl D and Holt J E, A practical method for tensile testing of apple tissues, *J Texture Studies*, 1983, **14,** 155.
3. Pitt R E, Stress-strain and failure characteristics of potato tissue under cyclic loading, *J Texture Studies*, 1984, **15,** 131.
4. Gray D and Hughes F C, *The Potato Crop*, by Haris, P.M. (ed), Chapman and Hall, London, 504.
5. Schoorl D and Halt J E, Cracking in potatoes *Texture Studies*, 1983, **14,** 61.

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CARBOHYDRATE CONTENT AND COMPOSITION OF SELECTED BEVERAGES

N.S. DODD AND M. GUPTE
Department of Post-graduate Studies and Research in Home Science
S.N.D.T. Women's University, Santa Cruz (West),
Bombay — 400 049, India.

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High performance liquid chromatography was used for identification and quantitation of sugars. The sugars identified in carbonated beverages and fruit based beverages were mainly glucose and fructose. Sucrose was also identified in some carbonated beverages and it was the predominant sugar in fruit based beverages. Glucose was the predominant sugar in most carbonated beverages where its concentration ranged from 3.4 to 7.6 g/100 g. Milk based beverages contained only lactose and sucrose. The total sugar content of fruit based beverages was significantly higher than carbonated and milk based beverages.

Soft drink beverages represent one of the largest single industrial uses of refined sugars. Recently, there has been a tremendous increase in the soft drink consumption because of rapid growth and development of the beverage industry in India[1] and their ready availability.

Soft drinks are about 90 per cent purified water and the other major ingredients are — sugar, flavourings, colours, acids and carbon dioxide. Sugar contributes to the sweetness necessary to balance the various ingredients and gives body and mouthfeel[2].

As there is limited information available in the literature about the content of sugars in various soft drink beverages available in the market, this study was undertaken to determine sugars in beverages.

Soft drink beverages were purchased from different markets; bottles or tetrapacks of the same beverage were pooled together. Laboratory analysis in duplicate was carried out on aliquots taken from pooled samples.

The samples of soft drinks were prepared by vacuum degassing using a magnetic stirrer while the fruit juice drinks and milk based beverages were homogenised using a blender. The soluble carbohydrates were extracted by the method of Vidal-Valverde *et al*[3]. The sample was boiled under reflux with 80 per cent ethanol. It was filtered through a no. 4 sintered glass funnel and the residue was extracted twice with 80 per cent ethanol until a negative Molisch test was obtained. All the ethanol extracts were evaporated under vacuum and the residues dissolved in distilled water and extracted with diethyl ether to remove colourants and other materials which could interfere in the analysis of sugars. The aqueous phase was evaporated to dryness under vacuum and the residue

TABLE 1. SOLUBLE CARBOHYDRATE (g/100g) COMPOSITION OF SELECTED COMMERCIAL SOFT BEVERAGES

Beverages	pH	Fructose	Glucose	Sucrose	Lactose	Total sugar
			Carbonated			
Thums Up	2.32	3.74	4.60	—	—	8.34
Double Cola	2.40	3.84	4.49	—	—	8.33
Campa Cola	2.35	3.64	4.74	—	—	8.38
Lemonade	2.73	2.87	3.12	—	—	5.99
Limca	2.70	4.96	4.79	—	—	9.75
Sprint	3.10	3.95	4.17	4.87	—	12.99
Rim Zim	2.43	1.66	3.49	—	—	5.06
Gold Spot	2.65	7.97	7.60	—	—	15.57
Campa	2.65	6.82	6.79	1.19	—	14.80
			Fruit based			
Frooti	3.41	6.57	5.86	4.20	—	16.63
Mangola	3.18	6.48	4.87	—	—	11.35
Volfruit Mango	2.93	5.46	9.06	0.35	—	14.87
Volfruit Orange	2.93	5.06	9.35	0.46	—	14.87
Pina Colada	3.03	6.27	5.26	—	—	11.53
Tango	3.15	7.93	7.52	0.74	—	16.19
Appy	3.33	8.26	6.09	2.94	—	17.29
			Milk based			
Energee	6.39	—	—	1.87	4.31	6.18
Great Shake	6.33	—	—	9.13	—	9.13
Amul Shake	5.89	—	3.41	—	2.05	5.46

finally dissolved in a fixed volume of distilled water. This was purified by using Sep Pak C_{18} reverse phase cartridges. The purified solutions were diluted with the mobile phase (acetonitrile/bidistilled water — 85/15, v/v) and filtered through a Millipore HA membrane. Carbohydrate composition was determined by using HPLC (Waters Associates Model) equipped with a 600 A pump and R-401 refractive index detector. The column used was Waters carbohydrates column.

The quantitation in the unknown samples was accomplished by comparing the sample peak area to the corresponding standards.

The mean carbohydrate content and composition of 20 soft drinks analyzed are shown in Table 1. It was noted that all the beverages were acidic but the carbonated (pH-2.3 to 3.1) and fruit based beverages (pH-2.9 to 3.4) were significantly more acidic than milk based beverages (pH-5.9 to 6.4). These values agree with the earlier reports.

The different sugars identified in the beverages were fructose, glucose, sucrose and lactose. Martin Villa *et al.*[4] also observed that most of the soft drinks contained glucose, fructose and disaccharide sucrose.

The total sugar content of 3 carbonated beverages — Sprint, Gold Spot and Campa (12.99, 15.57 and 14.80 g/100 g of sample respectively) were significantly higher than the levels observed in Cola beverages, Lemonade, Limca and Rim Zim. Our results are similar to those reported by Scott and Trick[5].

It was observed that glucose was the main carbohydrate present in most of the carbonated beverages, followed by fructose. Only 2 of the carbonated beverages — Sprint and Campa contained sucrose. Sprint which contained maximum amount of sucrose also had a slightly higher pH (3.10) compared to other carbonated beverages (2.32—2.73).

The total sugar content of the fruit based beverages was higher than in carbonated and milk based beverages, fructose and glucose were the 2 sugars present in all the fruit based beverages. Mangola and Pina Colada contained no sucrose, while amounts less than 1 g/100g were observed in Volfruit Mango, Volfruit Orange and Tango. The highest sucrose content was observed in Frooti (4.2g/100g) but it constituted only 25 per cent of the total sugars. Fructose was the main sugar in Frooti, Mangola, Pina Colada, Tango and Appy, whereas glucose was the predominant sugar in Volfruit Mango and Orange.

In the milk based beverages analysed, only Energee and Amul shake had lactose. The lactose content of Energee was 4.31 g/100 g or 70 per cent of the total sugars while Amul Shake contained 2.05 g/100g or 38 per cent of the total sugars. The other sugars in these 2 beverages differed. In Energee sucrose constituted the remaining 30 per cent of the total sugar while in Amul Shake, glucose was the predominant sugar (62 per cent). As Great Shake is soy based, it contained only sucrose (9.13 g/100g) but the total sugar content in it was significantly higher than the levels observed in Energee and Amul Shake.

From the results of the study, it was observed that there were significant variations in the sugar content of different beverages which ranged from 5.06 to 17.29 g/100 g and constitutes a significant source of energy.

The authors are indebted to Dr. A.N. Bhat, Dr. V.R. Shiratti and Mr. V.N. Nair for their technical guidance and assistance in carrying out the carbohydrate analysis at Hindustan Lever Research and Development Centre.

References

1. Manay N S and Shadaksharaswamy M, *Foods, Facts and Principles*, Wiley Eastern Limited, 1987. 165.
2. Woodroof J G, and Phillips G F, *Beverages: Carbonated and Non-carbonated.* The AVI Publishing Company, Inc Westport. Connecticut. 1974.
3. Vidal-Valverde, Valverde S, and Martin-Villa C. High performance liquid chromatographic determination of soluble carbohydrates in commercial drinks, *J Sci, Fd Agric 1985* **36**, 43.
4. Martin-Villa C, Vidal-Valverde C and Rojas Hidalgo, E. Soluble sugars in soft drinks, *Am. J Clin Nutr* 1981, **34**, 2151.
5. Scott F.W and Trick, K D. Carbohydrate content and caloric value of carbonated soft drinks, *Fd Chem.* 1980, **5**, 237.

OBITUARY

Dr. M.S. Narasinga Rao, aged 65 years, former Vice-President(HQ) AFST(I), passed away due to cardiac arrest on 2nd January 1990. He was the former Head of the Department of Protein Technology, CFTRI, Mysore and a renowned protein Chemist.

Mr, R. John Chandrashekar, a staff member of the AFST(I) Office, breathed his last suddenly on the 5th January 1990 at the age of 54 years. He had served the AFST(I) for over 15 years.

The association records the services rendered by them and sends its condolences to the members of the bereaved families.

BOOK REVIEWS

Dairy Engineering Technology and Engineering of Dairy Operation + SI Version: by C.P. Ananthakrishnan and N.N. Simha, Laxmi Publications, 2691, Baradari Bathmaran, New Delhi-110 006, 1987; pp 403; price: Rs.150/-

Dairy Technology is not a distinct Branch of Engineering like Chemical, Mechanical, Electrical, Civil etc., but combines all the major branches for this particular purpose. The Authors have introduced SI systems in Dairy Engineering and also combined some basic knowledge of Chemical, Microbiological and technological characteristics of dairy products.

The first five chapters, viz: Introduction, Basic concept, Workshop technology, Engineering Materials, and Fluid Flow present, the basic needs of the Dairy Technology concept. The chapters have been distinctly enlightened towards the requirements of the Engineering practices, and the needs of the workshop in the line of Dairy Engineering.

The sixth, seventh and eigth deal in pumps and piping, with the various types of pumps used in the dairy engineering, their fundamental operations, maintenance, trouble shooting problems and solution. Water technology provides sufficient notes and guidelines on the requirements of water for dairy plants, their system designs, effluents normally encountered with, their treatment, etc. Low temperature system, has been mainly availed to enlighten the importance of Refrigeration in the field of Dairy Engineering, various methods of Refrigeration systems in practice, and their practical application in this particular field.

The ninth chapter 'Some Unit Operation' deals with the various methods commonly employed in the process emphasising on the single step operations in the chain of production. The unit operation dealt with are, mechanical agitators, screening systems, sedimentation, separators, clarifiers, homogenization equipments, their usage, and applications. The tenth chapter has been devoted for the Electrical Technology, required for the Dairy motors, alternators, capacitors, etc:. The eleventh chapter' Heat flow and Heat exchanges' has been provided for the requirements of the heat load calculations normally associated in the Dairy Technology, different types of heat flows, design of the Heat Exchangers etc 'Steam Technology' reveals the type of Boilers used in the concerned field, their accessories, common methods of installation procedures, troubles encountered and their method of solving.

Next five chapters namely, Milk chilling equipments, Tanks, Fluid Milk Plant, Butter, Cheese and Ice cream equipments, Evaporators and Driers, have been exclusively devoted for the Dairy Technology in detail for the relevant applications. The following equipments and procedure have been enlightened viz:, different types of milk chilling plants, Plant & Machinery required for the Milk Chilling Centres, Storage tanks, Mobile tankers for the transport of milk, General features in the construction of the Dairy equipment, comparative appraisal of tanks, and vessels, pasteurisation process, different types of heat exchangers, and the associated equipment, ultra high temperature process equipment, different type of washers, conventional batch process of Butter making equipments, cheese making equipments, different methods of evaporators, driers, etc:. These chapters deal with the actual technology that is required for the Dairy Engineering. while the previous chapters have been devoted for the utilities of the Plant & Machinery in the Dairy Engineering

The eighteenth chapter deals with Instrumentation Technology used in the Dairy Engineering. The basic concepts of the various Instruments used, their construction features, maintenance, etc:, have been dealt in detail. The next chapter 'Plant Maintenance' has been exclusively devoted for the Plant practices for the best maintenance of the Dairy Plant, which include both preventive, and Breakdown maintenance. Next chapter 'Engineering Graphics' has been exclusively devoted for the Graphics that are normally used in the designs of the Dairy plants. with necessary information for the various code practices, convention, nomographics, etc:.

The twentyfirst chapter deals with Dairy Plants Layout and Design, factors governing the layouts, economics of the plants, and selection of the plants, operational schedules, methods of the construction of the plants, including flooring, lighting, draining and effluent treatments. The next chapter "Mechanised form operations" deals with various machinery generally covered under automatic operation, including conveyors of different forms, and the advantages of the automation of the systems. The last but one chapter 'Solar Energy Systems' deals with the different methods of utilising the solar radiation for the beneficial purpose in the field of Dairy Engineering both in heating and cooling systems.

The last chapter 'Regulation' followed by appendix provides the necessary standards used in the field, Govt Acts, Factory Acts etc, safety codes and practices, and different acts that come in the field of Dairy Technology which are required for the construction, operation, and maintenance of the Dairy Plants.

The text book provides the reader, engineer in practice and technologist a consolidated information in the field of Dairy Technology and is a good addition for any library.

P.K. RAMANATHAN
FOOD PLANT CONSULTANT, BANGALORE.

Hand Book on Anaerobic Fermentations: Edited by Larry E. Erickson and Daniel Yee Chak Fung, Marcel Dekker, Inc. 270, Madison Avenue, New York, N.Y 10016, 1988; pp 880; Price: Bound Illustrated $149.75 (US and Canada); $179.50 (All other countries).

This book is the third volume in the continuing series of books on Bioprocess Technology by the publishers. The book has 23 chapters in 6 sections written by specialists and covers the progress made in the different areas of Anaerobic microbiology. The first section has 3 chapters dealing with the methodology of culturing anaerobes, genetics and methodology of genetic manipulation of some anaerobes especially the Clostridia and information about some important anaerobic bacteria useful in Industry. Section II has 7 chapters dealing with process biochemistry, kinetics of growth and product formation, the role of the redox potential in anaerobic systems, the role of the membrane and the membrane processs in anaerobes, the role of proton motive force in anaerobic bacteria and aspects of product inhibition in anaerobic fermentations. It also has a chapter on one of the important anaerobic fermentations namely the acetone-butanol fermentation.

Section III deals with the bioenergetics and yields in anaerobic fermentations and product yields from such fermentations in the presence of electron acceptors other than oxygen. Section IV deals with data collection and analysis. Anaerobic environments are generally represented by mixed culture interactions and this aspect is well covered in section V with examples of mixed culture fermentations in nature. The interaction between hydrogen, formate and methanogens is also dealt with. Although not in the realm of anaerobic fermentations, this section also has a chapter on engineering flavour into fermented foods that is not only informative but also of practical use to food biotechnologists. Section VI deals with the design and application of anaerobic systems with special reference to degradation of toxic wastes, the design and functioning of various bioreactors, the start up dynamics and the control of anaerobic fermentations. Monitoring digestor stability and function is a major problem in anaerobic systems and this has been adequately dealt with in this section. Each chapter has very pertinent recent references for the benefit of the reader. The book can serve an excellent purpose both for the academics as well as for research scientists.

P. TAURO
H.A.U., HISSAR.

Fatty Acids in Industry: Edited by Robert W. Johnson and Earle Fritz, Marcel Dekker Inc, 270, Madison Avenue, New York 10016, 1989; pp: 667; Price: $150 (US and Canada); $180 (All other countries).

The book covers four important aspects about fatty acids such as Processes, Properties, Derivatives and Applications. It contains 23 chapters, dealing exhaustively on raw materials for fatty acids; Fat splitting and glycerine recovery; separation of fatty acids; Esterification; Polymerisation of fatty acids; Nitrogen derivatives of fatty acids; Polyoxyethylene esters of fatty acids; Dibasic fatty acids; Fat based emulsifiers; Fatty acids in synthetic Lubricants; Fatty acids in oil · field chemicals; Fatty acids in metal working Fluids; Fatty acids and amines in floatation; Fatty acids in Textiles; Fatty acids and Rosin in paper; Fatty acid derivatives in cosmetics; Pollution control in fatty chemical industries; and finally Biological and Toxicological properties of fatty acids and their derivatives.

Under raw materials, attention is drawn to Castor oil, Coconut oil, Soybean oil, Till oil and Tallow as some important raw materials for fatty acids, The fatty acid composition of the fats from these raw materials has been provided so that depending on the need of a specific fatty acid, raw material can be selected in addition to common fat splitting processes, Detail treatment has been given to pressure, catalytic high temperature, high vacuum and enzymatic splitting of fats. A detailed account of seperation of fatty acids has been given. Detailed description of plant and machinery for continuous distillation of fatty acids, which will be of much use in fatty acids industries have been given. After detailed account of hydrogenation and esterification, interesting aspects of Polymerisation of fatty acids have been thoroughly discussed. A certain amount of fundamental aspects of Polymerisation has been dealt with, by describing analysis and structure elucidation of polymerised products such as dimer, trimer etc. This helps in selection of polymers for their end use such as corrosion inhibition or other coatings. Polyoxyethylene esters of fatty acids as nonionic surfactants with low cost and low foaming properties find application in every day life. A detailed account of fatty alcohols has been made as some of them find use as plant growth hormones. A detailed course, including synthetic routes, manufacturing methods of branched chain fatty acids, which play a vital role in the manufacture of chemicals, coatings and metallurgy have been presented. These and other well documented informations about dibasic fatty acids which find useful applications in plasticizers, engine lubricants, adhesives and inks, together

with an exhaustive bibliography form a well distilled concentrate about fatty acids.

The Book contains useful information spread over 658 pages together with a good subject index. Nicely printed, with good get up, the book will definitely not only attract the attention of the readers, but also arrest it, when it is on the book shelf.

The book is a worth possessing asset for those people and industry dealing with Fatty Acids.

I felt very sorry on reading that one of the Editors Earle Fritzdied of Lung Cancer. Indeed, it is a great loss to Union Camp Corporation.

J.R. RANGASWAMY
C.F.T.R.I., MYSORE.

Dithiocarbamate Pesticides, Ethylenethiourea (ETu) and Propylenethiourea: A General Introduction: Environmental Health Criteria 78, Published by W.H.O., Geneva, 1988, pp: 140; Price: Sw. fr.15.

The monograph presents a general introduction to dithiocarbamates, ETu and PTu. The contents cover the following aspects about dithiocarbamates, ETu and PTu. Chemical properties and analytical methods; Source of human and environmental exposure. Environmental transport, distribution and transformation. Environmental levels and human exposure; Kinetics and metabolism; Effects on organisms in the environment; Effects on experimental animals and in vitro test systems; and Effects on man.

During documentation of analytical methods, the Task Force of WHO appears to have missed some of the extremely sensitive and easy methods as it has mentioned only an old method, which is based on the decomposition of dithiocarbamates. The method cited by the Task Force cannot be adopted tc micro-determination of residues due to inconsistencies and non-reproduceability.

Under section A: exhaustive account about dithiocarbamates regarding man-made source, Biotransformation, Photodegradation, fate of these compounds in foods before and after cocking, Market basket studies; Absorption, distribution and excretion have been detailed. The different routes of metabolism of these compounds in soil, plant and human have been very well brought out. The contents also deal with Toxicological aspects of the compounds for aquatic and terrestrial creatures, in addition to their effects on Reproductivity and Carcinogenicity. For all these effects, a comprehensive mode of action has been presented.

Under section B, the monograph covers all the above aspects with respect to ETu and PTu.

The reference section of the monograph, although exhaustive, is by no means complete as some of the recent works on their role in food preservation and connected probems have not found places; may be they were not available to them.

However, the Task Force of WHO deserves compliments for having compiled a monograph which finds a useful place on the shelf of any Library.

J.R. RANGASWAMY
C.F.T.R.I., MYSORE.

INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (in triplicate) should be typewritten in double space on one side of bond paper. They should be complete and in final form. The paper should not have been published or communicated for publication anywhere else. Research Notes should clearly indicate the scope of the investigation and the salient features of the results. Only *invited* review papers will be published.

2. The typescript should be arranged in the following order: Title (to be typed in capital and small letters for Research Papers and all capitals for Research Notes), Authors' names (all capitals) and Affiliation (capitals and small letters). Also give a short running title not exceeding 10 words as a footnote.

3. **Abstract:** The abstract should indicate the principal findings of the paper and typed in single space. It should not be more than 200 words and in such a form that abstract ng periodicals can readily use it.

4. Use names of chemical compounds and not their formulae in the text. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Footnotes especially for text should be avoided as far as possible.

5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables should be typed on *separate* sheets. Nil results should be indicatec and distinguished clearly from absence of data, which is indicated by '——' sign. Tables should not have more than *nine* columns.

6. **Illustrations:** Graphs and other line drawings should be drawn in Indian ink on tracing paper or white drawing paper preferably art paper not bigger than 20 cm (OY axis) × 16 cm (OX axis). The lettering should be twice the size of the printed letter. Photographs must be on glossy paper and must have good contrast; **three copies** should be sent.

7. **References:** Names of all the authors along with title of the paper should be cited. Abbreviations such as *et al.*, *ibid*, *idem* should be avoided. References should be serially numbered as superscripts in the order they are cited in the text and the same order should be maintained in the reference list. The titles of all scientific periodicals should be abbreviated in conformity with the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.

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   (*a*) *Research Paper*: Jadhav S S and Kulkarni P R, Presser amines in foods, J Fd Sci Technol, 1981, 18, 156.

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

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

   (*d*) *Proceedings, Conferences and Symposia Papers*: Nambudiri E S and Lewis Y S, Cocoa in confectionery, Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India, Mysore, May 1979, 27.

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

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

8. Consult the latest issue of the *Journal* for guidance. For "Additional Instructions for Reporting Results of Sensory Analysis" see **issue No. 1** of the Journal.

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