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(INDIA)

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RESEARCH PAPERS

Endo Polygalacturonase Lyase of *Streptomyces thermovulgaris* CR 42

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Streptomyces thermovulgaris CR42, an obligate thermophile isolated from forest soil, elaborated endopolygalacturonase lyase (endo PGL) as the only pectin degrading enzyme. Complete degradation of pectin added to the medium at 1% level was observed within 48 hr; aeration doubled the rate of enzyme production and 55°C was the optimal temperature for growth and enzyme production. Maximal enzyme was produced when the initial pH of the medium was 7.6, while no enzyme was elaborated at acidic pH (below pH 6.5.) Precipitation of the endo PGL by ammonium sulphate saturation at 70% gave 15-fold purification with 72% recovery; on further fractionation on DEAE cellulose column by stepwise increase in molarity of phosphate buffer (pH 7.0) a single active PGL peak was obtained in 0.2M buffer. Polyacrylamide gel electrophoresis of the purified fraction indicated the enzyme to be a homogeneous protein. The enzyme had a molecular weight of 37,200; it was activated by $CaCl_2.2H_20$ (0.5 mM) and was markedly suppressed by EDTA.

The production of pectolytic enzyme by mesophilic actinomycetes and their role in bioconversion of pectic polysaccharides in natural substrates has been studied by Billimoria and Bhat[1], Billimoria[2] and Kaiser[3]. Sato and Kaji[4] isolated from a plant strain of *Streptomyces fradiae* which produced pectate lyase. Although, thermophilic actinomycetes have been intensively investigated for protease[5,6], amylase[7,8] and cellulase[9-11] production, the pectinolytic activity of these organisms has not been studied.

The present paper describes the production and characteristics of endo polygalacturonase lyase (PGL) elaborated by an obligate thermophile *Streptomyces thermovulgaris* CR42.

Materials and Methods

Streptomyces thermovulgaris CR42 was isolated from forest soil collected from Borivili National Park near Bombay. It was maintained on McCarty's medium[12] at 28°C and preserved in sterile soil. The culture grew optimally at 55°C, sporulated within 48 hr and was identified according to the Bergey's Manual of Determinative Bacteriology[13].

Apple and citrus pectin (Green ribbon, 60-65 per cent esterified) were gifts from Obipectin Ltd., Switzerland. Pectic acid was prepared from citrus pectin as described by Rombouts[14]. Standard proteins, cytochrome C (type III from horse heart; 12,270), ribonuclease (13,500) lysozyme (17,500), trypsin inhibitor (21,500), ovalbumin (45,000) and bovine serum albumin (67,000) were purchased from Sigma Chemicals Ltd., U.S.A.

PGL assays: The enzyme was assayed both by estimating the formation of reducing groups and by viscosimetry. For reducing group assay, a mixture of 2.5 ml of 1 per cent pectin or pectic acid and 2.0 ml of 0.2M glycine-NaOH buffer (pH 9.6) was incubated with diluted enzyme (0.5 ml) at 55°C for 2 hr and the reducing groups formed were estimated by Somogyi's[15] method. The optical density was determined at 550 nm using Klett Summerson photoelectric colorimeter (green filter). One unit of PGL activity is defined as amount of the enzyme which releases 1 μ mole equivalent of reducing sugar/min. from 0.5 per cent pectin/pectate at 55°C and pH 9.6.

For viscosimetric assay, 5.0 ml of 1 per cent pectin was mixed with 4.0 ml of 0.2M glycine-NaOH buffer (pH 9.2) in Ostwald-Fenske viscometer (bulb 8.0 ml capacity) along with 1.0 ml of enzyme solution and incubated for 2 hr at 55°C. The per cent decrease in viscosity was determined as described by Kaiser[3]. A unit of viscosity reducing activity is defined as the amount of enzyme which decreases the viscosity of 0.5 per cent pectin/pectate solution by 50 per cent in 10 min at pH 9.2.

Protein determination: Protein concentrations were determined by the method of Lowry *et al.*[16] using bovine serum albumin as the standard.

Purification of enzyme: For production of PGL, *S.*

thermovulgaris was cultivated in medium containing: 5.0 g NaCl, 1.0 g $MgSO_4.7H_2O$, 0.04 g $CaCl_2,2H_2O$, 0.02 g $FeSO_4.7H_2O$, 0.01 g $ZnSO_4.7H_2O$, 2.0 g K_2HPO_4, 5.0 g casein hydrolysate (acid) and 5.0 g sodium polypectate or pectin in 1 l. distilled water; pH 7.2-7.4. The culture filtrate obtained after the growth of the organism for 48 hr at 55°C under shaken conditions (reciprocal shaker, 100 strokes/min) was concentrated 2-fold using infra red lamp at 32°C. Solid ammonium sulphate was added to the concentrated culture filtrate after cooling to 4°C to give 30 per cent saturation. The proteins which preciptated on keeping at 4°C overnight were centrifuged at 10,000 × G for 20 min and discarded. More ammonium sulphate was added to the supernatant to give 70 per cent saturation and the active protein obtained after centrifugation at 10,000 × G for 20 min, was dialysed against 2 l. of 0.001 M phosphate buffer (pH 7.0) with two consecutive changes and the enzyme lyophilised.

DEAE cellulose (Whatman DE 11, nominal capacity 1.0 meg/g) was treated successively with 1.0 N NaOH, water, 1.0 N HCl and again with 1.0 N NaOH. After thorough washing with distilled water, it was equilibrated for 24 hr with 10^{-3}M phosphate buffer (pH 7.0) and then packed in a column (1.9 × 50 cm). Ammonium sulphate precipitate enzyme (180 mg) was applied and eluted with a gradient of phosphate buffer, pH 7.0 by stepwise increase in molarity (10^{-3} to 1.0 M). 10.0 ml fractions were collected (1.0 ml/min) and tested for protein and enzyme activity.

Polyacrylamide gel electrophoresis: Acetylamide (3 × crystalline, Sisco Research Laboratory, Bombay), N, N'methylene bis acrylamide and TEMED (N-N-N' -N'-Tetramethyl-ethylene diamine) (BDH, Poole, England) were used without further treatment.

Disc electrophoresis was performed on 7 per cent polyacrylamide gel by the method of Davis[17] in both anionic (tris-glycine buffer, pH 8.3) and cationic (β alanine-acetic acid, pH 4, 3) systems. The electrophoresis was carried out for 2 hr with a current of 5mA/gel at 5-10°C using bromophenol blue as the indicator dye (0.5 ml of 0.001 per cent solution per 100 ml of tank buffer). The gels were stained with 1 per cent amido black and washed electrophoretically with 7 per cent acetic acid for 3 hr in a current of 6 mA/gel tube.

Molecular weight of endo PGL was determined by gel filtration using Sephadex G-75 (Pharmacia Fine chemicals, Sweden) by comparing the elution volume of enzyme with the elution volumes of standard proteins. The proteins were eluted with 0.01M phosphate buffer, pH 7.0, at a flow rate of 20 ml/hr.

Paper chromatography: Whatman filter paper No. 4 was used for ascending chromatography of the breakdown products of pectic acid by PGL. The substrate + endo PGL were incubated at 55°C for 24 hr and aliquots were removed at regular intervals, treated with, cation exchanger, Dowex 50W-x8, freeze dried and subsequently dissolved in water. 50 μl was spotted on the chromotography paper and chromatogram was run for 24 to 48 hr in the solvent system, ethyl acetate: pyridine: water: acetic acid (5:5:3:1 v/v). Monogalacturonic acid was used as the marker. The unsaturated products formed were located by spraying aniline hydrogen phthalate[18] and thiobarbituric acid reagent[19].

Results and Discussion

Growth and enzyme formation: The typical growth pattern and enzyme production is presented in Fig. 1. Enzyme production was observed to be a direct function of growth; within 48 hr, *S. thermovulgaris* completely utilised the pectin added to the medium in 0.5 and 1.0 per cent amounts, and maximum enzyme production occurred in 60 hr. The pH of the growth liquor fell to 6.8 in the early stages of growth from the initial pH of 7.5 and later shifted to 8.5: the decrease in pH could be due to the slower utilisation of the degraded products of pectin. On further growth and complete utilisation of the degraded products the pH shifted to alkaline side (8.5).

Physical factors influencing enzyme production: Aeration and agitation of the medium gave two fold increase in cell mass and PGL production; aeration of *Streptomyces, viridochromogenes*, however, was reported by Billimoria[2] to decrease enzyme production. The thermophile gave optimal enzyme yield at 55°C (Fig. 2).

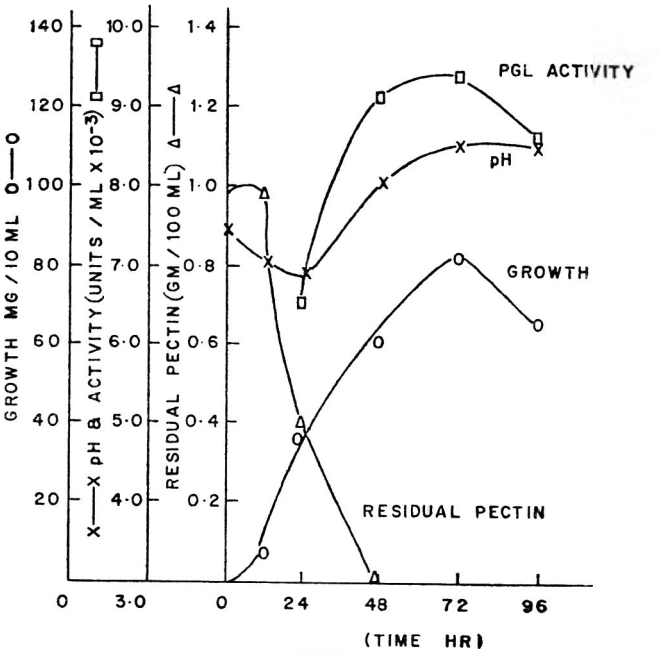

Fig. 1. Growth and endo PGL production by *S. thermovulgaris* CR 42.

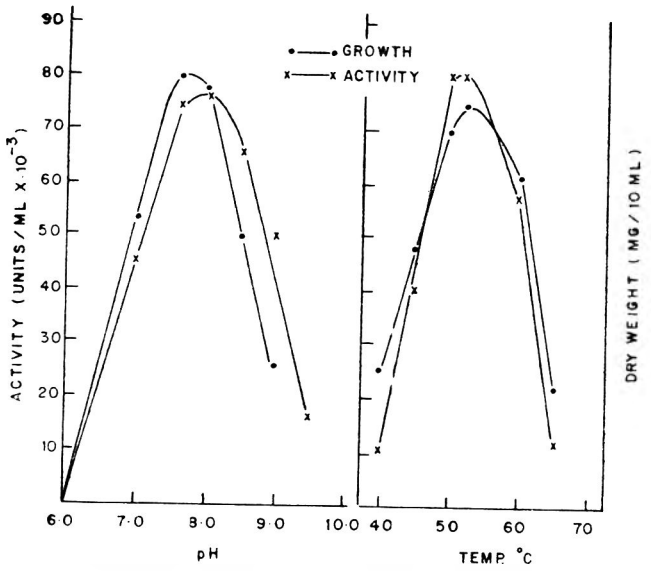

Fig. 2. Effect of pH and temperature on growth and endo PGL production by *S. thermovulgaris* CR42.

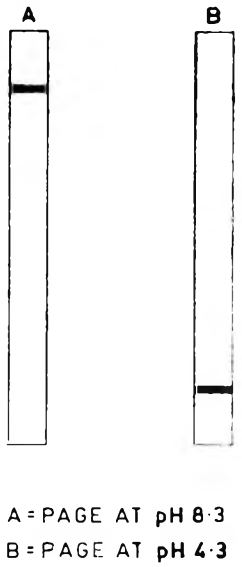

Fig. 4. Polyacrylamide gel electrophoresis of endo PGL at *a*) pH 8.3; *b*) 4.3.

S. thermovulgaris produced maximum PGL when the initial pH of the medium was adjusted to 7.5, while no enzyme was elaborated when the initial pH of the medium was below pH 6.5. Bateman[20] and Sherwood[21] had reported that in *Fusarium oxysporum*, polygalacturonase was produced in the acidic medium while lyase synthesis occurred at alkaline pH. Endo PGL and pectin esterase were induced in *Fusarium roseum* at pH 3.5, while endo PMG and endo PGL were induced at pH 6.5 in pectin medium[22]. But *S. thermovulgaris* produced a single enzyme at different pH levels; the low yields of enzyme at higher pH could be due to the simultaneous decrease in growth of the organism rather than due to the instability of the enzyme at this pH.

Purification of endo PGL: Partial purification of the enzyme could be successfully achieved by precipitating inactive proteins from the culture filtrate at 30 per cent saturation of ammonium sulphate. Seventy two per cent of the active enzyme could then be recovered on subsequent precipitation at 70 per cent ammonium sulphate saturation with a 15-fold purification. Fractionation of the ammonium sulphate precipitated enzyme on DEAE cellulose column gave six protein peaks; the protein eluted in 0.2M phosphate buffer (peak 4) was the only active protein (Fig. 3). Twenty three per cent of the loaded protein was eluted in this fraction and 48 per cent of the activity recovered. Purification of the enzyme achieved was 31-fold (Table 1). Polyacrylamide gel electrophoresis of the purified enzyme gave a single protein band, both in anionic and cationic systems indicating the homogeneity of the enzyme (Fig. 4).

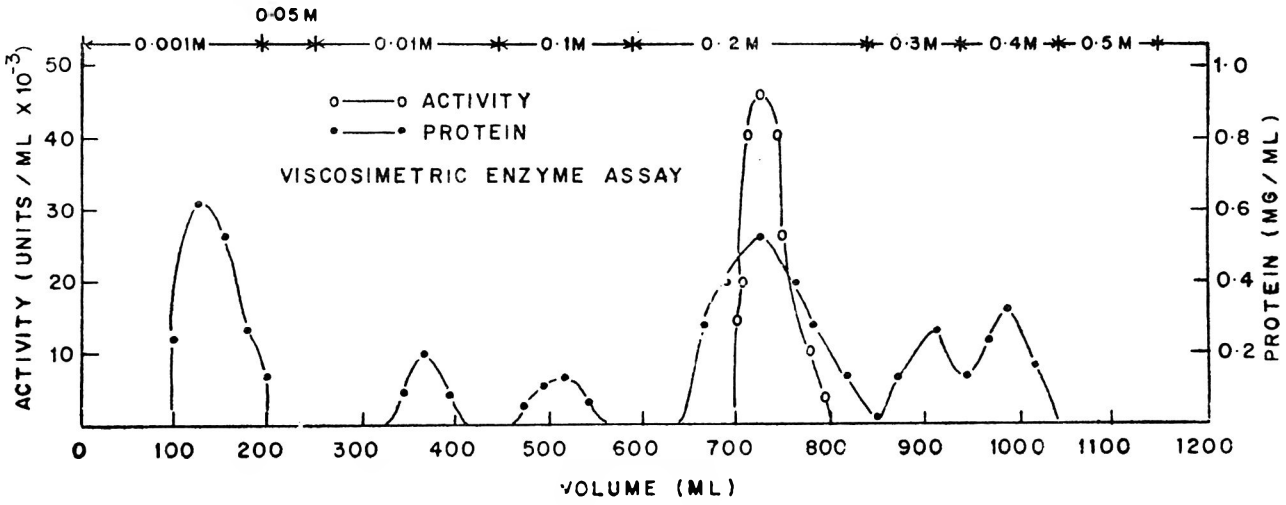

Fig. 3. Purification of endo PGL on DEAE cellulose column

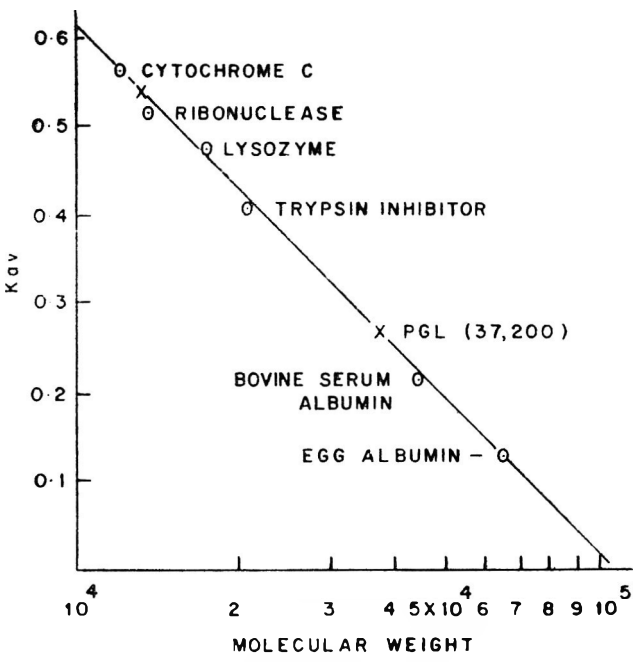

Fig. 5. Log of molecular weight of proteins and endo PGL against Kav.

Properties and characteristics: The molecular weight of the enzyme was estimated to be 37,200 which compares with the endo PGL obtained from fungal sources[23,24] (Fig. 5). Low concentrations (0.5 mM≤)

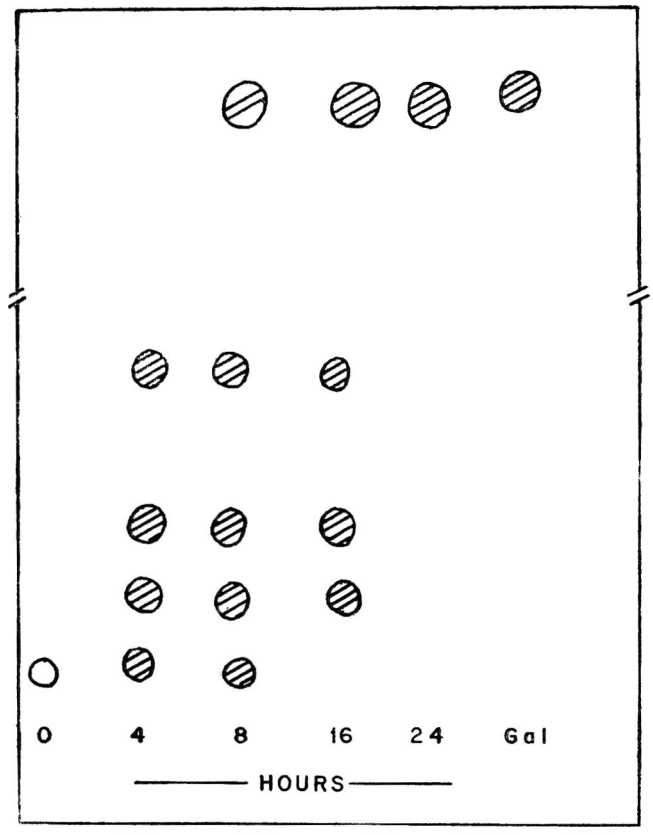

Fig. 7. Paper chromatogram of the breakdown products of pectic acid by the action of endo PGL.

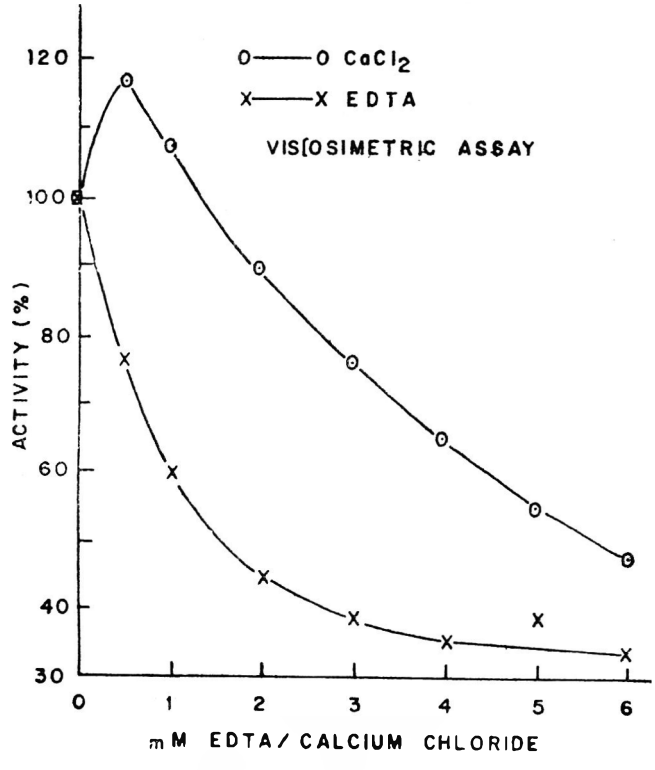

Fig. 6. Effect of EDTA and calcium chloride on endo PGL activity.

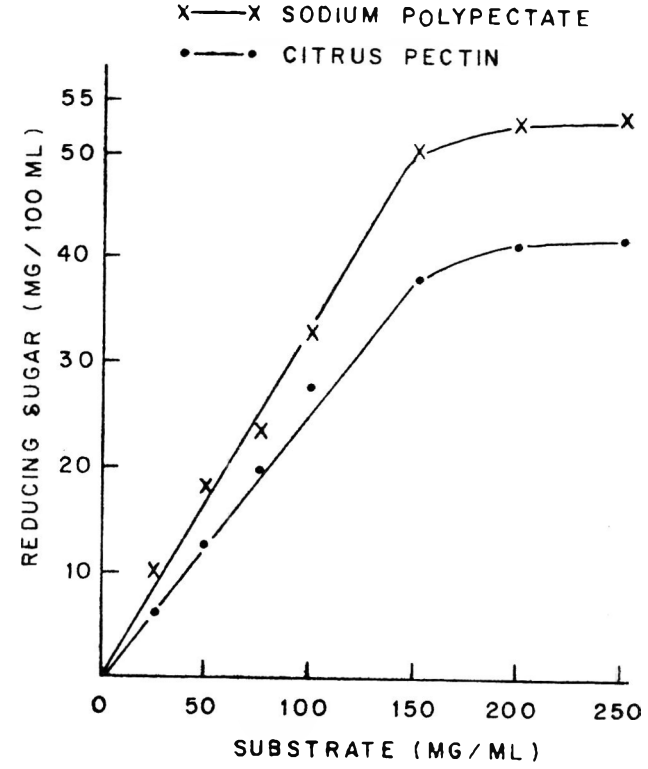

Fig. 8. Effect of substrate concentration on the reaction rate of endo PGL of *S. thermovulgaris* CR42.

TABLE 1. PURIFICATION OF ENDO-PGL

Step	Volume (ml)	Total protein (mg)	Total activity X10^{-3} (units)	Units/ml X10^{-3}	Sp. activity (X10^{-3}/mg)	Purification (fold)	Yield (%
Metabolic filtrate	2000	3840	210,000	105	54.7	1.0	100
IR Concn	1000	3840	210,000	210	54.7	1.0	100
$(NH_4)_2$ SO_4 ppt. (70%) saturation)	100	184.3	151,200	1512	820.4	15.0	72
DEAE Cellulose	100	42.7	73,500	735	1721.3	31.5	35
Sephadex G-75	18	24.0	42,000	2333	1750.4	32.0	20

Assay Reducing group formation

of calcium chloride in the assay mixture increased the enzyme activity, while EDTA suppressed it (Fig. 6). Paper chromatography of the breakdown products of pectic acid gave four distinct spots with the thiobarbiturate reagent which indicated the production of unsaturated high molecular weight products; but on longer incubation (16 hr), the samples from assay mixture gave single spots with Rf value lesser than that of the authentic monogalacturonic acid sample (Fig. 7). This indicated the initial random splitting of the substrate by the enzyme, followed by the formation of monomeric products. The pectic acid lyase nature of the enzyme was confirmed by enzyme assay at 232 nm using Beckmann Spectrophotometer D2^{25}. The enzyme was observed to prefer pectic acid to pectin, but failed to act on polymethylated pectin. Thus, the enzyme was classified as endo polygalacturonase lyase. E.C. 4.2.2.1.

Michaelis Menton costant (Km) of the lyase was observed to be 0.2 and 0.97 per cent sodium polypectate (NaPP) and pectin, respectively, while Vmax were 4.6 and 2.8 μ moles/min/mg of protein, respectively, for NaPP and pectin (Fig. 8).

Acknowledgement

The authors thank Dr G. B. Nadkarni, Head, Biochemistry Division, and Dr S. R. Padwal-Desai, B.A.R.C., Bombay, for providing facilities for spectrophotometric assay and the U.G.C., India, for financial assistance to NRR.

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The Synergistic Effect of Aflatoxin B_1 and Ochratoxin A in Rats

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Reduced gain in body weight was the most consistent clinical change noticed in rats fed with toxins, especially with purified diet. Generally, the livers of rats fed with aflatoxin B_1 were fatty, whereas the kidneys of ochratoxin A fed rats were reduced in size and weight. The haematology picture and enzyme levels did not show any significant difference between various groups.

Histologically, the groups fed with both toxins showed hyperchromatic nuclei and nucleoli, clumping of cytoplasm and slight necrosis of hepatic cells. The collecting tubules showed degenerative changes of the epithelial cells. Some of these cells were anaplastic with hyperchromatic large nuclei. No such abnormality could be seen in rats fed with either aflatoxin B_1 or ochratoxin A alone.

Many fungi that contaminate foods and feeds are capable of producing more than one toxin and also a number of fungi may produce multiple toxins on a given substrate. Substances other than mycotoxins may enhance toxicity and carcinogenicity of certain mycotoxins. These mycotoxins and other promoter substances may exert synergistic toxic reaction. Systematic work on mycotoxin synergism is of recent origin[1,2]. A combination of aflatoxin B_1 and T-2 toxin exhibited a distinct increase in toxicity in mice[3], whereas with the toxin pair rubratoxin B and aflatoxin B_1, toxicity was expressed only, if the animals were fed on sublethal levels of aflatoxin B_1 during the trial period[4]. A synergistic lethal response was observed in acute toxic interaction of ochratoxin and penicillic acid in mice[5]. The other acute toxicity studies where synergistic lethal response was observed are, ochratoxin plus rubratoxin B in infant rats[6] and ochratoxin A plus citrinin in beagle dogs[7].

A synergistic growth inhibition was noted with rubratoxin B plus aflatoxin B_1 in guineapigs[8] and in infant rats[6]. Richard *et al.*[9] investigated the effect of ochratoxin in combination with aflatoxin on compliment activity in guineapigs, but found no synergistic toxic response.

In a chronic toxicity study on two or more structurally similar metabolites like aflatoxin B_1 and B_2, Ayres *et al.*[10] found distinct synergism in inducing trout liver tumors. In an initiator promoter procedure study with aflatoxin B_1, T_2 toxin and diacetoxyscirpenol in the mouse-skin tumor test, aflatoxin B_1 initiated the tumor[3]. Kanisawa and Suzuki[11] in a chronic study with ochratoxin A and afltoxin B_1 in mice, found aflatoxin B_1 to affect synergistically the liver carcinogenicity of ochratoxin A. Wyatt *et al.*[12] on the other hand observed no synergistic toxic effect in broiler chicks when maintained on dietary aflatoxin (2.5 ppm) and rubratoxin B (500 ppm) for three weeks. Cyclopropenoid fatty acids and gossypol in cottonseed elicit tumor formation only in the presence of aflatoxin B_1[13]. In another study, the short term exposure of rats to chemical renal carcinogens followed by long term feeding of citrinin induced an increase in renal tumors[14].

The hepatotoxic carcinogen aflatoxin B_1 and nephrotoxic ochratoxin A are the toxins elaborated by species of *Aspergilli*, which are common storage moulds infecting a wide variety of food and feed commodities. Both the toxins frequently occur on the agricultural commodities in India[15,16]. The present study is on the possible synergistic effect of aflatoxin B_1 and ochratoxin A in rats. This was done with a view to simulate natural levels of food contamination that may commonly prevail under tropical post harvest conditions, and to evaluate the probable toxic effects that these mycotoxins may cause in man and animals.

Materials and Methods

Male Wistar rats (50 albino weanling) were statistically grouped into five groups as follows: (*i*) 1/20 LD_{50} aflatoxin B_1; (*ii*) 1/20 LD_{50} ochratoxin A; (*iii*) 1/20 LD_{50} (aflatoxin B_1 along with ochratoxin A); (*iv*) 1/40 LD_{50} (aflatoxin B_1 along with ochratoxin A) and, (*v*) The control group. The LD_{50} was taken as 7 mg/kg body weight for aflatoxin B_1 and 22 mg/kg body weight for ochratoxin A. The mycotoxins were dissolved in

TABLE 1. THE GAIN IN WEIGHT AND RELATIVE ORGAN WEIGHTS OF RATS MAINTAINED ON NATURAL DIET*

Treatment	Gain in wt. (g)	Av. of relative organ wt. (g/100 g of body wt.) Liver	Kidney
Stock diet (control)	173.0[a]	3.74[b]	0.69[a]
1/20 aflatoxin B_1	178.2[a]	4.73[a]	0.70[a]
1/20 ochratoxin A	180.6[a]	3.59[b]	0.48[b]
1/20 (aflatoxin B_1 + ochratoxin A)	162.4[a]	4.07[b]	0.42[b]
Standard deviation (11 df)	±14.7	±0.37	±0.07

Means of the same column followed by different letters differ significantly at 5% level according to Duncan's New Multiple Range Test

*Natural diet (g/100 g diet); wheat four, 26.66; Ragi flour, 26.66; Bengalgram, 26.67; refined groundnut oil, 10.00; casein, 6.67; calcium carbonate, 0.67; Shark liver oil, 0.67; and salt mixture, 2.00

Note: The group 1/40 LD_{50} (aflatoxin B_1 + ochratoxin A) was not maintained for this experiment.

50 per cent ethanol at various levels and 0.1 ml was fed to each rat along with the diet in a semi-solid state. The control rats were fed each with 0.1 ml of 50 per cent ethanol along with the diet. The mycotoxin level was varied every week as with the change in body weight. The semi-solid diet was completely consumed by the rats. The urine of rats was analysed for creatine and creatinine[17] at periodic intervals. On autopsy, the blood was collected for haematology and the samples were analysed for (*i*) serum glutamic oxalacetic transaminase (SGOT)[18] (*ii*) serum glutamic pyruvic transaminase (SGPT)[18] and (*iii*) creatine phosphokinase (CPK)[19]. The liver and the kidney were also subjected to histopathological observations. The organ weights and enzyme estimates were statistically analysed.

The above feeding trials were conducted with purified as well as with natural diets each for a period of 4 months.

Results

Growth retardation was the most consistent clinical change seen in the rats fed ochratoxin A singly or in combination with aflatoxin B_1 only with purified diet. The kidney and liver changes included generalized paleness in the experimental groups. Generally, livers of animals fed aflatoxin B_1 were fatty and enlarged. In the ochratoxin A fed groups kidneys were reduced in size; this was more evident in the combined toxin group, where they were small, pale and unequal in size-one flattened and other smaller (compensatory hypertrophy suspected). Statistically significant decrease in kidney weight was recorded in combined toxin group (Tables 1 and 2). The haematology results and enzymes did not show any significant differences between the various groups.

Histology: The liver and the kidney sections from experimental animals show the following histopathological changes (Table 3). Livers showed cytoplasmic vacuolations, centrilobular fatty infiltrations and slight bile-duct proliferation in aflatoxin B_1 fed animals. The groups fed with both the toxins showed hyperchromatic nuclei and nucleoli, clumping of cytoplasm and slight necrosis of hepatic cells. The anaplastic and hyperchromatic nuclei, necrosis and bile-duct proliferation were more pronounced in the combined toxin groups. Kidneys of experimental animals showed normal morphology of capsules, glomeruli and blood vessels. Slight disturbances of renal tubules and slight enlargement of the collecting tubular cells were observed in ochratoxin A fed rats on purified diet. The combined toxin groups (both 1/40 LD_{50} and 1/20 LD_{50}) show the following pathology: groups of collecting tubules show

TABLE 2. THE GAIN IN BODY WEIGHT AND THE RELATIVE ORGAN WEIGHTS OF RATS MAINTAINED ON PURIFIED DIET*

Treatments	Gain in wt. (g) Mean ± SEM		Av. of relative organ wt. (g/100 g body wt.) Liver Mean ± SEM		Kidney Mean ± SEM	
Control[a] (ethanol)	206.3 ±2.7		3.63 ±0.22		0.54 ±0.01	
1/20 LD_{50} aflatoxin B_1	197.8	X	4.49	Y	0.56	Y
1/20 LD_{50} ochratoxin A	150.8 ±4.9 (8 d.f.)	Y	3.70 ±0.17 (14 d.f.)	Z	0.54 ±0.01 (14 d.f.)	Y
1/20 LD_{50} (aflatoxin B_1 + ochratoxin A)	127.2	Z	4.91	Y	0.37	Z
1/40 LD_{50} (aflatoxin B_1 + ochratoxin A)[a]	179.7 ±7.9		4.19 ±0.10		0.48 ±0.01	

[a]Two rats each in the control group and the experimental group 1/40 LD_{50} (aflatoxin B + ochratoxin A), died due to heart puncture. These two groups were omitted for statistical analysis.

Means of the same column followed by different letters differ significantly at 5% level according to Duncans New Multiple Range Test.

*Purified diet (g/100 g); Corn starch, 72; casein, 15; salt mixture, 2: vitaminized starch, 1; vitaminized oil, 1; Refined groudnut oil, 9.

TABLE 3. HISTOPATHOLOGICAL CHANGES IN LIVER AND KIDNEY OF RATS MAINTAINED ON VARIOUS DIETS

Treatment	Liver		Kidney	
	Natural diet	Purified diet	Natural diet	Purified diet
1/20 LD_{50} aflatoxin B_1	HN(0/5) BDP(1/5) CLFI(4/5)	HN(0/5) BDP(1/5) CLFI(5/5)	Normal	Normal
1/20 LD_{50} (aflatoxin B_1 + ochratoxin A)	HN(3/4) BDP(2/4) CLFI(4/4)	HN(3/4) BDP(1/4) CLFI(4/4)	SCT(3/4) ENEC(4/4)	SCT(2/4) ENEC(4/4)
1/40 LD_{50} (aflatoxin B_1 + ochratoxin A)	—	HN — BDP — CLFI(5/5)	—	SCT(1/5) ENEC(4/5)
1/20 LD_{50} ochratoxin A	Normal	Normal	SCT(1/5) ENEC(0/5)	SCT(1/5) ENEC(1/5)
Control	Normal	Normal	Normal	Normal

HN = Hyperchromatic nuclei; BDP = Bile duct proliferation; CLFI = Centrilobular fatty infiltration; SCT = Swelling of collecting tubules; ENEC = Enlargement of nuclei of epithelial cells

The figures in the parenthesis indicate the ratio between the number of rats showing the specific histopathological change and the number of rats taken.

degenerative changes of the epithelial cells (Fig. 1). one or two abnormal cells are seen lining these tubules. These cells are of anaplastic type with hyperchromatic large nuclei (Fig. 2). At places, mitosis is also seen (Fig 3). Fibroblastic proliferation is seen in between some of these tubules (Fig. 4). It appears that metaplastic changes are occurring in these tubules associated with increased fibrosis, containing a few inflammatory cells.

Discussion

Except the decreased weight gain in the toxin fed animals on purified diet as has been observed by several other workers[6,8], the experimental animals were almost normal. There was no mortality. The aflatoxin fed rats showed enlarged and fatty livers whereas kidneys of ochratoxin A fed rats weighed significantly less. No differences could be seen in creatine and creatinine of both blood and urine as well as in serum SGOT, SGPT and CPK of the experimental and control animals.

A significant histopathological observation was made in case of kidneys of rats fed with a combination of 1/20 LD_{50} dose each of aflatoxin B_1 and ochratoxin A. The kidneys of these animals showed degenerative changes of the epithelial cells, a few abnormal cells lining the tubules with enlarged nuclei, mitosis and fibroblastic proliferation in between some of these tubules. In case of animals fed either of these mycotoxins at the 1/20 LD_{50} level, no such abnormalities were seen in the kidneys. A chronic synergistic effect was thus exerted. This is true irrespective, of whether the rats are fed with purified or natural diet. The enlargement of the nuclei of the kidney epithelial cells seems to be associated with the reduced weight of the kidney in rats fed with combined toxins. It may be recalled here that aflatoxin B_1 not only induces tumors in rat liver[20] but also causes renal epithelial neoplastia in rats fed with 1 ppm of aflatoxin B_1 for a period of 5 months according to Epstein *et al.*[21] Similarly, ochratoxin A is known to cause impairment of and lesions in proximal convoluted tubules of kidney in rats[22,23] as well as mild liver degeneration[24] and fatty infiltration[25]. In the present study, it appears that ochratoxin A may be an initiator in causing the anaplastic cells with large hyperchromatic nuclei in kidney epithelial cells and aflatoxin B_1 may act as a promoter. Hence the type of pathology induced by the combined mycotoxins is perhaps synergistic in nature.

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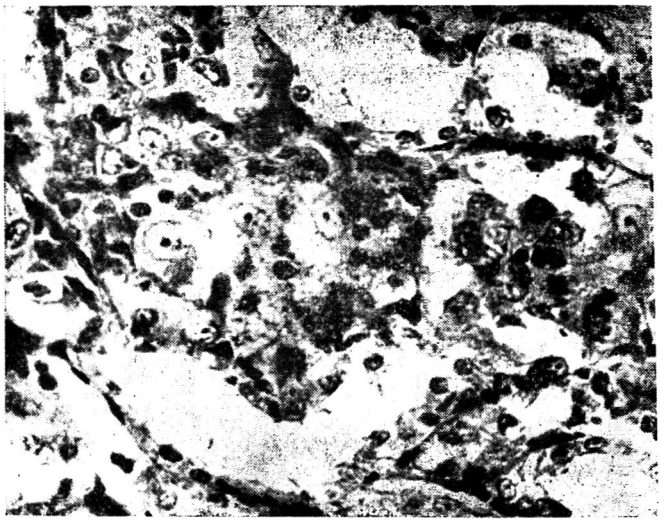

Fig. 1. Groups of collecting tubules showing degenerative changes in the epithelial cells (H & E X 650).

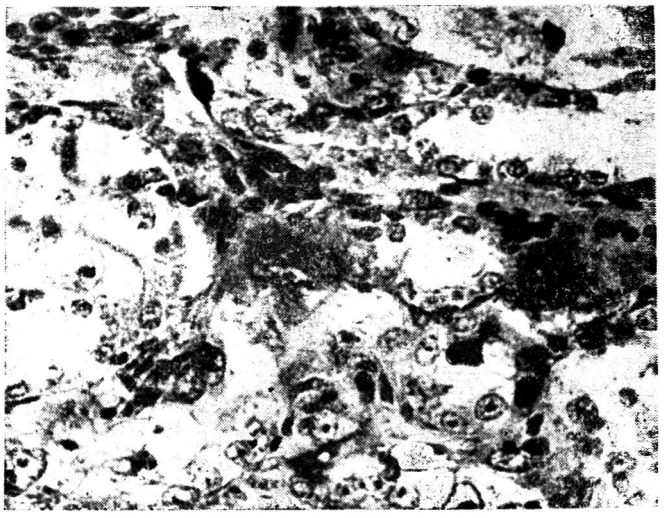

Fig. 2. A few anaplastic type cells with hyperchromatic large nuclei are seen lining some of the collecting tubules (H & E X 650).

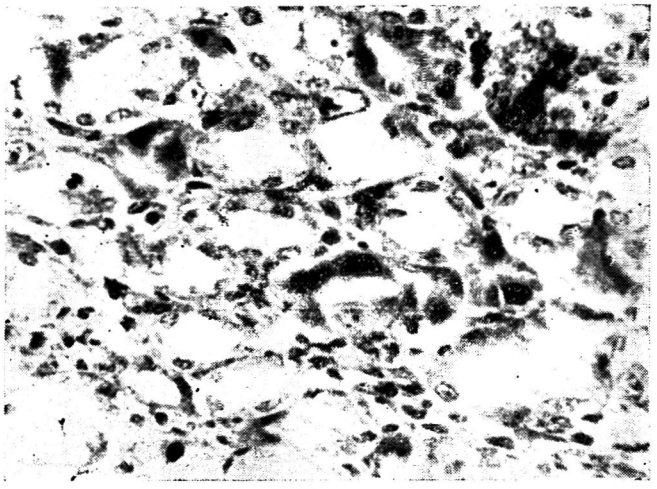

Fig. 3. At places, mitosis is clearly seen (H & E X 650).

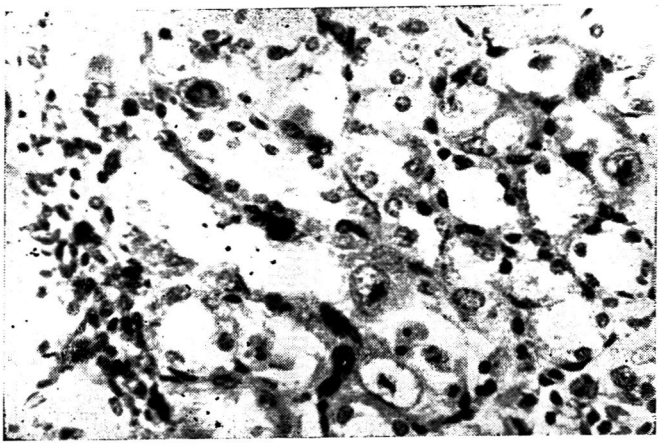

Fig. 4. Fibroblastic proliferation is seen in between some of the collecting tubules (H & E X 650).

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Studies on Some Quality Aspects of Semolina from Different Types of Wheats

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Three particle size ranges -20, +28; -28, +36; -36, +46 mesh were selected for studying quality aspects of laboratory millied semolina from soft aestivum ('Pissi local'), medium hard aestivum (WG-357), durum ('Bijaga yellow') and dicoccum ('Jave') wheats. Total ash and protein content in semolina gradually decreased with decrease of particle size in all the types of wheats. Semolina from soft aestivum wheat was highly inferior in cooking quality compared to semolina from other wheats. Its water uptake rate, cooking tolerance and water uptake capacity were very low. Semolina from durum and dicocuum had high cooking tolerance. Roasting (i) improved the cooking quality of semolina from soft and medium hard aestivum and durum wheats—maximum improvement was observed in soft aestivum wheat, and (ii) had a slight adverse effect on the cooking quality of dicocuum semolina.

Wheat semolina forms an important basic raw material in the preparation of several Indian traditional foods, especially, sweet and savoury, breakfast as well as snack foods. Many varieties of semolina popularly known as *Bansi*, white and *Samba* in various categories of particle sizes are marketed for use in the preparation of *Upmav*, *Kesaribath* (*Shira*), *Chiroti*, *Ladu*, *Semia* (Vermicelli) etc. Roasting of semolina is a popular practice in many households for preparation of semolina based food items. However, no information is available in the literature on the comparative functional qualities of different types of semolina and also their suitability for specific end uses. Hence studies were undertaken on some quality aspects of laboratory milled semolina from different types of wheats, keeping in view the particle size distribution patterns of commercial semolina samples. The results are presented in this paper.

Materials and Methods

Materials: Commercial semolina samples procured from Mysore (India) market were of the following types:

Commercial name	Type of wheat	Category of particle size
Bansi	Durum	coarse, medium coarse, fine and very fine.
White	Aestivum	medium coarse, fine and very fine
Samba	Dicoccum	very coarse

Following commercial wheats were used for the laboratery studies.

Variety	Type	Hardness	Place of cultivation
(Pissi local)	Aestivum	soft	Madhya Pradesh
(WG 357)	Aestivum	medium hard	Punjab
(Bijaga yellow)	Durum	extra hard	Karnataka
(Jave)	Dicoccum	hard	Tamil Nadu

Physical characteristics of wheat: The weight of 1000 kernels (grains) as well as the length and breadth of 100 grains selected at random were determined. Hardness of 20 kernels was determined in triplicate, using the Grain Hardness Tester (Ogawa Seiki Co., Tokyo).

Laboratory semolina milling: Wheat samples were milled for semolina in a Buhler Laboratory Mill (MLU-202) using only the break system by disconnecting the pneumatic conveyor pipe leading to the reduction roll system. Based on the preliminary trials for getting semolina of widely varying particle size ranges (20-60 mesh), the following procedure was adopted.

'Bijaga yellow' and 'Jave' were conditioned to 18 per cent moisture for 4 hr, whereas 'WG 357' and 'Pissi local' were conditioned to 15 and 14 per cent moisture respectively. Besides this, 0.5 per cent water was added 30 min before milling as described by Black and Bushuk[2].

Clearances of 0.5 and 0.3 mm for break rolls B2 and B3 respectively were found optimum for getting semolina of the desired particle size. The overtailings from finer sieves at the three break rolls were collected as semolina. Semolina was also recovered by sieving the bran fraction. These were purified by winnowing off adhering bran under controlled air currents and divided into different fractions based on particle sizes, in a Buhler Plansifter. The fractions used for studying physico-chemical and cooking quality characteristics were −20, +28; −28, +36; and −36, +46 mesh.

Bulk density and particle size distribution: By filling a 500 ml measuring cylinder with 200 g of semolina, bulk density was determined and expressed as g/ml. Two hundred grams of semolina was subjected to sieving for 5 min in a Buhler Plansifter using sieves of 20, 28, 36, 46 and 60 mesh. For commercial *Samba* semolina, sieves used were 6, 10, 14, 20 and 28 mesh.

Total ash and protein: Total ash both in whole wheat and semolina was determined according to AACC procedure[1] and crude protein (N × 5.7) by microkjeldhal method.

Cooking quality: To a 100 ml beaker containing 50 ml of water previously heated in a boiling water bath for 5 min, 5 g of semolina was added and cooking continued for 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 and 6.0 min. Using a Buchner funnel covered with watch glass, cooked semolina was drained for 5 min and weighed immediately. The water uptake was calcuated as percentage of weight of semolina. The cooked product was visually examined for the completion of cooking, as indicated by the disappearance of the opaque central core in the semolina granule, and also stickiness and other textural characteristics by hand feel. The maximum cooking time at the end of which the cooked product started (*i*) disintegrating, i.e., losing its granular texture, or (*ii*) becoming pasty or sticky, was taken as cooking tolerance. The water uptake at the end of cooking tolerance period was taken as water uptake capacity.

Roasting of semolina: Semolina, spread as 0.5 cm thick layer in an aluminium tray, was roasted in an air circulation oven at 150°C. Based on trials for different periods ranging from 3 to 15 min roasting for 8-10 min gave a product of desired colour and aroma. Hence different semolina samples were roasted for 8-10 min.

Results and Discussion

Physico-chemical characteristics of commercial semolina: Of the different commercial semolina analysed, only two extreme patterns (samples A and B) for the particle size distribution are presented in Fig. 1. In the coarse *Bansi* semolina, the major fraction was in the range of −14, +28 mesh. In *Bansi* and white semolina, the major fractions were −28, +36 mesh in medium coarse

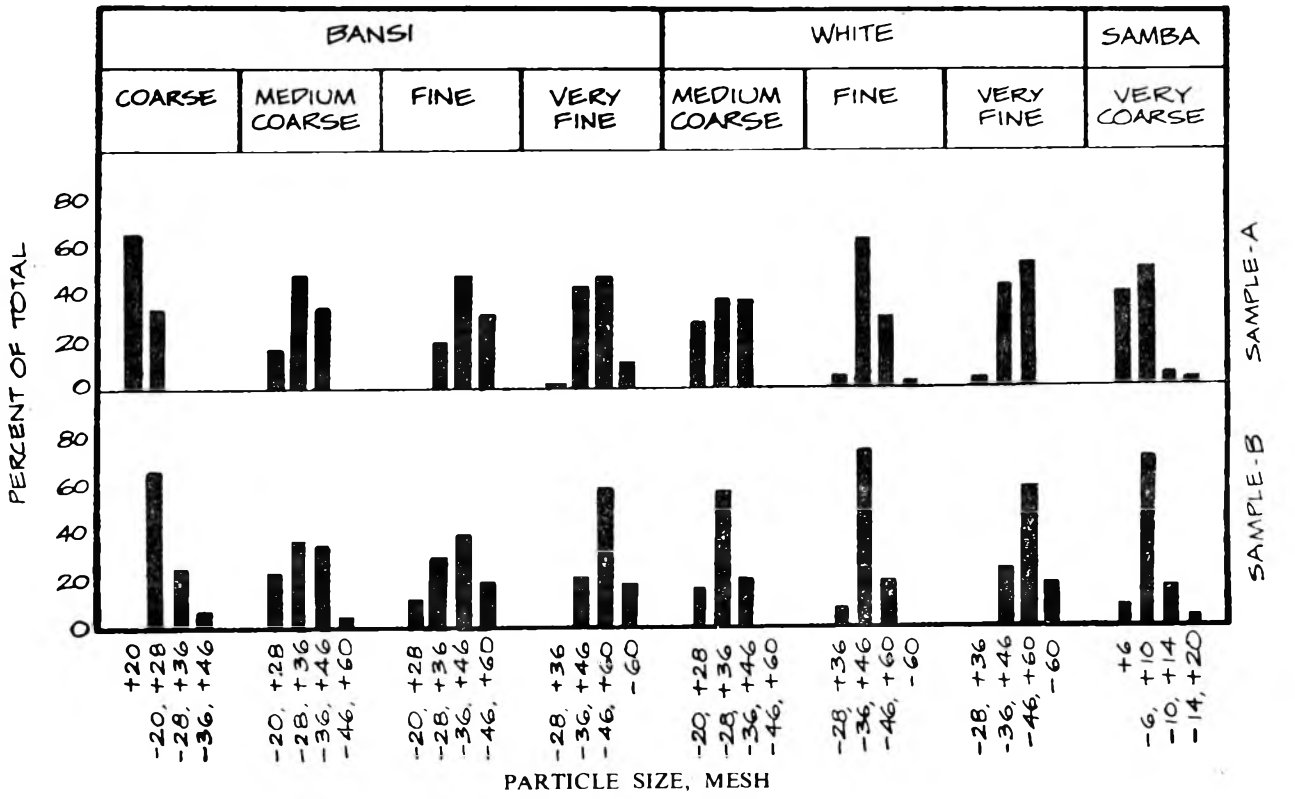

Fig. 1. Particle size distribution in commercial semolina samples.

TABLE 1. SOME PHYSICO-CHEMICAL CHARACTERITSTICS OF WHEATS USED FOR LABORATORY SEMOLINA MILLING

Wheat variety	1000 Kernel wt. (g)	Length/ breadth ratio	Hardness (kg/grain)	Total ash* (%)	Protein* (N×5.7) (%)
'Pissi local'	47.4	2.22	8.8	1.21	10.1
'WG-357'	43.6	2.13	14.9	1.42	11.0
'Bijaga yellow'	43.5	2.67	15.2	1.57	16.0
'Jave'	31.8	3.44	10.8	1.76	15.6

*on 14% moisture basis

category, −36, +46 mesh in the fine category and −46, +60 mesh in the very fine category. In *Samba* semolina which was very coarse, the major fraction was in the range of −6, +10 mesh. Most of the samples contained a major fraction which accounted for 40-60 per cent of the total particles; this is indicative of lack of uniformity in particle size distribution.

It is interesting to note that the range for total ash content of white semolina (0.41-0.59 per cent) was approximately half that of *Bansi* semolina (0.78-1.31 per cent) and one third to one fourth that of *Samba* semolina (1.51-1.62 per cent). This may be attributed to the traditional methods adopted for milling of *Bansi* and *Samba* semolina as compared to the roller milling adopted for white semolina.

TABLE 2. DISTRIBUTION OF TOTAL ASH AND PROTEIN IN LABORATORY MILLED SEMOLINA*

Wheat variety	Semolina colour	Total ash (%)				Protein (%) (N×5.7)			
		−20** +46	−20 +28	−28 +36	−36 +46	−20** +46	−20 +28	−28 +36	−36 +46
'Pissi local'	Dull white	0.53	0.73	0.49	0.48	8.2	8.8	8.1	8.0
'WG − 357'	Dull white	0.60	0.80	0.56	0.55	8.9	9.2	9.0	8.7
'Bijaga yellow'	Bright creamy yellow	0.70	0.97	0.64	0.59	13.6	14.5	13.5	13.2
'Jave'	Dull brownish	0.99	1.42	0.88	0.78	13.0	14.1	12.7	12.5

*on 14% moisture basis
**composite sample

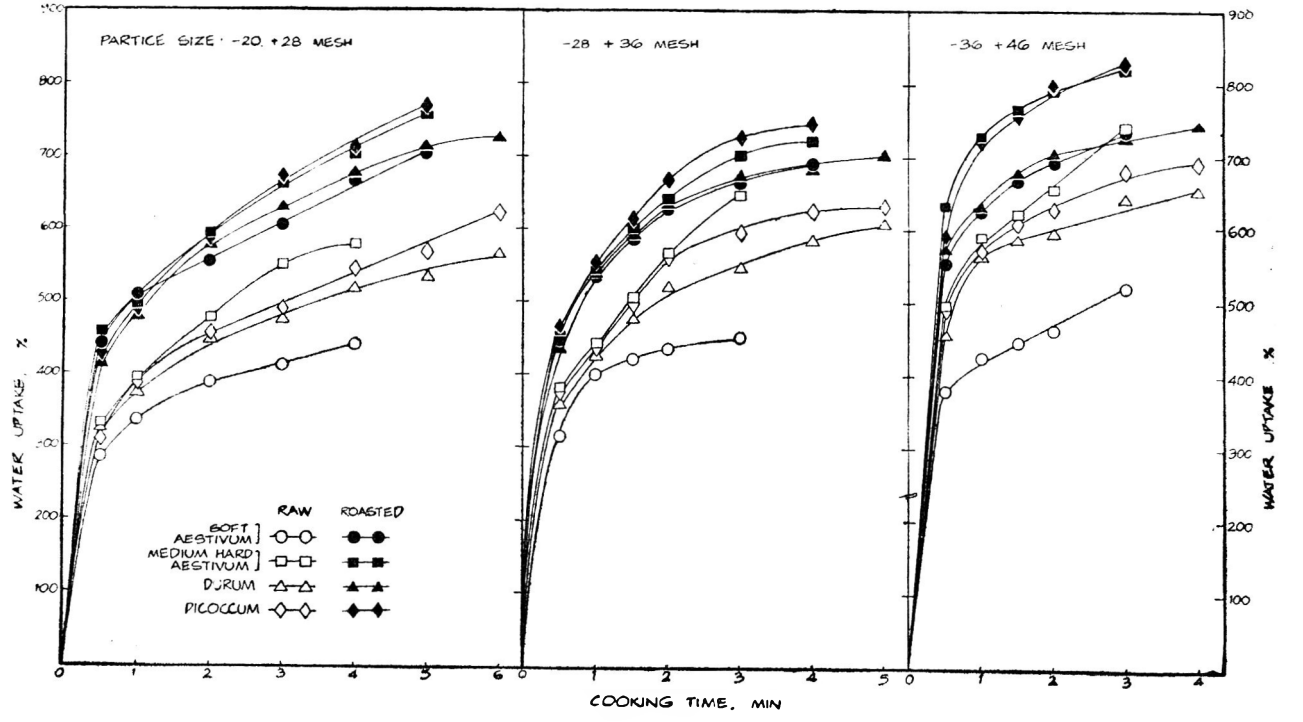

Fig. 2. Effect of cooking time on the water uptake of raw and roasted semolina from different wheats.

Physico-chemical characteristics: Some important physico-chemical characteristics of the wheat varieties as well as semolina milled therefrom are given in Tables 1 and 2. Bulk density of semolina samples ranged between 0.56 and 0.59. Ash content increased in the following order: 'Pissi local', 'WG-357', 'Bijaga yellow' and 'Jave'. The relatively high ash values for 'Jave' semolina may be due to the variation in structure, shape and size of the grain leading to a less efficient bran separation, as compared to other wheat varieties. The absence of mechanical purifying system in the laboratory mill was possibly responsible for relatively higher ash content of coarse semolina. In the semolina milled from the same variety, the protein content decreased gradually with the decrease in particle size.

Cooking time vs water uptake by semolina: The effect of cooking time on the water uptake of raw and roasted semolina for the particle size ranges of – 20 +28; – 28 +36 and – 36 +46 accounting for major proportion of most of the commercial semolina samples is presented in Fig. 2. Among the raw samples, the rate of water uptake for semolina from 'WG-357' variety was the highest, while that of 'Pissi local' was the lowest. The rate of water uptake increased with decrease in particle size of semolina in all the wheat varieties.

Roasting of semolina increased the water uptake rates; maximum increase was in '*Pissi local*'. The wide differences in the water uptake rates of semolina from different wheat varieties narrowed down appreciably by roasting.

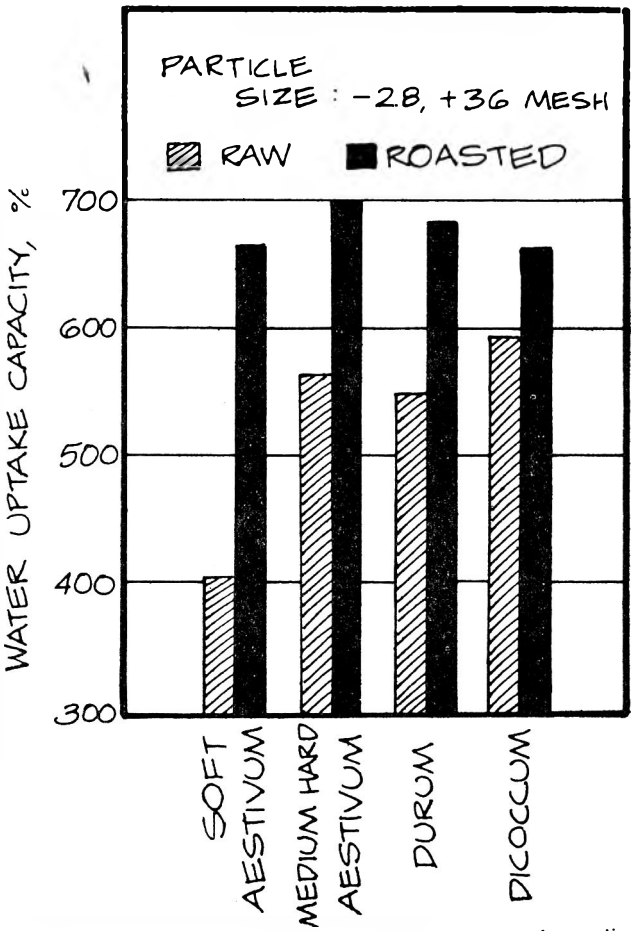

Fig. 4. Water uptake capacities for raw and roasted semolina from different wheats.

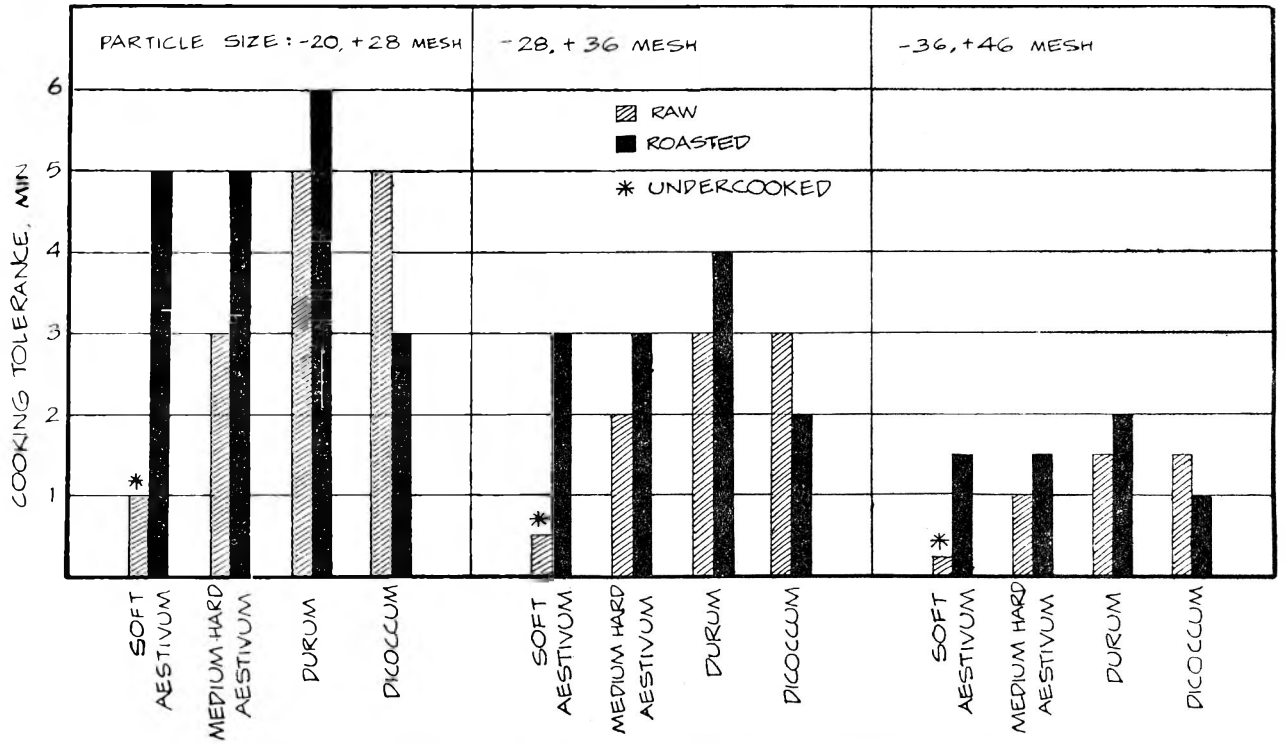

Fig. 3. Cooking tolerances for raw and roasted semolina from different wheats.

Cooking tolerance vs variety of wheat: In practice, cooking tolerance is important for avoiding undesirable textural changes such as development of pastiness, stickiness, etc. in cooked semolina product. Among the varieties the raw semolina from 'Pissi local' had the lowest cooking tolerance and became pasty and sticky, even before it was cooked completely (Fig. 3). Raw semolina from both 'Bijaga yellow' and 'Jave' had the maximum cooking tolerance, while that of 'WG-357' had a slightly lower value. Roasting of semolina from 'Pissi local' brought about highly significant improvement in its cooking tolerance values, comparable to raw semolina from 'Bijaga yellow' and 'Jave'. Roasting of semolina from 'WG-357' and 'Bijaga yellow' also improved significantly their cooking tolerance; on the other hand, a slight adverse effect was observed in the case of 'Jave'.

Water uptake of semolina vs variety of wheat: Water uptake for raw and roasted semolina in the medium particle size range (– 28, +36 mesh, are shown in Fig. 4, the values for the other two particle size ranges being similar. Raw semolina from 'Pissi local' showed very low water uptake while those of the other three varieties had more or less comparable values. It is interesting to note that roasting increased significantly the water uptake capacities for semolina from all varieties of wheats, thus resulting in higher yield of the cooked product, which is a desirable cooking characteristic. The increase was maximum in the case of semolina from 'Pissi local', thereby making it comparable to the other semolina.

These results indicate that the semolina from durum wheat ('Bijaga local') has the best cooking quality followed by dicoccum ('Jave'). Semolina from soft aestivum wheat ('Pissi local') was very poor. Roasting improved the cooking quality of semolina from soft aestivum wheat followed by medium hard aestivum (WG-357') and durum wheats. Roasting, however had a slight adverse effect on dicoccum semolina.

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Drying-cum-curing of Freshly Harvested High Moisture Paddy by Roasting with Hot Sand

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Sand roasting of freshly harvested high moisture paddy in a mechanical roaster for 1 to 1½ min at 95 to 155°C removed 8-10% moisture in the paddy. Tempering of the roasted paddy for 1½ to 2 hr. healed the cracks to some extent which were formed during roasting. The rice processed in this method exhibited characteristics similar to that of cured or parboiled rice. Paddy with high initial moisture (>22%) is better suited for parboiling while those with low moisture (18 to 21%) can be cured using this roasting equipment.

Drying of freshly harvested high moisture paddy is a serious problem in places where the harvesting coincides with the onset of monsoon. Several methods like short-term preservation using chemicals such as common salt, urea, etc.[1,2,3] contact method of drying using paddy husk, husk powder, graded earth etc,[3] have been tried. However, due to certain technological and local problems, these methods could not be applied in villages. Mechanical drying was found to be very costly. If the drying was combined with curing (steaming or roasting followed by tempering for eliminating the pasty cooking character of rice from fresh paddy) or even parboiling, the drying cost may be partly absorbed by such processing. Studies at IRRI[4,5] and PPRC[6] as well as the experience of the 'Sela'[7] process of parboiling have indicated that drying-cum-starch-cooking can be superimposed in wet paddy. With this background, the possibility of using a mechanically operated sand-roaster (used for commercial puffing of Bengal gram) for drying-cum-curing of fresh paddy has been investigated and reported.

Materials and Methods

The roasting unit employed was a commercial gram-

roaster developed by Shunmugam Pillai[8]. Paddy could be roasted continuously at the rate of about 3 quintals per hr, with hot sand at a required temperature for a period of 1 to 1½ min and recovered. Different varieties of freshly harvested paddy at moisture levels ranging from 18 to 26 per cent were roasted at temperatures varying from 95 to 155°C and tempered in metallic bins for 1-2 hr at room temperature followed by spreading in open air for cooling. Samples withdrawn at different intervals during tempering were shade dried for studies on the curing, cracking, milling and cooking properties as well as colour of rice obtained from it. Moisture of paddy was determined as per the method of Indudhara Swamy *et al.*[9].

Cracking was detected in a paddy crack detector[10], milling, curing and cooking qualities were determined as per the methods described earlier[11,12]. The effect of varying the mesh size of sand for roasting and also the sand:paddy ratio during roasting were also studied.

Results and Discussion

Data on the effect of sand roasting and subsequent tempering on the proportion of cracked paddy grains are presented in Table 1.[5] Roasting with sand considerably reduced the moisture levels ranging from 5-10 per cent. It is however, seen that passage of paddy through the roaster for a short period of 1 to 1½ min increases the temperature of paddy to 106°C and a great increase in the proportion of cracked grains at all temperatures of roasting irrespective of variety or moisture content at the time of roasting. Tempering the roasted paddy in closed bins considerably healed these cracks in both the varieties examined. Tempering for 1-2 hr is desirable to bring down the proportion of cracked grains to a level comparable with that of the untreated paddy. Further studies were, therefore, conducted after giving 1½ to 2 hr tempering period to the roasted paddy.

Results of such studies on two varieties of paddy are presented in Table 2. The higher the roasting temperature (115°-155°C), the greater was the reduction in the moisture content (about 8-10 per cent) of paddy.

The proportion of cracked grains in the roasted paddy after tempering depended on the initial moisture content of the paddy. With paddy having initial moisture of about 18 per cent, high roasting temperature resulted in reduction of about 8 per cent moisture causing larger proportion of cracked grains (15-18 per cent). Better results can be obtained if the roasting temperature for such paddy is kept lower (around 95°C) with a tempering for 1 to 2 hr. For paddy with higher moisture content (>22 per cent) a slight beneficial effect is noticed on cracking and milling even at higher roasting temperatures. Paddy with initial moistures above 25 per cent and roasting at 140 to 155°C gives parboiling effect, without affecting milling quality even when tempering is not done. These results are in conformity with those obtained by IRRI scientists[4,5]. The breakage of paddy during milling followed a pattern quite parallel to the extent of cracked grains. 'CH-45' variety paddy which had white core in the endosperm was, however, found to stand high roasting temperature better than other varieties even at a lower moisture without detriment to its milling breakage. An explanation for this behaviour could be the relatively higher affinity for water and higher equilibrium moisture content of chalky varieties as indicated by earlier work[13].

TABLE 1. EFFECT OF SAND ROASTING AND SUBSEQUENT TEMPERING ON CRACK FORMATION IN PADDY

Sand-paddy roasting		Tempering			Cracked grains
Temp (°C)	Final moisture (%)	Period (hr)	Moisture (%)	Temp (°C)	(%)
Paddy var. 'CH 45' (Pre-roast moisture 18%)					
155	10.8	Nil	—	—	60
—	—	0.5	—	NR	43
—	—	1.0	9.1	105	34
145	11.8	Nil	—	—	92
—	—	0.5	—	NR	64
—	—	1.0	10.5	100	28
135	13.2	Nil	—	—	92
—	—	0.5	—	—	42
—	—	1.0	12.3	100	21
125	14.5	Nil	—	—	92
—	—	0.5	—	NR	35
—	—	1.0	13.7	95	15 (31.6)
—	—	Control	—	—	5 (41.6)
Paddy var. 'CH 45' (Pre-roast moisture 22.8%)					
140	14.9	Nil	—	—	74
—	—	0.5	—	NR	67
—	—	1.0	14.4	100	16
150	12.2	Nil	—	—	69
—	—	0.5	—	NR	57
—	—	1.0	12.2	102	24
—	—	Control	—	—	(42.0)
Paddy var. 'BS' (Pre-roast moisture 20.3%)					
130	12.9	Nil	—	—	67
—	—	1.0	11.2	95	18 (16.9)
—	—	2.0	10.1	85	11 (12.9)
—	—	Control	—	—	6 (20.6)

Figures in the parentheses indicate milling breakage. Paddy to sand ratio is 1:25: sand size —25+44 BSS; NR: not recorded.

TABLE 2. EFFECT OF SAND ROASTING OF PADDY ON CRACKING, MILLING BREAKAGE AND CULINARY PROPERTIES

Variety	Roasting temp. (°C)	Tempered paddy moisture (%)	Cracked grains (%)	Milling breakage %	Capacity of rice	Bulk vol. of cooked rice*	Cooking characteristics	Acceptability
CH 45 (18%)	125	13.5	15.0	31.6	DO	435	F	A
	135	11.5	19.0	31.5	PT	435	F	A
	145	9.1	18.0	32.3	PT	435	F	A
	Non-roasted	—	5.0	41.6	BO	370	P	NA
IR 20 (18.5%)	95	14.6	9.0	19.2	O	415	F	A
	140	10.3	15.0	27.0	O	420	F	A
	Non-roasted	—	0.0	16.0	BO	360	P	NA
IR 20 (21%)	110	9.6	18.0	19.8	O	420	F	A
	135	10.1	21.0	32.0	O	420	F	A
	Non-roasted	—	0.0	16.0	O	360	P	NA
CH 45 (22.7%)	140	12.4	14.0	23.5	PT	445	F	A
	150	10.5	13.0	26.4	T	425	VF	A
	Non-roasted	—	5.0	41.6	BO	370	P	NA
IR 20 (25%)	105	12.9	4.0	10.5	DO	425	F	A
	125	11.6	10.0	9.4	T	430	F	A
	150	11.2	5.0	9.2	T	420	VF	A
	Non-roasted	—	5.0	9.2	O	360	P	NA
IR 20 (26.2%)	105	17.1	3.0	2.7	PT	420	F	A
	150	11.3	4.0	6.8	T	410	VF	A
	Non-roasted	—	5.0	9.2	O	360	P	NA

Figures in parentheses indicate moisture of paddy prior to roasting.

*Volume of cooked rice ml/100 g of raw rice.

BO—Bright opaque; O—Opaque; DO—Dull opaque; PT—Partially translucent; T—Translucent.; F—Fluffy like old rice; P—Pasty; VF—Over fluffy like parboiled rice; A—Acceptable; NA—Nota cceptable.

CH 45 was tempered for 1.5 hr, and IR 20 was tempered for 2 hr.

Paddy to sand ratio was 1:25 and sand size - 25+44 BSS.

The rice from the roasted samples were non-pasty and had a higher bulk volume ranging from 410 to 445 ml per 100 g of rice after cooking and resembled stored old rice in their cooking characteristics, whereas the untreated control rice cooked to pasty consistency and had a low bulk swelling volume ranging from 360 to 370 ml per 100 g rice. The visual appearance of the milled rice from the roasted sample depended on the initial moisture content of the paddy and roasting temperature. Lower the roasting temperature and lower the initial moisture content of paddy, more opaque was the rice. High moisture and high roasting temperature caused slight or even full translucency, resulting in full or near parboiling effect.

The mesh size of sand used for roasting as well as the proportion of sand to paddy during roasting have also been found to affect the drying rate and consequently the milling characteristics of the paddy. Too fine a sand (<-44 BSS) because of its higher surface area, rapidly withdrew the moisture from the paddy and resulted in very high crack formation and milling breakage. Similarly high paddy to sand ratio (1:60) caused greater heat transfer and rapid drying and resulted in increased cracking and breakage. Use of medium-fine grade sand (-25+44 BSS) and a paddy to sand ratio of 1:25 gave optimum results.

These results have indicated that sand roasting of paddy followed by 1-2 hr tempering can be practised to get opaque or translucent rice having improved cooking characteristics and free from pastiness. The product is comparable to cured rice or parboiled rice prepared by standard techniques. Paddy with high initial moisture (>22 per cent) is better suited for

parboiling, while low moisture paddy (18-21 per cent) can be employed for preparing 'cured' rice.

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Effect of Long-term Feeding of a Coconut Oil-based Diet and a Combined Coconut Oil-fish Diet on Serum Cholesterol and Fatty Acid Composition of Different Organs of Albino Rats

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The influence of oil sardine (*Sardinella longiceps*) and coconut oil together on the fatty acid composition of various organs and on the serum cholesterol level of albino rats was studied. Fatty acid pattern of the different organs was affected to varying extents by the dietary fatty acids. The level of arachidonic acid in the liver was found to be independent of the dietary supply of this acid, but appeared to be related to the level of linoleic acid in the diet. Dietary sardine was found to be very effective in lowering the serum cholesterol level even in the presence of a cholesterol-elevating agent such as coconut oil. This effect can be due to the high content of polyunsaturated acids in fish lipids as well as the nature of fish proteins.

The type of dietary fat, especially the relative proportions of saturated and polyunsaturated fatty acids, are believed to influence blood lipic levels and the degree of atherosclerosis. Many experimental and epidemiological studies have indicated a relationship between the incidence of atherosclerosis and serum cholesterol levels[1]. Truswell[2] is of the opinion that cholesterol in plasma is in equilibrium with other tissue pools of cholesterol, and that the concentration of total cholesterol in plasma must be taken as the index of metabolically-important cholesterol in the body. Moreover it is the only index that correlates strongly with the risk of pathological atherosclerosis.

Dietary polyunsaturated fatty acids are known to be effective in lowering serum cholesterol levels[3]. Fish and other marine oils are rich in polyunsaturated fatty acids and therefore, can act as strong hypocholesterolemic agents[4-8]. These findings are of special significance to regular consumers of fish. In Kerala, oil sardine is available almost throughout the year at a comparatively low price and forms part of the daily diet for a majority of the population of the region.

Alongside, the dietary fat used is almost exclusively coconut oil. Though earlier workers have elucidated the cholesterol-lowering effects of fish or fish oils, their effect in the presence of hypercholesterolemic agents has not been studied. It was therefore, thought of interest to investigate the effect of a combination of fish and coconut oil on the serum cholesterol level of rats. In addition, the effect of dietary fatty acids on the fatty acid pattern of the liver, heart, kidney, skeletal muscle and fat deposit in the stomach was also studied.

Materials and Methods

Three-week old male albino rats (Sprague-Dawley strain) were used in three groups of five rats each. Details of the diet of each group are given in Table 1. Cholesterol, at 1.0 per cent level, was included in the diets of all the three groups to ensure severe hypercholesterolemia. Half the protein for the third group was obtained from oil sardine, which also provided half the dietary fat in the form of sardine oil. Stock diet without oil and fish was first prepared and the required amounts of oil and fish were then mixed daily. The diet was supplied to the animals at the rate of 10 g (dry wt) /rat/ day initially, and later on this was increased to 20 g/rat/day. Test diets were fed for 90 days. At the end of this period the animals were starved for 24 hr and sacrificed. Blood from each animal in a group was collected for the determination of total cholesterol. The animals from each group were dissected and the heart, liver, kidney and skeletal muscles and the fat deposit in the stomach, were collected for analysis. Each type of tissue from the animals of a particular group was collected together and analysed.

TABLE 1. COMPOSITION OF TEST DIETS GIVEN TO THE THREE GROUPS OF RATS

	Group 1 %	Group 2 %	Group 3 %
Glucose	50.0	50.0	50.0
Wheat flour	25.0	25.0	25.0
Casein	16.0	16.0	8.0
Groundnut oil	5.0	—	—
Coconut oil	—	5.0	2.5
Salt mixture[a]	2.0	2.0	2.0
Shark liver oil	1.0	1.0	1.0
Cholesterol	1.0	1.0	1.0
Oil sardine (dry wt basis)	—	—	10.5[b]
Vitamin mixture[c] (g/kg)	1.0	1.0	1.0

[a]Hubbel, *et al*[28].

[b]Equivalent to 8% protein and 2.5% fat

[c]Chapman *et al*[29].

Lipids from the tissues were extracted with 2:1 (v/v) chloroform-methanol[9] and the chloroform layer was washed by the method of Folch *et al*[10]. The chloroform layer was dried with anyhydrous sodium sulphate and evaporated to dryness. Fatty acid methyl esters of the lipids were prepared by saponification with 0.5 N methanolic KOH followed by treatment with methanolic BF_3[11]. Extraction of lipids and all subsequent operations were carried out in nitrogen atmosphere. The methyl esters were anlysed on a gas chromatograph (Toshniwal), using a flame ionization detector and strip chart recorder, on a column of stainless steel, 6 ft × 0.25 inch o.d. packed with 10 per cent silar 10 C (Applied Science., U.S.A.) on Anakrom ABS, 110-120 mesh (Analabs). Identification of peaks and quantitative estimation of components were carried out as described by Viswanathan Nair and Gopakumar[12]. Fatty acid composition of the feeds fed to the three groups of rats were determined similarly.

Blood samples collected were centrifuged at 2500g at 4°C for 10 min to separate the serum. Total cholesterol in the serum was determined by the method of Sackett[13].

Results and Discussion

Fatty acid compositions of fat in the feeds given to the three groups of rats are shown in Table 2. The feed

TABLE 2. FATTY ACID COMPOSITION (% BY WEIGHT OF TOTAL FATTY ACIDS) OF FEED

Fatty acids	Groundnut oil	Coconut oil	Fish + coconut oil
8:0	0.4	7.5	3.3
10:0	0.3	5.9	2.7
12:0	1.7	20.8	10.2
14:0	1.2	19.6	10.6
14:1	-*	0.3	0.7
16:0	13.8	11.9	14.8
16:1	1.7	1.2	9.3
18:0	5.4	5.2	7.0
18:1	38.5	16.3	16.3
18:2	28.7	9.0	9.6
18:3	3.3	0.9	1.0
20:1	—	1.2	0.9
20:2	—	—	1.9
20:4	—	—	1.3
20:5	—	—	6.2
22:1	5.1	—	—
22:6	—	—	4.3
C_{16} and lower sat. acids	17.4	65.0	41.6
Total C_{18}	75.9	31.4	33.9

*- Not present

containing coconut oil (group 2) was characterised by high proportions (65 per cent) of lower saturated acids, 8:0 to 16:0. This group of acids constitute only 17.4 per cent in the diet of the first group of rats, while for the third group of rats it was 41.6 per cent. Four 18-C acids taken together were the major component fatty acids (75.9 per cent) in the feed containing groundnut oil, and about 38 per cent of this was linoleic acid. In contrast, the other two groups received only one-third of this proportion of linoleic acid.

Fatty acid compositions of the liver, heart, kidney, skeletal muscle and mesenteric fat of the three groups of rats are shown in Table 3. Table 4 gives the distribution of the various acids based on the degree of unsaturation and chain length. Long-chain poly-unsaturated acids were present to a significant extent only in the lipids of organs and not in either skeletal muscle or depot fat; these two being somewhat similar in their fatty acid composition.

Dietary fatty acids have influenced to some extent the fatty acid pattern of all the rat tissues studied during this investigation. 12-C and 14-C acids were present to maximum extent in the group fed coconut oil. Similarly the tissues of rats fed groundnut oil had the highest levels of 18-C acids. Highest levels of 20:5 and 22:6 acids were found in the liver and heart of the rats given fish oil as part of dietary fat. All these indicate a definite shift in the fatty acid profile of the lipids of the various tissues towards the fatty acid pattern of the diet. Beare and Kates[14] showed that the fatty acid composition of the liver triglycerides of rats was altered rapidly by changes in dietary fat. A fairly high proportion of the fatty acids in storage depot fats might also have come from dietary fat, but changes in the composition of these occur rather slowly[15].

The proportion of arachidonic acid in rat liver and heart, however did not depend on its availability in the diet. Rats from groups 1 and 2 received no arachidonic acid at all from the diet, while for the third group, this acid constituted 1.3 per cent of the total dietary fatty acids. Yet this group of rats had the lowest proportion of 20:4 in the lipids of liver and heart among the three groups. Groundnut oil fed rats, which had the highest level of dietary linoleic acid, also showed the highest level of 20:4 acid, and the two seem to be directly related. Diets rich in linoleate have been shown to elevate the

TABLE 3. FATTY ACID COMPOSITION (% BY WEIGHT) OF VARIOUS TISSUES OF RATS FED CHOLESTEROL ALONG WITH GROUNDNUT OIL, COCONUT OIL, AND COCONUT OIL+OIL SARDINE AS SOURCES OF DIETARY FAT

Fatty acids	Liver			Heart			Kidney			Skeletal muscle			Depot fat		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
10:0	-*	—	—	—	—	0.1	0.1	0.3	0.1	—	—	—	0.1	0.3	0.2
12:0:	0.2	1.0	0.4	0.6	3.4	1.6	1.5	3.9	2.4	1.3	5.9	3.8	1.6	5.9	3.7
12:1	0.1	—	—	—	0.3	0.5	—	0.2	—	—	0.2	0.2	—	0.2	0.3
13:0	—	0.1	0.1	0.1	—	0.3	0.1	0.3	0.2	—	0.2	0.3	—	0.3	0.1
14:0	0.8	1.7	1.0	2.2	3.6	2.4	2.9	4.4	3.7	3.0	6.0	5.2	2.7	6.2	4.7
14:1	0.2	0.9	0.4	0.9	1.5	1.2	0.7	1.3	0.9	0.5	0.9	1.0	—	1.2	0.9
15:0	0.1	—	—	—	0.5	0.3	0.2	0.5	0.3	0.1	0.2	0.3	0.4	0.4	0.2
16:0	18.1	18.1	20.3	20.2	19.5	19.0	27.9	24.2	25.6	26.0	25.7	25.9	24.4	24.7	26.0
16:1	4.4	5.6	4.5	5.9	6.8	7.5	9.0	8.4	8.6	8.6	10.7	13.3	9.8	9.0	11.8
17:0	0.3	0.1	0.3	0.4	0.6	0.4	0.2	0.5	0.4	0.1	0.4	0.6	0.2	0.5	0.5
18:0	14.0	14.0	16.6	13.5	11.5	11.5	7.3	7.7	8.4	4.8	4.2	5.0	4.7	4.2	4.6
18:1	24.3	25.4	18.0	29.3	27.1	25.8	28.8	32.1	32.5	40.2	34.8	32.7	39.8	34.9	34.1
18:2	17.3	12.4	10.8	18.1	13.4	13.8	15.9	7.4	7.6	13.0	8.4	8.8	14.6	8.6	9.9
18:3	1.1	1.0	1.0	0.2	0.8	0.5	1.3	1.0	0.8	0.5	0.8	1.0	0.3	0.5	0.4
20:1	0.8	0.7	—	0.4	0.8	0.6	—	1.1	0.6	1.0	0.9	1.1	1.4	2.6	1.0
20:4	15.6	14.0	12.0	8.1	7.9	6.7	3.5	6.4	5.9	—	—	—	—	—	—
20:5	—	1.0	4.1	—	—	1.0	—	—	—	—	—	—	—	—	—
22:1	1.4	0.7	0.8	—	0.5	0.6	0.7	0.1	—	0.3	0.4	0.6	—	0.5	0.8
22:5	—	—	1.7	—	0.3	1.8	—	—	—	0.1	0.3	0.3	—	—	—
22:6	1.1	2.9	8.0	—	1.8	4.6	—	—	—	—	—	—	—	—	—
Unidentified	0.2	0.4	—	—	—	—	—	0.6	2.0	—	—	—	—	—	—

* - Not present. 1, Groundnut oil; 2, Coconut oil; 3, Coconut oil+oil sardine.

TABLE 4. DISTRIBUTION OF FATTY ACIDS (% BY WEIGHT) ACCORDING TO DEGREE OF UNSATURATION AND CHAIN LENGTH IN THE TISSUES OF RATS FED GROUNDNUT OIL, COCONUT OIL AND COCONUT OIL+OIL SARDINE

Falty acids	Liver			Heart			Kidney			Skeletal muscle			Depot fat		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Saturated	33.7	35.4	38.7	37.0	39.1	35.6	40.1	41.8	41.1	35.5	42.9	41.4	34.1	42.5	39.9
Monoenoic	31.2	33.3	23.8	36.4	37.0	35.9	39.2	43.2	42.6	50.6	47.9	48.9	51.0	48.4	48.9
Polyunsaturated	35.1	31.3	37.6	26.4	24.2	28.4	20.7	14.5	14.3	13.5	9.2	9.8	14.9	9.1	10.3
C_{12}	0.7	1.0	0.4	0.6	3.7	2.1	1.5	5.7	2.4	1.3	6.1	4.0	1.6	6.1	4.0
C_{14}	1.0	2.6	1.4	3.1	4.1	3.6	3.5	4.1	4.6	3.5	6.9	6.2	2.7	7.4	5.6
C_{16}	22.5	23.7	24.8	26.1	26.3	26.5	36.9	32.6	34.2	34.6	36.4	39.2	34.2	33.7	37.8
C_{18}	56.7	52.8	46.4	61.1	52.8	51.6	53.3	48.2	47.3	53.7	44.0	42.5	59.4	48.2	49.0
C_{20}	16.4	15.7	16.1	8.5	8.7	8.3	3.5	7.2	6.5	1.0	0.9	1.1	1.4	2.6	1.0
C_{22}	2.7	4.0	10.5	–*	2.7	7.0	0.7	0.1	—	0.4	0.7	0.9	—	0.5	0.8

* – Not present. 1, 2, and 3 as under Table 3.

level of all w6 long-chain unsaturated acids in certain rat tissues[16]. Walker[17,18] has shown that corn oil supplementation of a fat-free diet resulted in increased levels of w6 acids in the total lipids of rat liver and heart. It is known that the desaturation of linoleic acid is the rate-limiting step in the synthesis of arachidonic acid and rat has a highly efficient mechanism to achieve desaturation.[19] The level of arachidonic acid in liver is of special significance because it is known that the cholesterol-esterifying system of rats have some specificity for arachidonate[20-23]. Swell and Law[24] have shown that conversion of cholesterol arachidonate to a polar product is substantial and that this may be a major pathway in cholesterol metabolism in rats. It is highly significant that the proportion of arachidonic acid was higher in rats which were receiving no arachidonic acid or other higher polyunsaturated fatty acids from the diet (but high amount of linoleic acid), than in animals receiving a dietary supply of these acids. The findings of the present study show that availability of linoleic acid or other precursors of arachidonic acid is a very important factor in cholesterol metabolism in rats.

Total cholesterol content of the serum of the three groups of rats are shown in Table 5. The group fed coconut oil had a substantially higher level of total blood cholesterol than the other two groups. When oil sardine was included in the diet to replace half the coconut oil, the serum total cholesterol was lower than even that for groundnut oil-fed group. Variations in the blood cholesterol levels within groups were minimum in the coconut oil-fed group and maximum in the fish-coconut oil-fed group and the difference in blood cholesterol levels between these two groups was highly significant (at 1 per cent level, $F=137.8$). This clearly shows that dietary oil sardine is very effective in lowering serum cholesterol levels in rats. It is known that fish oils or whole fish are good hypocholesterolemic agents[6,8] The present findings clearly demonstrate that fish is effective in lowering the serum cholesterol levels of rats in the presence of a highly cholesterol-elevating agent such as coconut oil.

TABLE 5. TOTAL SERUM CHOLESTEROL IN THE THREE GROUPS OF RATS

Groups fed	Total serum cholesterol (mg/100ml) (Mean±SD)	Coeff. of variation
Groundnut oil	93.1±4.2	4.28
Coconut oil	137.5±4.6	3.55
Coconut oil+fish	93.8±4.9	5.22

The mechanisms involved in the cholesterol-lowering or elevating effects of dietary fats are not well understood. Many factors seem to be involved. Reviewing the role of fat in cholesterol metabolism, Truswell[2] states that saturated fatty acids, especially of lower chain length, exert a strong cholesterol elevating effect. The present findings with coconut oil also indicates this observation. Crocker *et al.*[25] found that a combination of hydrogenated coconut oil and cholesterol apparently decreased the ability of guinea pigs to incorporate polyunsaturated fatty acids into cholesterol esters. Since the transport form of cholesterol in these are esters[26], inhibition of their formation naturally results in accumulation of cholesterol.

Rats fed fish as part of the diet behaved differently. Even though the proportion of lower saturated fatty acids in the feed was almost 2.5 times that in the groundnut oil-based feed, the serum total cholesterol level was actually slightly lower than for the latter group. Adding

fish to the diet changes the nature of both the fat and protein components of the diet, and the lower serum cholesterol level could be a result of the combined effect of these two factors. Lipid-depressant activities of marine oils could be due to a combination of factors, including total unsaturation, type of unsaturation, and proportions of specific polyunsaturated fatty acids[6]. Apart from their role in the transport of cholesterol[25], polyunsaturated fatty acids have a tendency to cause cholesterol to accumulate in liver, thus lowering the cholesterol level in serum[25]. Moreover, cholesterol esterified with polyunsaturated acids are more readily catabolised and disposed off than its esters with saturated acids. Thus the higher proportion of characteristic long-chain polyunsaturated fatty acids in fish appears to be one of the factors that has helped to bring down serum cholesterol level of the rats, given a fish-based feed.

Table 1 shows that half the protein given to rats in group 3 was casein and the other half came from the oil sardine. This might also have exerted some influence on the metabolism of cholesterol. Findings on the hypocholesterolemic effects of fish oil *vis-a-vis* whole fish are not always in agreement. Sen *et al*[8]. found that whole oil sardine fish were more effective in lowering serum cholesterol levels in rats than the oil pressed out of them, but Peifer *et al*[6]. have reported that whole fish and an equivalent quantity of oil had equal hypocholesterolemic effects. Sen*et al*[8]. conclude that a high proportion of oil in the diet may have an overwhelming effect and that other influences are masked by this. Chevallier[27] points out that in order to study the effect of any agent on cholesterol metabolism, it is necessary to consider the absorption coefficient of cholesterol under the given conditions. This absorption coefficient is lower when diet is poor in casein, whatever the proportion of lipids, than when the diet is rich in casein. With diet poor in casein, the faecal excretion rates of cholesterol are higher than with corresponding diets rich in casein. Thus it seems reasonable to conclude that substitution of one half of casein with fish protein in the diet might also have played a role in bringing down the cholesterol level in the present studies.

The finding that the oil sardine fish is capable of lowering substantially the serum cholesterol levels in rats when fed along with diets containing strong cholesterol-elevating agents such as coconut oil is of great significance in the diet of the people of Kerala. The present practice of inclusion of a moderate amount of fish in the diet, might well mitigate any harmful hypercholesterolemic effects of coconut oil.

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Gulabjamun from Khoa Powder

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Gulabjamun—a khoa-based sweet was prepared using fresh as well as stored cow and buffalo khoa powder. Cow khoa powder had a characteristic khoa-like flavour as against slightly cooked flavour in the case of buffalo khoa powder. The body and texture of the *gulabjamuns* prepared from cow's milk khoa powder were soft and spongy, while those from buffalo milk khoa powder were hard and compact. Cow khoa powder stored for 120 days could still be used for the preparation of acceptable quality *gulabjamuns*, whereas fair quality *gulabjamuns* could be made from buffalo khoa powder stored for 75 days.

Khoa used as a base material for the preparation of *gulabjamun* (a sweet prepared from milk solids and fat) has short storage life even at refrigerated temperature. Hence the scarcity of khoa is felt specially during summer months. Thus any increase in the shelf-life of khoa will obviate the shortage. Attempts to increase the keeping quality of khoa by various means such as irradiation[1-4] and gas packing[5,6] have met with limited success. Patel and De[7] reported the manufacture of buffalo milk khoa powder of substantial shelf life for the preparation of *Peda*. The present work was undertaken to compare the suitability of cow and buffalo khoa powder for the preparation of *gulabjamuns*.

Materials and Methods

Preparation of khoapowder: The khoa powder samples were prepared using milk from the Institute's experimental dairy after standardization to 4.0 per cent and 5.0 per cent fat for cow and buffalo milk respectively, by the method of Patel and De[7] with slight modification as shown in Fig 1.

Preparation of gulabjamuns: (*i*) *Control*—The control *gulabjamuns* were prepared from standardized milk khoa by the conventional method as delineated by Gill and De[8].

(*ii*) *Experimental*[8]*:* To 100 g of khoa powder 10 g of *maida* and 1 g baking powder was added and mixed dry. To this mix, 25-30 ml of water was added and the mix worked into dough, which was then formed into balls and fried in ghee. The fried balls were then soaked in sugar syrup[8].

Sensory evaluation: The khoa powder and the *gulabjamun* samples were subjected to sensory evaluation by a selected panel of judges, using a 9-point hedonic scale. The khoa powder samples were analysed for fat and moisture[9], protein[10], ash[11], lactose (by difference), free fat, solubility index and bulk density[12].

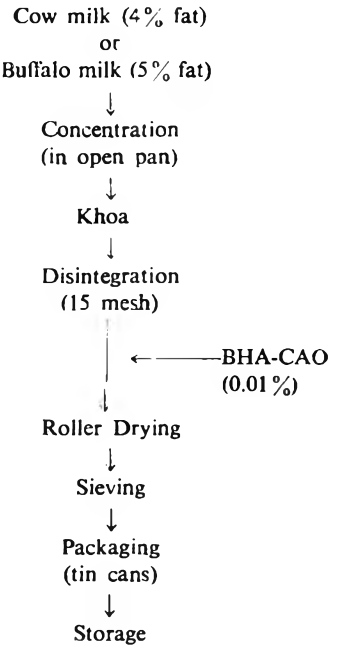

Fig. 1. Flow diagram for the manufacture of khoa powder from cow or buffalo milk.

Results and Discussion

The khoa powders from both cow milk and buffalo milk were of good quality and acceptable (Table 1), although the latter showed slightly cooked flavour. Our results regarding quality of khoa powder are similar to that reported by Patel and De[7] for buffalo khoa powder. The chemical characteristics as revealed by chemical analysis showed that cow milk khoa powder (CKP) and buffalo milk khoa powder (BKP) contained, moisture 3.0 and 2.9 per cent; fat 31.3 and 33.9 per cent; protein 24.1 and 27.0 per cent; lactose 36.8 and 30.9 per cent; ash 4.8 and 5.3 per cent; solubility index

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TABLE 1. SENSORY SCORE OF KHOA POWDER (HEDONIC SCALE)

Type of khoa powder	Colour	Body & texture	Flavour
Cow	8.0	8.0	8.0
Buffalo	8.0	8.0	7.0

TABLE 2. CHARACTERISTICS OF *GULABJAMUN* PREPARED FROM KHOA POWDERS

Type of Khoa powder	Flavour	Body & texture	Sensory score	Remarks
Cow	Khoa like	Soft and spongy	8.0	Acceptable
Buffalo	Slight khoa	Hard and compact	6.0	Crust hard not spongy
Fresh khoa	Typical of khoa	Soft and spongy	9.0	Excellent

13.0 and 14.5 ml; bulk density (*i*) loose 0.33 and 0.27 g/ml; (*ii*) packed 0.43 and 0.35 g/ml and free fat (as percentage of total fat) 58.70 and 64.20 respectively. These data for BKP compare favourably with those obtained by Patel and De[7].

The suitability of the prepared khoa powders for preparing *gulabjamun* shows that an acceptable quality of *gulabjamuns* (Table 2) could be made from cow milk khoa powder which had flavour similar to that of *gulabjamuns* made from fresh khoa. On the other hand, the *gulabjamuns* prepared from BKP had only slight flavour of fresh khoa *gulabjamuns* and had hard body and lacked characteristic spongy texture. The crust was hard and had a tendency to peel-off. Although Patel and De[3] used their BKP for the preparation of *Peda*, they also prepared *gulabjamuns* which possessed hard body and non-spongy texture (unpublished report).

The stored khoa powders were used for the preparation of *gulabjamuns* at an interval of 15 days and the organoleptic scores (overall acceptability) are given in Table 3. The organoleptic score for *gulabjamuns* made from CKP stored for 120 days shows that fairly good quality *gulabjamuns* could be prepared, assuming that a score of 6.0 (liked slightly) is considered as minimum acceptable. The acceptability score for *gulabjamuns* prepared from BKP stored for 75 days at room temperature was 5.7 (Table 3) which is in close agreement with the result obtained by Patel and De[7] for the acceptability score of *Peda* (6.0) made from BKP stored at room temperature for a period 75 days.

Acknowledgement

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TABLE 3. SENSORY SCORE OF *GULABJAMUN* MADE FROM STORED KHOA POWDERS

Type of khoa powder	Storage temp. (°C)	Storage period, (days)							
		0	30	45	60	75	90	105	120
Cow	* 16-30	8.0	8.0	8.0	8.0	7.5	7.0	7.0	6.5
	37±1	8.0	7.0	6.6	5.8	5.0	4.0	3.0	1.0
Buffaloes	* 16 - 30	6.0	6.0	6.0	6.0	5.7	5.5	5.5	—
	37±1	6.0	5.6	5.4	5.0	4.0	2.0	2.0	—

* Room Tempreature

Metanil Yellow Binding with Some of the Foodstuffs

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Metanil yellow, C.I. acid yellow 36 (13065), the sodium or calcium salt of m—[p-(anilinophenyl)azo] benzene sulphonic acid, is one of the most commonly used water soluble non-permitted azo dyes to colour the foodstuffs, even to pulses with a false presumption, that it could be washed out in pre-cooking washings of red gram dal (*Cajanus cajan*). The experiments on the binding capacity and extractability of metanil yellow from coloured dal, rice, casein and starch showed that it could not be washed out completely even after repeated washings. This is due to the deep penetration and firm binding of the dye with grain constituents. Preliminary study indicates that the binding capacity of the dye is more with protein than with starch.

Metanil yellow (C.I. Acid yellow 36, 13065), the sodium or calcium salt of m-[p-(anilinophenyl) azo] benzene sulphonic acid, is the most commonly used water soluble azo dye to colour various eatables[1]. Our recent comparative study on the use of colours in foodstuffs collected from rural and urban markets confirms that this is the most frequently encountered colour[2]. Besides finding use in a variety of eatables, this dye is occasionally employed to colour raw red gram dal (*Cajanus cajan*) to make it look brighter to customers. The use of metanil yellow in raw pulses is not taken seriously because of the false presumption that the dye being water soluble, will be completely eliminated during household pre-cooking washings. An enquiry from Director General of Health Services, Govt. of India, in this regard prompted us to confirm if the colour could be easily and completely washed away during pre-cooking washings of the grains. The outcome of the experiments on the binding capacity and extractability of metanil yellow from red gram dal, rice, casein and starch has been described in this communication.

Materials and Methods

Red gram (Arhar or Tur dal) (*Cajanus cajan*), Rice (*Oryza sativa*), technical grade casein and starch samples were purchased from the market. Metanil yellow was procured from M/s Vesco Products Ltd., Calcutta.

Sample preparation: The aqueous solution of metanil yellow was mixed with 5.0g of the food material at 0.02, 0.1, 0.5 and 1.0 per cent concentrations. For complete soaking, the sample was kept for 12 hr at room temperature, dried and used in the present experiment.

Water washing and ethanol extraction of the sample: Samples of dal, rice, casein and starch were washed with 10-folds of water each time till the washings became colourless. Number of the washings were recorded. To get the maximum extraction, the samples were subjected to soxhlet extraction using 80 per cent ethanol. The residual materials were powdered and reextracted in soxhlet with 80 per cent ethanol. Effects of repeated hot water washings and single large volume overnight soaking with water were also seen.

The amount of metanil yellow was estimated in water washings and ethanolic extracts by the method of Srivastava *et al.*[3]

Histological studies: Sections of water soaked dal were cut at 8 μ thickness with freezing microtome and stained with HCl for the presence of metanil yellow which gets changed from yellow to violet in acidic medium.

Results

Red gram dal: 49.4 to 61.1 per cent of the dye could be separated by combined 30 water washings and 22.7 to 38.3 per cent dye was extracted by 80 per cent ethanol. Water and alcoholic extraction of metanil yellow decreased as the concentration of dye in dal increased from 0.02 to 1.0 per cent. Total recovery of the dye from both the processes was 71.1 to 99.4 per cent (Table 1).

Rice: The recovery pattern of metanil yellow from coloured rice was almost the same as in dal (Table 2). However, from rice samples not more than 66.9 to 88.2 per cent metanil yellow could be recovered from both water and 80 per cent ethanol washings. In 29 water washings, 14.6 to 60.5 per cent of the dye was extracted. Further, extraction with 80 per cent ethanol could remove 19.8 to 85.5 per cent of metanil yellow.

Casein: Though the recovery of metanil yellow

TABLE 1. RECOVERY OF METANIL YELLOW FROM COLOURED DAL

Metanil yellow (%)	Dye recovery		Total recovery (%)
	Water (%)	80% ethanol (%)	
0.02	61.1	38.3	99.4
0.10	59.9	23.7	83.6
0.50	58.7	25.4	84.1
1.00	49.4	22.7	71.1

TABLE 2. RECOVERY OF METANIL YELLOW FROM COLOURED RICE

Metanil yellow (%)	Dye recovery		Total recovery (%)
	water (%)	80% ethanol (%)	
0.02	0.0	85.5	85.5
0.10	14.6	66.8	81.5
0.50	60.5	27.5	88.2
1.00	47.0	19.8	66.9

TABLE 3. RECOVERY OF METANIL YELLOW FROM COLOURED CASEIN (TECHNICAL GRADE)

Metanil yellow (%)	Dye recovery		Total recovery (%)
	water (%)	80% ethanol (%)	
0.02	30.0	70.0	100.0
0.10	35.5	63.6	99.1
0.50	85.3	13.3	98.6
1.00	93.3	6.6	99.9

TABLE 4. RECOVERY OF METANIL YELLOW FROM COLOURED STARCH (SOLUBLE STARCH)

Metanil yellow (%)	Dye recovery		Total recovery (%)
	water (%)	80% ethanol (%)	
0.02	91.5	8.5	100.0
0.10	96.9	3.1	100.0
0.50	96.1	2.7	98.8
1.0	93.0	3.2	96.2

from coloured casein was 100 per cent from combining both the processes, it required 84 water washings for complete leaching of the colour (30.0 to 93.3 per cent). The leaching of the dye increased as the concentration of metanil yellow increased (Table 3). The amount of dye extracted by 80 per cent ethanol ranged from 6.6 to 70.0 per cent.

Starch: Recovery of the dye from coloured starch was from 91.5 to 96.9 per cent by 20 water washings. Remaining dye could be extracted by 80 per cent ethanol (3.2 to 8.5 per cent). In this case also all the dye was recovered by combining both the processes (Table 4).

Effect of soaking and hot water washings: Washing and soaking of grains overnight with a single large volume of water could only remove a fraction of the dye (average 10 per cent). Hot water washings, although could remove more of the dye in first few washings, had no additional advantage as far as the complete extraction was concerned. Even after 30 elevated temperature (60-70°) water washings, almost 40 per cent of the dye still remained bound with the dal/rice as was the case with cold water washings.

Histological studies: Examination of the cotyledon of *Cajanus cajan* (Fig. 1 and 2) revealed that metanil yellow penetrated inside and was bound with starch granules.

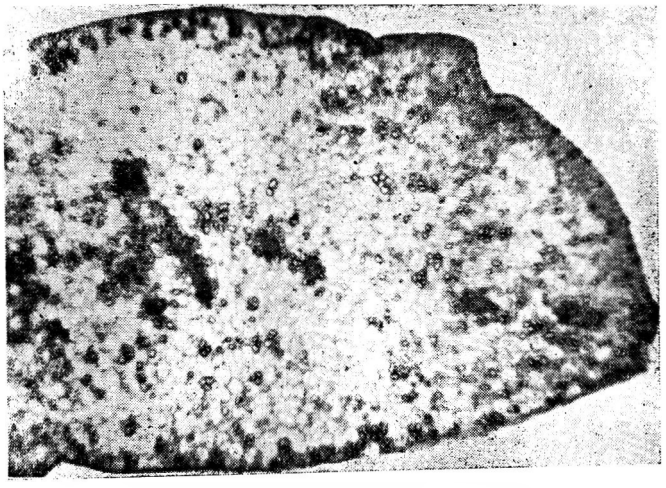

Fig. 1. Section of the control cotyledon of *Cajanus cajan*.

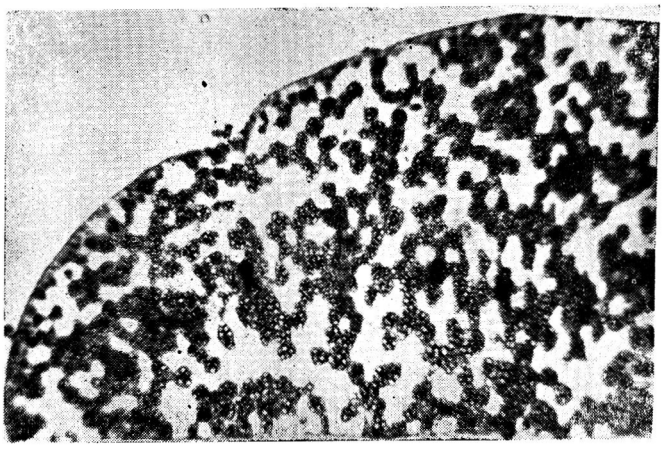

Fig. 2. Section of the coloured cotyledon of *Cajanus cajan* (stained with HCl.). Dark metanil yellow coloured granules are visible.

Discussion

In routine household cooking, rice and pulses are washed only once or twice with water before use. Our studies showed that as many as 30-40 water washings could not wash out all the dye. The colour could not be completely removed even after washing with 80 per cent ethanol. The recovery of the dye is always lesser at the higher concentrations, perhaps due to its deep penetration and firm association with grain constituents. Our experiments show that recovery of metanil yellow by water washing from casein is less than that from pure starch. Also, it took over 84 water washings to extract out all the leachable dye from casein compared to 20 washings required in case of starch. This may be interpreted to mean that the dye binding capacity of protein is more than that of pure starch.

The differences observed in the extraction profile of dye from dal and rice to those noted in isolated pure casein and starch may suggest that histological texture and other bioconstituents of grain do play a role in the binding and extractability pattern of the dye. It is, thus, obvious that metanil yellow once added to pulses or rice even if washed several times during pre-cooking washings can enter the body and produce harmful effects.[4]

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Microwave Technique for Detection of Milk Powder Loss Through Spray Dryer Exhaust Air

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Studies on absorption of microwaves in milk powder are reported. The investigations were carried out in an experimental set up at x-band of frequencies (8-12.4 GHz). For a given flow rate, the microwave absorption at 8.6 GHz and temperatures 35°C and 85°C was found to be 0.35 dB and 0.30 dB respectively. It was also noted that microwave absorption increases with frequency for a given particle size. Therefore, this principle of microwave absorption is, further, found suitable for detecting milk powder loss through spray dryer exhaust air.

In a milk spray dryer, concentrated milk of about 48 to 52 per cent concentration is sprayed with the help of pressure or disc atomizer in a drying chamber. Hot air having temperatures from 150-300°C is used as drying medium as it presents less chances of contamination. Powder particle size varies from about 5 to 200 microns[1].

The powder from the exhaust air is separated with the help of cyclone separators, bag filters and wet scrubbers. The cyclone separator is more effective in separating large particles. It separates about 98 per cent of the particles above 20 microns in diameter and about 90 per cent of all particles of 10 microns in diameter and larger[2]. A properly designed cyclone is capable of separating upto 99 per cent solids depending upon the size[1]. The efficiency can be increased by employing many cyclones in parallel. The bag filters are often employed as secondary collectors after cyclone separator. The bag filters can separate particles as small as 0.1 micron size. The collection efficiency can be as high as 99 per cent under proper operating conditions[1]. The wet scrubbers have very high efficiency of separation. However, there is a possibility of contamination and product deterioration[1]. Another means of separating entrained powder particles is electrostatic precipitation. Gunjal[3] in his experiments on electrostatic precipitation found that the maximum efficiency of separation at the applied voltage of 35 kv was 99 per cent.

Fine particles usually upto 50 micron size go with the outlet air. In general, the loss of powder through exhaust, in spray drying plants, ranges from 0.5 to 3 per cent of production rate.

At the current price of milk powder, even the smallest quantity of milk powder loss would amount to a considerable annual financial loss. For instance, a dryer producing skim milk powder at the rate of 48 t. a day over a year of 300 working days with an exhaust loss of 1 per cent, gives a financial loss of about rupees 21.6 lakhs. Thus exhaust losses from all the milk spray drying plants amount to a direct loss of more than one crore rupees per year to the country.

In addition to the financial aspects, the air pollution requirements are becoming more strict. Emission standards of 100 ppm (100 mg/m^3 air) could well be enforced in the future. This is equivalent to a collection efficiency of 99.7 to 99.8 per cent[4]. Conventional cyclones cannot achieve this level of collection efficiency. This powder loss also creates a problem of dust in the building and leads to unhygienic environment in the plant.

The magnitude of powder loss cannot accurately be estimated if it is not measured in the exhaust air. If the powder loss, through the exhaust air, is increased due to certain reasons such as faulty operation of the dryer, it will escape notice if an accurate technique is not available for measuring the loss.

Dornyei *et al*[5]. have developed an instrument for measuring powder losses in spray drying installations. The instrument incorporates a cyclone probe which is inserted into the air outlet vent of the installation, as well as Prandt l tube which is used to measure the dynamic pressure of the gas at various points across the diameter of the outlet vent. The specific powder concentration of the air (g/m^3) is determined by dividing the amount of powder captured in a special receptacle and filter by the amount of gas sucked through the cyclone probe. Powder losses/hr are then calculated by multiplying the specific powder concentration of the air by the amount of air discharged per hour.

From the available literature, it was confirmed that no such instrument is available in India by which the loss of powder in the exhaust air may be directly and continuously measured. The microwave absorption technique which is simple and economical was selected for the measurement of powder loss in this study.

The study was conducted with particle size above 100 microns only. Particles less than 100 microns were in lump and could not be separated in different size groups by the Laboratory sieves. No effort is made to draw a conclusion from this study about the particle size less than 100 microns. The paper merely suggests a technique. However, to arrive at some definite conclusion for the particle size less than 100 micron, studies have to be conducted.

Materials and Methods

A suitable circular wave guide (Fig. 2) was designed and fabricated. A block diagram of experimental set-up used for the measurement of the microwave absorption in milk powder is shown in Fig. 1. The following assumptions were made for the measurement of microwave absorption in the milk powder.

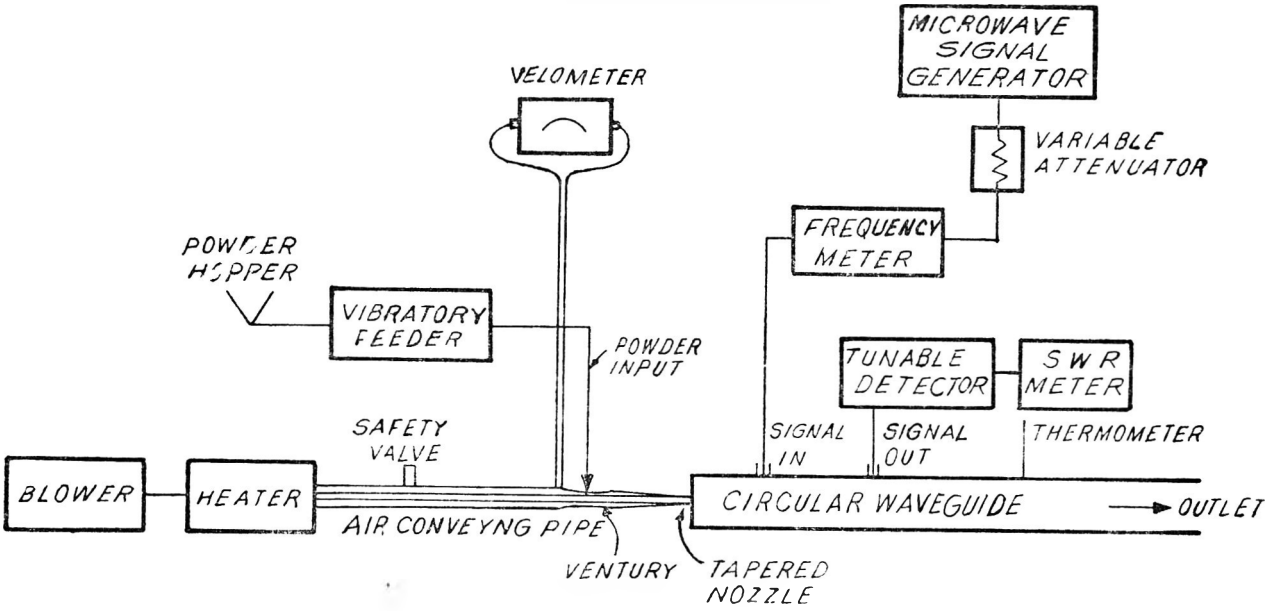

Fig. 1. Block diagram of the experimental set up used for the measurement of the microwave absorption in milk powders or any other dust like materials.

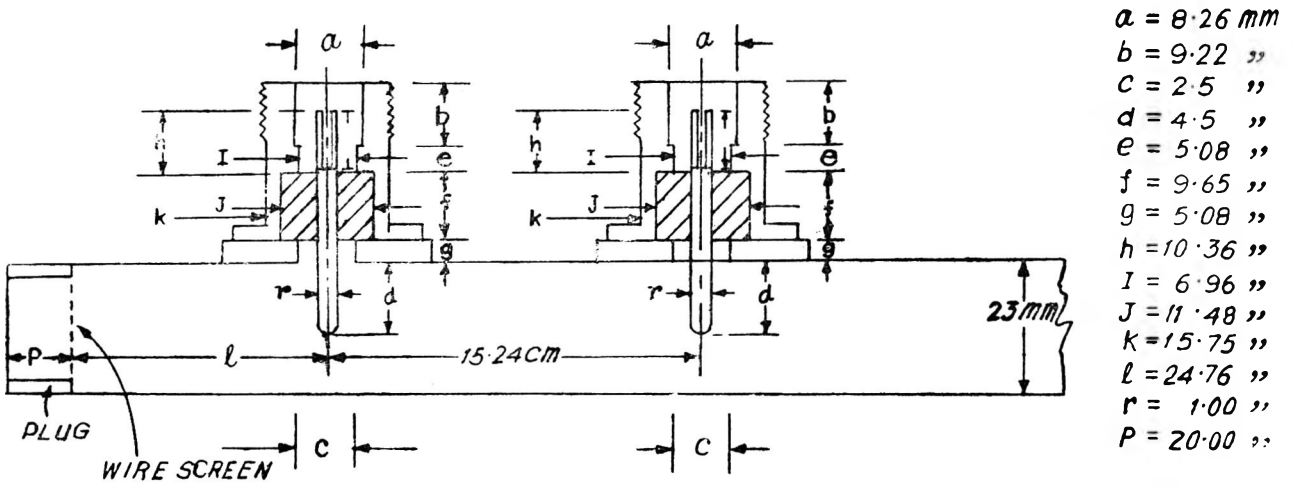

Fig. 2. X-band circular waveguide with coaxial N-type female B N C connectors

The concentration of powder in the air was constant which was insured by maintaining a constant powder feed rate and air flow rates.

The absorption in the air contained in the waveguide was assumed to be negligible. Though the atmosphere of the earth contains many gases which absorb microwaves in varying amounts, only oxygen and water vapour produce significant attenuation. The energy absorbed by these gases is converted to heat and is lost.

The factors affecting microwave absorption in powder are (*i*) amount of powder, (*ii*) temperature, (*iii*) powder particle size, and (*iv*) relative humidity. The variables studied are:

Concentration, p.c. : 0.608, 0.365, 0.260, 0.202, 0.159
Temperature, °C : 35, 45, 55, 65, 75 and 85.
Average particle size, microns : 100, 164, 184 and 205.

These studies were conducted on skim milk powder, whole milk powder and baby food (milk) powder.

The standing wave ratio meter (a high gain amplifier) was used to measure the absorption (Fig. 1). Klystron frequency was set to various values such as 8.6, 8.9, 9.2 and 9.5 GHz and at each frequency the absorption of the microwave signal was measured between two connectors separated by a distance about of 2 λg for various values of powder concentration in the air.

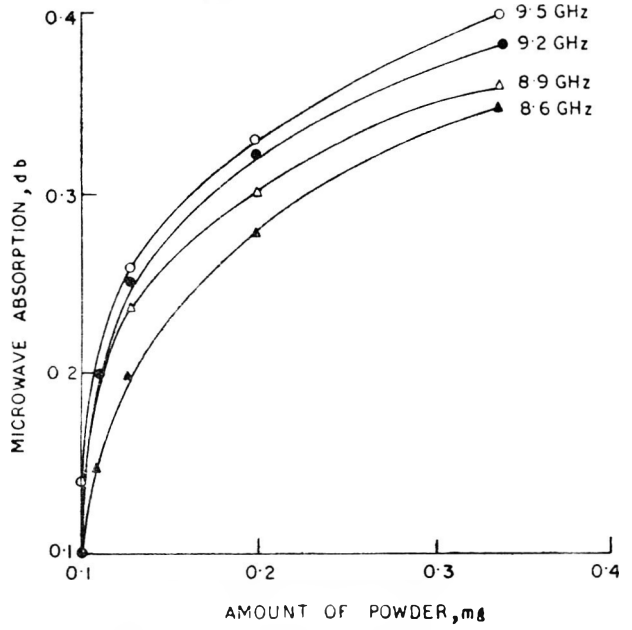

Fig. 3. Variation of microwave absorption by skim milk powder of average particle size of 100 microns with its mass at different frequencies and at 35°C.

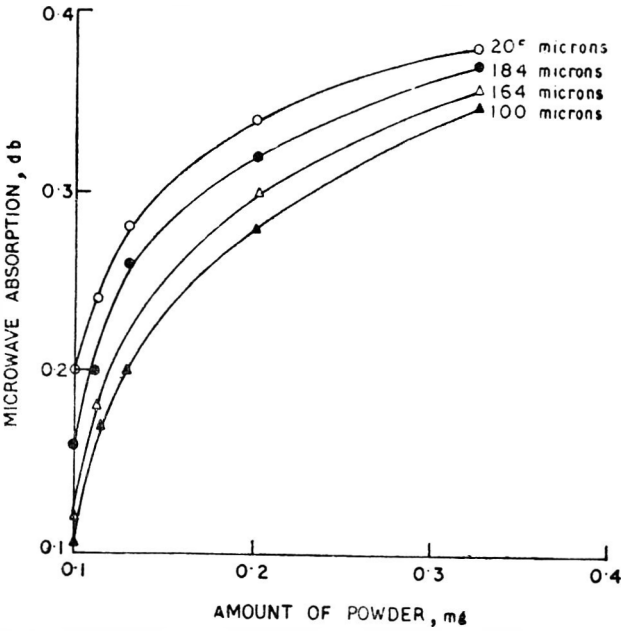

Fig. 4. Variation of microwave absorption by skim milk powder of different particle sizes at 35°C and at microwave frequency of 8.6 GHz.

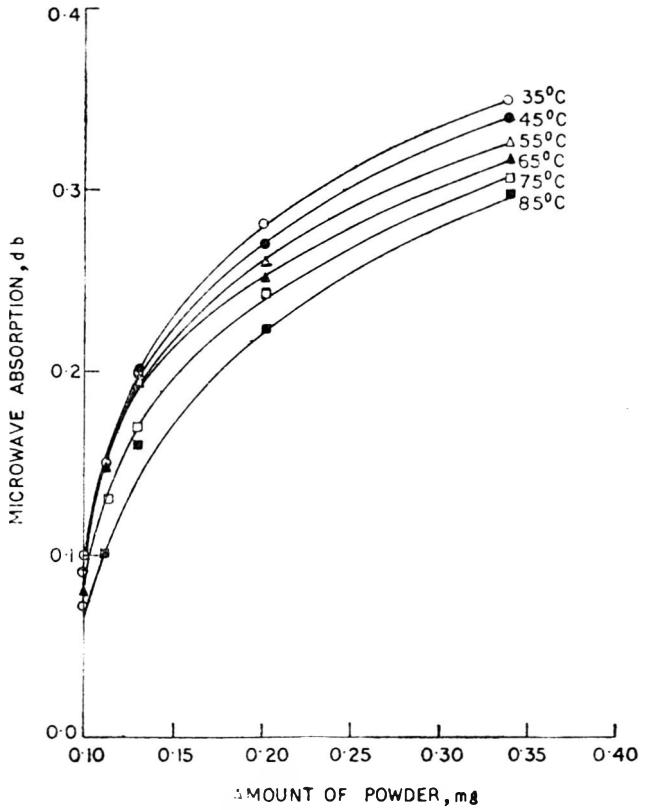

Fig. 5. Variation of microwave absorption by skim milk powder of average particle size of 100 microns at different temperatures and at microwave frequency of 8.6 GHz.

Each concentration was tested for the effect of temperature on microwave absorption at a frequency of 8.6 GHz only (Fig. 4).

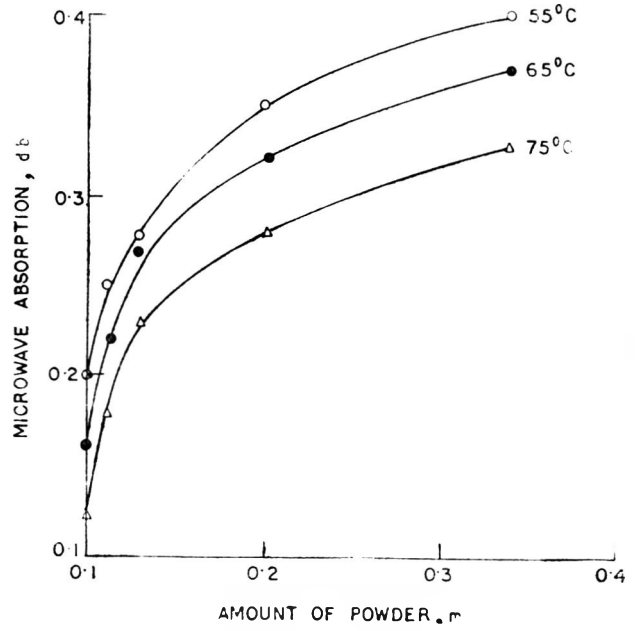

Fig. 6. Variation of microwave absorption by whole milk powder at different temperatures and at 8.6 GHz. microwave frequency.

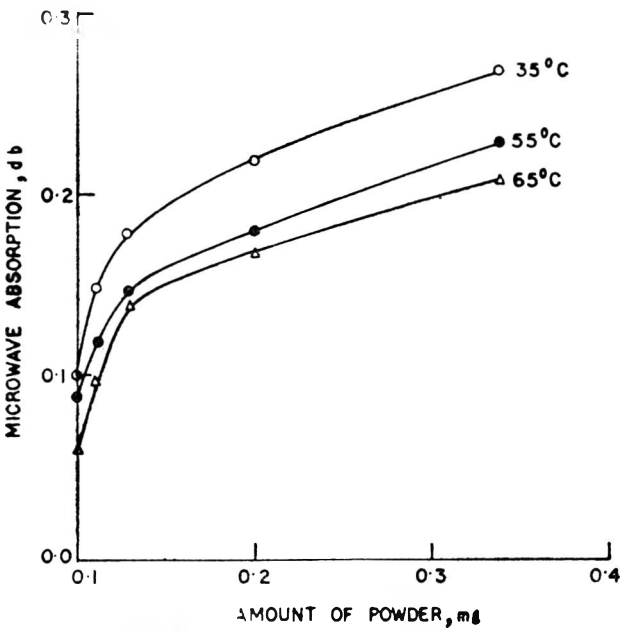

Fig. 7. Variation of microwave absorption by baby-food (milk) powder at different temperatures and at microwave frequency of 8.6 GHz.

To get a constant flow of the powder for a particular concentration, the blower was put on to give a measured air flow and this air was charged with the milk powder through an orifice which was coupled to a vibratory feeder.

To measure absorption of the signal through the powder flowing down the waveguide, the SWR meter, was used. The meter was peaked by tuning the probe detector. The dB scale of this meter was used to measure the power level and hence the absorption of signal. Since the absorption measured was a difference in power levels at the fixed connectors, the results were not affected by pure air flow at high speed and humidity.

The absorption measured was less for higher air flow rates and more for lower air flow rates as it should be. The amount of power was less in the first case and more in the second and hence the data were physically reliable.

Results and Discussion

The curves plotted for the average values of microwave absorption by skim milk powder at different temperatures and frequencies are shown in Figs. 3,4 and 5. The curves are of second order illustrating that absorption of microwave in milk powder is not linear with the amount of powder. The curves plotted for the average values of microwave absorption by whole milk powder and baby food at 8.6 GHz frequency and at different temperatures are shown in Figs 6 and 7 respectively. An analysis of the data reveal that these are related in the form $y=a+bx+cx^2$

Effect of temperature on microwave absorption: As shown in Fig.5 for the air temperatures of 35, 45, 55, 65, 75 and 85°C the corresponding values of microwave absorption are 0.35, 0.34, 0.33, 0.32, 0.31 and 0.30 dB respectively for the same amount of milk powder at 8.6 GHz frequency. It shows that the micowave absorption decreases as the temperature of air increases. It was also true at 9.2 GHz and 9.5 GHz frequency as shown in Fig. 3.

Effect of frequency on microwave absorption: From Fig. 2, it is evident that for the values of frequency of 8.6, 8.9, 9.2 and 9.5 GHz the corresponding values of microwave absorption are 0.35, 0.36, 0.38 and 0.40 dB respectively. It shows that the microwave absorption in skim milk powder increases as the operating frequency is increased.

Effect of particle size on microwave absorption: As shown in Fig. 4, for the values of average particle sizes of 100, 104, 184 and 205 microns the corresponding values of microwave absorption by skim milk powder are 0.35, 0.36, 0.37 and 0.38 dB respectively for the same amount of powder. It may, therefore, be concluded that the microwave absorption increases with the particle size.

Design of powder loss detector in a powder plant: Assuming a spray dryer exhaust pipe of 50 cm radius, the operating frequency band can be calculated as follows.

Calculation of operating frequency band: In a hollow circular waveguide TE_{11} mode is dominant. For TE_{11} mode, the cut off wave length and cut off frequency is

$$\lambda c\ TE^{6}_{11} = 3.412\ a$$

$$fc\ TE_{11} = \frac{u}{\lambda c} = \frac{3\times10^{10}}{3.412\times50} = 176\ \text{MHz}$$

where, u is the velocity of e.m. waves, cm/sec.
λc is the cut off wave length, cm;
fc is the cut off frequency, sec^{-1}

For next higher order mode TMol
λc TMol $= 2.61\ a^{6}$

$$fc\ \text{TMol} = \frac{3\times10^{10}}{2.61\times50} = 230\ \text{MHz}$$

Therefore, safe operating frequency could be taken to be =200 GHz.

Therefore, safe band of frequencies would be 10 per cent around 200 MHz and hence frequency band will be 190—210 MHz. It is therefore, assumed that over this frequency band waveguide loss will not vary with frequency. So operating frequency will be 0.190 to 0.210 GHz.

To fix the separation between the probes the guide wave length at 200 MHz would come out to be 2.35 metres.

Calculation for exhaust pipe having any diameter can be made by above mentioned procedure. The above calculation shows that for the plants where the exhaust pipe diameter ranges from 100 to 50 cm, a study of the absorption of frequencies between 100-300 MHz is to be made, so that proper instrumentation namely a dial of red or green signalling indicator may be fitted on the control panel. It is suggested that an average power of 10 watts at 100-300 MHz will be enough and will not require very sophisticated detecting equipment.

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Kinetics of Water Vapour Sorption by Wheat Flour from Saturated Atmosphere

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Kinetics of moisture absorption by wheat flour from saturated atmosphere was studied. The absoroption process followed first order kinetics in the difference ($m-m_e$). The process rate constant obeyed Arrhenius law with a discontinuity between 343 and 348 K. The corresponding activation energies had the same order of magnitude as those for adsorption and hydration. Except for the discontinuity between 343 and 348 K, calculated values of equilibrium moisture content were proportional to water vapour pressure raised to a fractional power. The heat of condensation did not vary much with temperature and had about the same value as that for water.

Conditioning flours by absorption of water vapour from saturated atmospheres is an important step common to processing of many flour based foods. Baking characteristics are believed to be related to flour hydration properties. In addition, such an absorption process is also important from the stand point of flour storage. Therefore, an understanding of the absorption process kinetics is of considerable practical utility, both in the design of moistening process and of related equipment. Such a kinetic model could also be used for convenient evaluation of equilibrium moisture content in a reasonably short time.

Most of the published material on water vapour absorption by wheat flour relates to equilibrium moisture content and only a few to the kinetic aspect. Earlier studies[1,2] on vapour absorption process have been limited to room temperature and humidities below saturation. Udani *et al*[3] presented the first mathematical model for the sorption kinetics. They proposed that

$$\frac{m - m_o}{m_e - m_o} = 1 - \exp(-t/R) \qquad \ldots.(1)$$

where m represents dry basis moisture content with subscripts o and e standing for the initial and equilibrium values respectively; t stands for time and R is the absorption rate characteristic constants. None of the studies considered the effect of temperature. Eq(1) implies that

$$\frac{dm}{dt} = \frac{1}{R}(m_e - m) \qquad \ldots.(2)$$

Eq(2) is not a diffusion model unless the surface resistance is very high compared to diffusive resistance so as to be controlling the rate process. In the experiments of Udani *et al*[3]. this could not have been so. If it is considered that hydration of the surface of the flour particle is the first step preceding entry into the main body, then m in eq (2) should be replaced by m' representing point moisture content on the surface of the particle, because all sorption sites are not equally accessible to the surrounding atmosphere. If the flour particles were extremely small or so porous that all surface sorptive sites were equally available, then m' will approximately equal m' and eq (1) will hold.

Materials and Methods

Studies were conducted at 100 per cent relative humidity and at eleven temperature levels in the range of 293 to 368 K including the end points. Flour samples were prepared by grinding one kg of cleaned wheat (variety 'RR-21', from 1975 harvest) on a Brabender Quadrumat mill. The ground material was passed through a 600 μ sieve and after thorough mixing divided into a large number of samples each weighing 40 g. Each individual sample was sealed in a separate polyethylene envelope and stored at room temperature in an empty desiccator. Out of 40 g, about 12 g were utilized for moisture sorption studies and the rest for initial moisture content determination.

The experimental set up used for the study has already been described by Narain *et al*[4]. A schematic diagram of the set up is given in Fig. 1. The temperature of the test chamber could be maintained within ± 0.5°C. Saturated atmosphere was created by evaporation from

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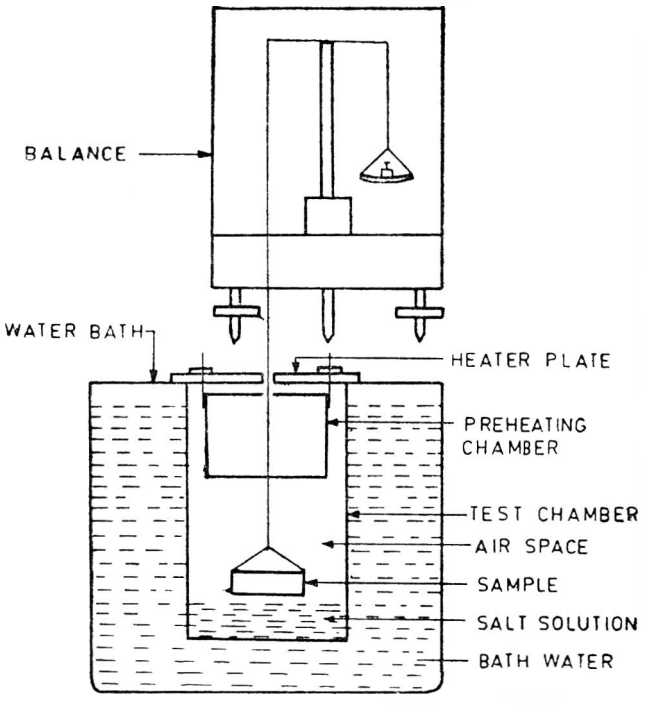

Fig. 1. Schematic diagram of measuring system.

a free surface of distilled water maintained in the test chamber. At such high humidities any contact of vapours with surfaces or objects even at slightly low temperature than vapours would cause condensation. To avoid this, test samples were heated in the preheater to about 2°C higher than the test chamber temperature. This heating took about 10 to 20 min. The condensation on the inner top surface of the test chamber could be avoided by keeping that surface about 2°C above the test chamber temperature.

Before starting each experiment, the constant temperature bath and the heaters of the preheating chamber were put on. After the temperatures had stabilized at the desired level an additional 30 min. lapsed before the sorption process was allowed to be started. All this took about 2 hr and included the 10 to 20 min time for preheating the sample. For each experiment 12 g sample were loosely spread on very fine stainless steel wire basket, 8 cm $\times$8cm $\times$2 cm to give about 4 mm thick layer of flour. Each sample after reaching the desired preheat temperature was weighed *in situ* and immediately lowered into the test chamber. This weight was taken as weight at zero time. Knowing the initial moisture content and weight before preheating, the moisture content at zero time could be readily calculated. All intitial moisture contents were determined by standard air oven method.

Results and Discussion

When a solid absorbs moisture from an atmosphere of air-water vapour mixture, water molecules must move through the gas phase to reach the solid gas interface and then move into the solid. Gas phase diffusivities of water molecules are much larger than those obtaining in the solid phase. Therefore, even in the absence of convective effects, availability of vapour molecules in the neighbourhood of solid is not going to be the limiting factor for the moisture absorption process. Factors governing the rate process must lie with the solid phase. If the rate of moisture absorption by the solid was to be a diffusion controlled process, then a relation of the form of eq (1) cannot describe the kinetics. This rules out moisture diffusion in the solid as the rate controlling step.

Solids such as flour particles are microporous gels. Moisture absorbed causes the hydration of the substance of the solid. Being porous and small in size, most of the internal sorptive sites could be considered equally exposed for hydration; internal moisture gradients, if any, could be neglected. If it is further assumed that hydration of the substance of the solid, which is the rate controlling step, follows a first order kinetics in the difference $(m - m_e)$ then the equation

$$\frac{dm}{dt} = k(m_e - m)$$

or

$$\frac{m_e - m}{m_e - m_o} = \exp(-kt) \qquad \ldots.(3)$$

should describe the moisture absorption process. The symbol k represents the rate constant for hydration. Eq (3) has the same form as Eq (1). Analysis of the data showed that Eq (3) could describe the moisture absorption kinetics of wheat flour with considerable accuracy. Sample weights could predicted within an error of 1.73 per cent for all the cases.

By definition of dry basis moisture content

$$\frac{m_e - m}{m_e - m_o} = \frac{W_e - W}{W_e - W_o}$$

Where W_e, W_o and W are sample weights corresponding to moisture contents m_e, m_o and m respectively. Therefore, eq(3) could also be written as

$$\frac{W_e - W}{W_e - W_o} = \exp(-kt) \qquad \ldots.(4)$$

Working with W rather than m avoids carrying over of any error that may have inadvertantly entered in determination of the initial moisture content corresponding to zero time. Therefore, all calculations were made using sample weights.

If the time interval $(t_{n+1} - t_n)$ between two successive weight measurements W_n and W_{n+1} is always the same, then from Eq(4)

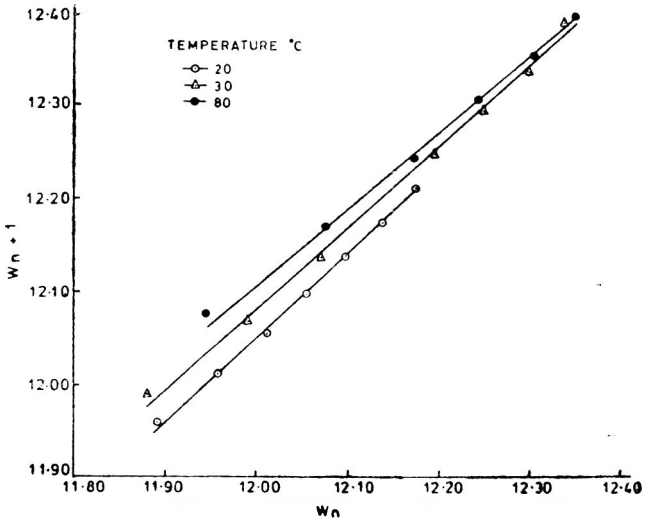

Fig. 2. A sample plot of moisture absorption data in terms of equation (6).

$$\frac{W_e - W_{n+1}}{W_e - W_n} = \exp - k(t_{n+1} - t_n) = \text{constant} \quad \ldots.(5)$$

In the present case the difference $(t_{n+1} - t_n)$ was always 0.25 hr. Substituting this value in eq (5) and simplifying, the following relation is obtained

$$W_{n+1} = W_n Z + W_e (1-Z) \quad \ldots.(6)$$

Where $Z = \exp(-0.25k)$

A data set which satisfies eq(4) must also satisfy eq(6). A sample plot of the present data in terms of the eq(6) is given in Fig. 2. It is readily seen that the data closely follow eq(6), therefore eq(4). Since eq(6) is linear in the unknown parameters W_e and k, it could be used for their evaluation. However, if the quantity 0.25 k is small (exp $-0.25\ k$ near unity), errors in determination

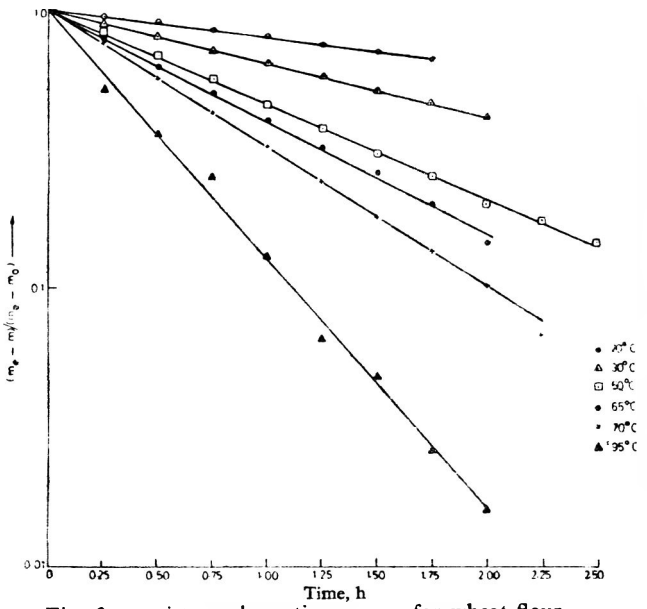

Fig. 3. moisture absorption curves for wheat flour.

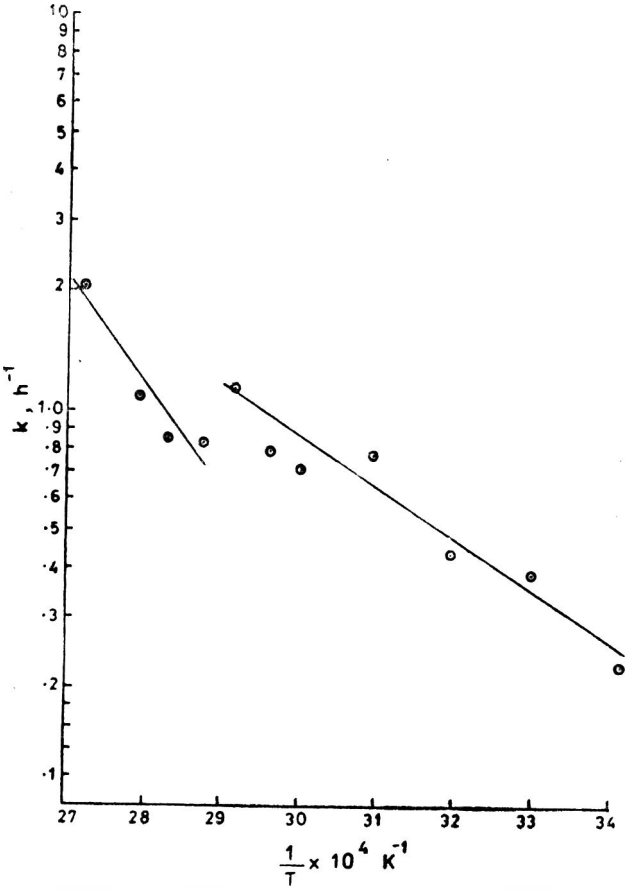

Fig. 4. Effect of temperature on the rate constant.

of Z would get considerably magnified when antilog of Z is taken to find the rate constant k. This would also cause large errors in evaluation of W_e because of the factor (1– Z). Therefore, the parameter W_e was evaluated using the procedure of Issacs and Gaudy[5]:

$$W_e = \frac{W_n W_{n+\varepsilon} - (W_{n+2\varepsilon})^2}{W_n + W_{n+\varepsilon} - 2(W_{n+2\varepsilon})} \quad \ldots.(7)$$

The quantities W_n, $W_{n+\varepsilon}$ and $W_{n+2\varepsilon}$ are any three sample weights which are equally spaced over the time domain of the data set with corresponding time interval being ε. For obvious reasons ε should be as large as possible. Actually eq (7) follows from eq (5) using any three W values that are at equally spaced time intervals. After calculating W_e, the rate constant k was evaluated by applying the method of least squares to the logarithmic form of eq (4). From W_e known, m_e could be readily calculated.

Change in flour moisture content as a function of time for various temperatures is shown in Fig. 3. The solid lines drawn through the data points represent eq (4). Clearly, the first order kinetic model of eq (3) is being obeyed. This supports the physical argument used in developing eq (3) as a reasonable overall description of the physics of moisture absorption by wheat flour.

TABLE 1. VALUES OF THE RATE CONSTANT AND EQUILIBRIUM MOISTURE CONTENT AT VARIOUS TEMPERATURES

Temperature T, K	Rate constant k, h^{-1}	Equilibrium moisture content m_e, % (d.b.)
293.0	0.230	22.36
303.0	0.395	21.43
313.0	0.437	21.02
323.0	0.783	18.06
333.0	0.718	18.64
338.0	0.798	19.71
343.0	1.149	17.30
348.0	0.842	19.81
353.0	0.866	18.45
358.0	1.080	17.65
368.0	2.068	15.54

The hypothesis is further strengthened by the examination of the temperature dependence of k and m_e that follows.

The rate constant k and equilibrium moisture content m_e were both found temperature dependent. Their values at different temperatures are given in Table 1. Variation of k with temperature is shown in Fig. 4. Except for temperatures between 343 and 348 K, it followed Arrhenius law. Thus for $343 \leqslant T \geqslant 348$ K

$$k = k_o \exp\left(-\frac{E}{RT}\right) \quad \ldots.(8)$$

The pre-exponential factors and activation energies were evaluated for the linear portions of Fig. 4. They were,

$T \leqslant 343$ K : $k_o = 6.97 \times 10^3$: E = 5.96 k-cal/g-mole

$T \geqslant 348$ K : $k_o = 2.42 \times 10^7$: E = 11.96 k-cal/g-mole

Both the activation energies have the same order of magnitude as found for physical adsorption and hydration of biological materials. Some of the typical values are: 4.53 k-cal/g-mole for tobacco[6], 11.12 k-cal/g-mole for dehulled soybean[7], 14.17 k-cal/g-mole for whole soybean[7] and 14.01 k-cal/g-mole for pigeon pea grains[7]. The activation energy for high temperature portion of the curve is almost the same as described by Becker[8] for hydration of whole wheat grain and by Bushuk and Winkler[1] for adsorption by wheat flour. The break in Fig. 4 suggests a change in the characteristics of the flour constituents between 343 and 348 K. Based on data of Bedi *et al.*[9] on whole wheat grain the starch fraction in the flour used comes to about 70 per cent. In addition, the sorptive capacity of starch is highest amongst the various wheat flour consituents[11]. Therefore, any change in the nature of this fraction must significantly influence the overall sorption behaviour of wheat flour. Ungelatinized wheat starch has comparatively low hydration capacity[10]. Gelatinization of the starch

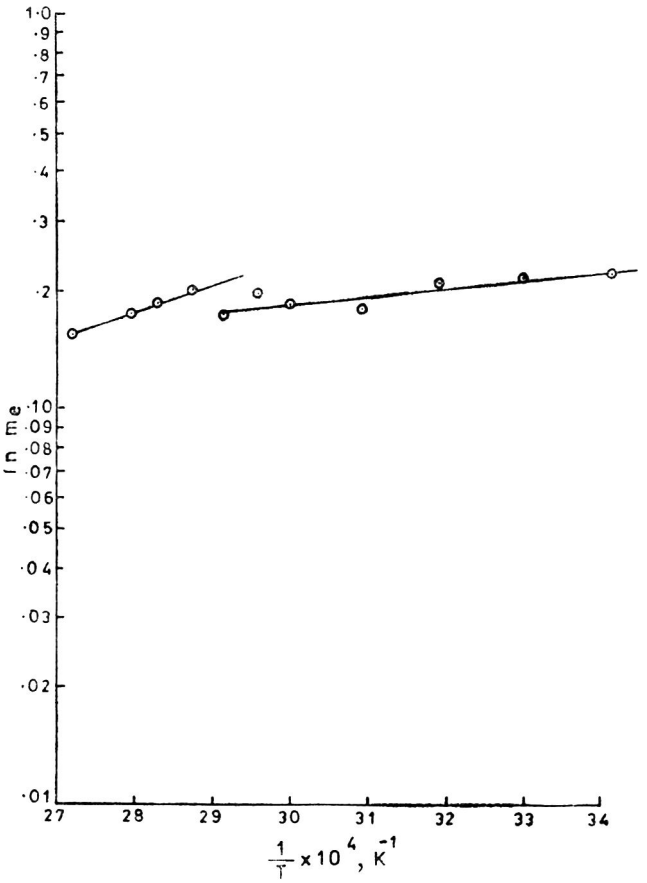

Fig. 5. Effect of temperature on equilibrium moisture content.

is known to occur at about 335 K[11]. At still higher temperatures, further changes occur that influence its hydration[12]. Therefore, it appears that the starch of the flour sample under consideration underwent continued transformations even between 343 to 348 K at which stage its nature stabilized. This might be responsible for the break in Fig. 4. It is possible that other constituents of flour may also have contributed; but considering the preponderance of starch in wheat, it is reasonable to assume that the overall behaviour of the flour is essentially a reflection of starch.

Variation of m_e with temperature is shown in Fig. 5. The m_e versus T relationship also shows a break over about the same temperature range as in case of k. Outside this range, the relationship follows the equation

$$m_e = A \exp(B/T) \quad \ldots.(9)$$

where A and B are constants having the values

$T \leqslant 343$ K : A = 3.685×10^{-2}, B = 531.74 K

$T \geqslant 348$ K : A = 2.444×10^{-3}, B = 1529.11 K

Over the range where eq (9) holds, m_e decreases with the increase in temperature which is in accordance with general behaviour of food materials. In the zone of break this behaviour is reversed. In the light of the explanation given earlier for temperature response of

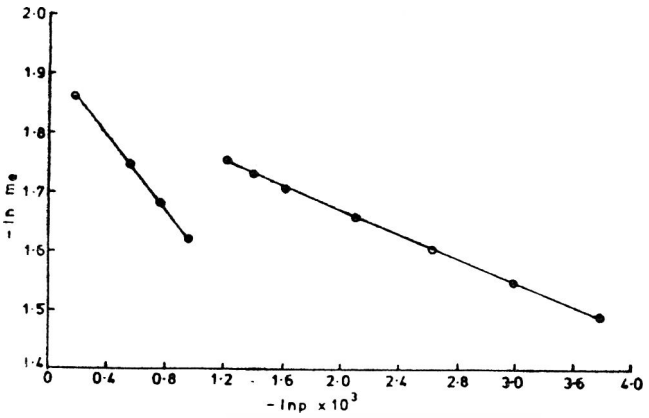

Fig. 6. Effect of water vapoir pressure on equilibirium moisture content.

k this break is to be expected for exactly the same reasons. The steepest increase in equilibrium moisture content occurs over 343 to 348 K. This ties in with the temperature of 338 K near which, sharpest increase in the water holding capacity of starch has been reported[10].

Thus equilibrium between a substance and its vapour pressure follows Clausius-Clapeyron equation

$$\frac{\partial P}{\partial T} = -\frac{L}{(V - v)T} \qquad \ldots.(10)$$

where P is the saturation pressure of the vapour; V, *v*, and L stand for volume per unit mass of the substance in vapour phase, volume per unit mass of the substance in condensed phase, and heat of condensation per unit mass respectively. Here substance under consideration is water vapour which condenses when takenup by wheat flour. Under the usual assumption that $V >> v$ and the vapour behaves as ideal gas

$$\frac{\partial ln\ P}{\partial(1/T)} = \frac{L}{R} \qquad \ldots.(11)$$

Effect of vapour pressure on equilibrium moisture content is shown in Fig. 6. The linear portions of the curve obey the equation

$$m_e = NP^n \qquad \ldots.(12)$$

where N and *n* are constants. Substituting for P in eq (11)

$$\frac{\partial ln\ m_e}{\partial\,(1/T)} = \frac{Ln}{R} \qquad \ldots.(13)$$

Eq (13) is nothing but a differential form of eq (9). Clearly

$$B = \frac{Ln}{R} \qquad \ldots.(14)$$

The values of N and *n* of eq (12) were

$T \leqslant 343$ K : $N = 15.37 \times 10^{-2}$, $n = -10.295 \times 10^{-2}$

$T \geqslant 348$ K : $N = 14.75 \times 10^{-2}$, $n = -30.45 \times 10^{-2}$

From values of B and *n* the corresponding values of the heat of condensation come to 10.263 and 9.978 *k*-cal/g-mole. These values are almost the same as reported by Bushuk *et al*[1]. for their wheat flour at moisture contents more than 16 per cent. The values are also quite close to 10.129 *k*-cal being heat of condensation for each g-mole of pure water averaged over the temperature range of the present study.

If eq (9) is written as

$$m_e = A \exp\left(\frac{Ln}{RT}\right) \qquad \ldots.(15)$$

The product L *n* could be interpreted as activation energy for the condensation process. These values come to 1.06 and 3.04 *k*-cal/g-mole for $T \leqslant 343$ K and $T \geqslant 348$ K respectively. It is not clear as to why these values should differ so much from one another. These values are very much smaller than respective activation energies associated with eq (8). The difference of these activation energies for repective temperature ranges are 4.88 and 8.92 *k*-cal/g-mole. If a change consists of many intermediate steps then the activation energy associated with the rate constant is the resultant value of activation energies for individual steps. Vapour molecules first adsorbed on the surface must further penetrate within the molecular structure of the complex macromolecules that comprise the solid. Almost all food materials capable of swelling on hydration can be considered as gels. This penetration must subsequently cause solubilization of the gel structure which results in swelling. The activation energy measured must be the resultant of all these steps. Since there is considerable difference between the over-all values and those for condensation, it follows that energy barriers to be crossed for steps subsequent to adsorption are much steeper. This implies that temperature induced changes discussed earlier should have their maximum effect on steps subsequent to surface adsorption.

Acknowledgement

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ERRATA

I. In the article "Residues of phorate. carbaryl and endosulfan in peas by V. S. Kavadia and B.L. Pareek, *this Journal* 1981, Vol. 18, No. 4, page 149–151.

Page 149 in Abstract, line 1: '15 kg' should read as '1.5 kg'.

Page 149, column 2 para 3, line 5: '40 min' should read as '30 min'.

II. In the article "Prevention of germination and spoilage of submerged paddy by B. Venkateswarlu and V. Somasundara Rao, *this Journal* 1981, Vol. 18, No. 4 Page 161–163.

Page 163, Table 2, 'Column 2: 'Heated' should read as 'Heaped'.

RESEARCH NOTES

CLARIFICATION OF LIME JUICE BY CELLULASE OF *PENICILLIUM FUNICULOSUM*

Cellulase of *Penicillium funiculosum* was used for the clarification of lime juice. Under optimum conditions of pH (4.0-4.5), temperature (30-40°C), and enzyme concentration (1.64 FPU), turbidity was reduced to more than 90%. The product was clear with the original flavour of fresh lime juice after 18-24 hr incubation.

Use of pectinolytic enzyme in the preparation of lime juice cordial has been reported by Sreekantiah *et al*[1]. The turbidity in the citrus fruit juice is largely due to the cell walls which are cellulosic in nature. Cellulase is reported[2] to be useful in the clarification of the juice. An attempt has, therefore, been made to clarify lime juice by using the cellulase of *Penicillium funiculosum*, a true cellulase producing culture isolated in this laboratory[3,4]

The cellulase enzyme of *P. funiculosum* was obtained by the method described in the earlier publication[5]. The activity of enzyme was determined by using filter paper as a substrate and the reducing sugar formed in the system was measured colorimetrically by Somogyi's method[6]. The activity of enzyme was expressed in terms of filter paper units (FPU) described by Mandels and Weber[7]. The enzyme filtrate was also tested for pectinase activity by Kaiser's method[8], which showed negligible activity between pH range 2.2 to 8.1 and at temperatures 30, 40 and 50°C.

Fresh limes (*C. aurantifolia*) weighing each about 28 g were cut into two halves and the juice (about 20 ml from each lime) was extracted by squeezing. The juice was diluted to 50 ml with distilled water and the pH was adjusted to various levels, viz., 2.2 to 5.6 by adding sodium hydroxide solution. Thirty ml of juice was taken in each flask and to that 10 ml of acetate buffer of respective pH was added. Ten ml of cellulase enzyme of different concentrations ranging from 0.82 to 3.2 FPU was added to find out optimum concentration of the enzyme required for clarification. The enzyme added juice was incubated at three temperatures, viz., 30, 40 and 50°C for periods ranging from 2 to 32 hr. At the end of the incubation period, the reduction in turbidity was measured colorimetrically at 520 *nm* and the readings were compared with the initial values.

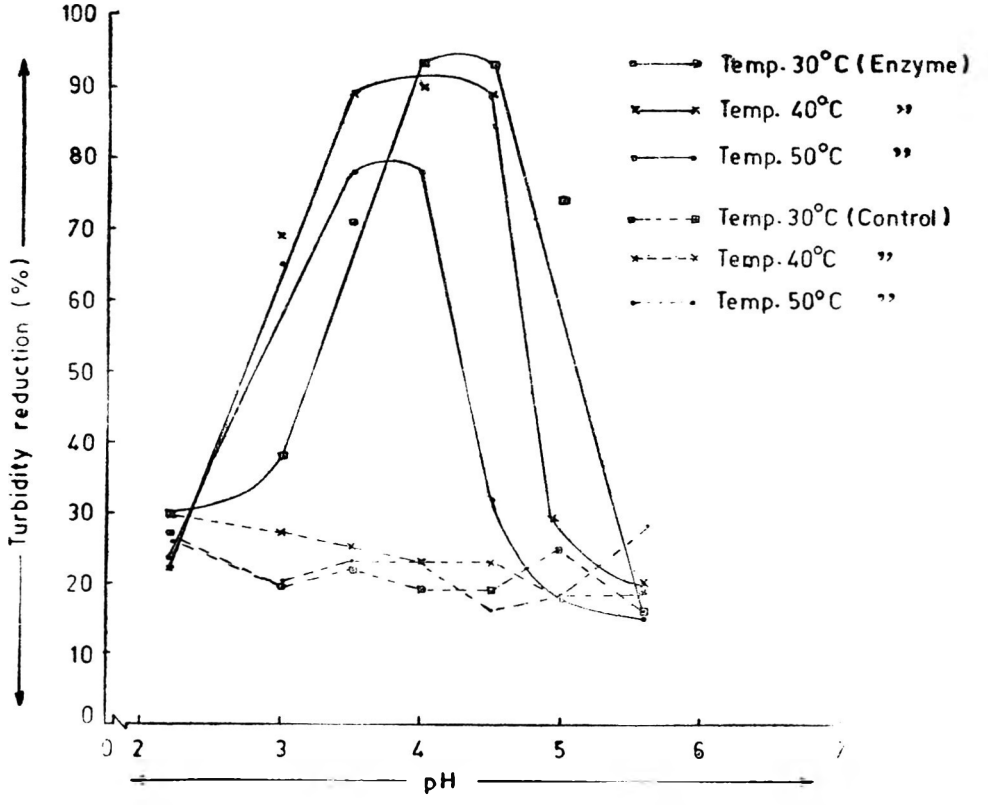

Fig. 1. Clarification of lime juice at different temperatures and pH.

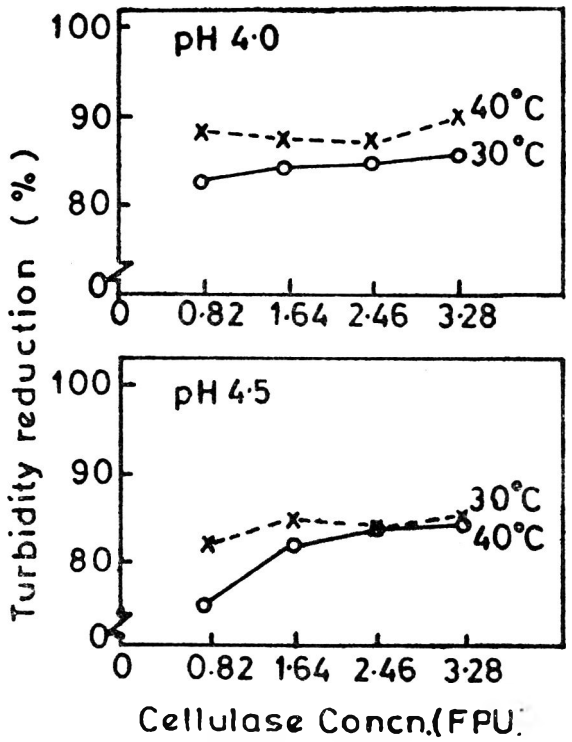

Fig. 2. Clarification of lime juice at different concentrations of cellulase.

Enzyme kinetic studies revealed that the maximum turbidity reduction of lime juice (over 90 per cent) took place at pH 4.0 to 4.5 at 30 and 40 °C, when cellulase of 1.64 FPU was used (Fig. 1). Undiluted juice also showed similar results. At 50 °C, the turbidity reduction was around 80 per cent. It was observed that for the first 18 hr, there was rapid reduction in turbidity. The rate of turbidity reduction almost levelled off beyond this incubation period. Further, it was also noted that even 0.82 FPU of the enzyme was adequate and effective for the clarification at 30 and 40 °C (Fig. 2).

The above results indicate that cellulase of *P. funiculosum* can be used effectively for the clarification of lime juice to yield a clear product with original flavour of fresh lime juice. Maximum turbidity reduction viz., over 90 per cent can be obtained at pH 4.0 to 4.5, temperature 30 to 40 °C, time of incubation 18 to 24 hr and enzyme concentration 1.64 FPU.

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QUALITY ASPECTS OF COMMERCIAL RAW MANGO POWDER (*AMCHUR*)

Six market samples of amchur were analysed for chemical composition. They did not contain sodium chloride, ascorbic acid or SO_2. Wide variations were observed in water and alcohol insoluble material, non-enzymatic browning and tannin contents. Two types of insects and three strains of *Aspergillus* were also observed.

Raw mango powder, popularly known as *amchur* is widely used in vegetable curries in India to add flavour and taste and give body to the gravy. The raw material for *amchur* consists of immature green mangoes, harvested or dropped during rains and storms. They are washed, peeled, sliced, dried in the sun and powdered[1]. Due to the heterogeneity of the raw material and the lack of suitable storage and packing procedures, the product varies widely in its colour and composition. Being hygroscopic, it picks up moisture during storage and many a time is infested with fungal species besides being prone to insect attack. Literature survey revealed a lacuna in the knowledge on the quality aspects of market samples of *amchur* and hence the present investigation.

Six market samples of *amchur* in 200 g packages were collected from different marketing centres in Delhi. The samples were homogenized and analysed for moisture, acidity, sulphur dioxide, water and alcohol insoluble solids, sodium chloride and tannins according to the methods given in AOAC[2]. Ascorbic acid was determined by the method as given by the Association of Vitamin Chemists[3]. Reducing and total sugars were estimated by the Lane & Eynon method[4] and the non-enzymatic browning determined by the procedure standardised by Thorat *et al*[5]. The insect species present in

TABLE 1. CHEMICAL COMPOSITION OF DIFFERENT MARKET SAMPLES OF RAW MANGO POWDER

Particulars	sample number					
	1	2	3	5	5	6
Moisture (%)	8.0	5.9	8.1	8.3	6.5	5.1
Acidity (%) (as anhydrous citric acid w/w)	17.8	15.0	16.8	14.4	15.5	15.6
Total Sugars (%)	13.3	12.1	11.1	11.7	12.5	13.4
Reducing sugars (%)	10.7	10.6	8.7	8.3	8.5	10.8
Tannins (%) as tannic acid	4.3	4.8	3.1	4.1	4.5	3.7
Water insoluble solids (%)	44.4	46.6	45.1	56.4	40.4	53.4
Alcohol insoluble solids (%)	29.5	31.7	32.5	33.5	30.5	28.5
Non-enzymatic browning*	14.3	12.1	16.5	14.7	16.3	14.0

*OD_{420} multiplied by dilution factor

Sodium chloride, ascorbic, acid and SO_2 were not present in any of the samples.

the samples were isolated and identified at the Division of Entomology, I.A.R.I., New Delhi. The moulds were isolated by streak plate method[6] on potato-Dextrose Agar medium. The isolates were identified by the methods given in the Manual of *Aspergilli*[7].

As seen from Table 1 there was a considerable variation in the chemical composition of *amchur* procured from different sources. The sample No. 1 with high moisture also contained the highest acidity. Further this sample contained higher proportions of sugars as compared to four other samples. All the samples were devoid of ascorbic acid and none of them contained either sulphur dioxide or sodium chloride. There was wide variation in the water and alcohol insoluble materials, non-enzymatic browning and also tannins. This may be due to varietal differences in mango, variation in the maturity stage of fruits used and the method of storage practised for the raw material and the finished product. The high levels of non-enzymatic browning could also be ascribed to the total absence of anti-oxidants like ascorbic acid or sulphur dioxide in these specimens.

Further, it is evident from Table 2 that the market samples harboured two types of insects viz. (*i*) Fig moth, *Ephestia cautella* (Wlk) and (*ii*) Saw-toothed Grain beetle, (*Oryzaephilus surinamensis* L.). The samples were also found to be infected with *Aspergillus niger* van Tieghem, *Aspergillus candidus* L., and *Aspergillus flavus* L. It is presumed that these insects and moulds were either derived from the raw material and persisted through the process of dehydration or they gained access to the product during storage.

The aforesaid study reveals a wide variation in the physico-chemical composition of the market samples of *amchur* and the extent of insect and microbial contamination in them. There is thus a need to standardize the procedure for preparation, drying, packaging and storage of the raw mango powder.

TABLE 2. INSECT AND MOULD SPECIES FOUND IN DIFFERENT MARKET SAMPLES OF RAW MANGO POWDER

Particulars	Sample number					
	1	2	3	4	5	6
Insects						
Ephestia cautella (*Wlk*)	+	+	+	+	+	+
Oryzaephilus surinamensis L.	—	—	+	—	+	+
Moulds						
Aspergillus niger van Tieghem	+	+	+	+	+	+
Aspergillus candidus L.	+	+	—	+	—	+
Aspergillus flavus L.	—	+	+	+	+	—

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PRODUCTION OF CARBONYLS DURING SPONTANEOUS OXIDATION OF COW AND BUFFALO MILKS

Carbonyl compounds produced during spontaneous oxidation of cow and buffalo milks were estimated. The total carbonyl and mono carbonyl content of milk fat increased during spontaneous oxidation of milk while the ketoglyceride content remained the same. However, the increase in carbonyl content did not correspond with that of TBA values. Though there was a marginal increase in total and monocarbonyl contents, the increase in n-alkanal class was substantial, which may be responsible for the off-flavour in the spontaneously oxidized milk.

Spontaneous oxidation of lipids in milk has been considered as one of the major problems in the dairy industry[1]. Potter and Hankinson[2] reported that 23 per cent of 3000 samples developed oxidized flavour after 24 to 48 hr storage. Carbonyls, mainly aldehydes, have been shown to be responsible for off-flavour in milks[3]. These are formed from the oxidation of polyunsaturated fatty acids in milk fat. In the present study different carbonyls were isolated and estimated from milk fat of fresh and spontaneously oxidized milks, to understand the nature and levels of these compounds produced during spontaneous oxidation of cow and buffalo milk.

Spontaneously oxidised milk was prepared by following the procedure of Parks *et al*[4]. Milk samples collected directly in clean glass bottles were heated at 65°C for 6 min, cooled and stored at 5±1°C for about 7-8 days during which they attained oxidized flavour[1]. Butter obtained from the cream of fresh and spontaneously oxidized milk was melted at 100°C and filtered to get clear milk fat. The extent of spontaneous oxidiation of milk was measured by thobarbituric acid (TBA) values by the method of King[5]. Total carbonyls, monocarbonyls and ketoglycerides were isolated and estimated as 2,4-dinitrophenyl hydrazones by the method of Schwartz *et al*[6]. Monocarbonyls were further separated into classes by thin layer chromatography on MgO: celite plates[7].

The quantities of total carbonyls, monocarbonyls and ketoglycerides, the percentage proportion of monocarbonyl classes and the TBA values in cow and buffalo milk fat are given in Tables 1 and 2 respectively. The total carbonyls and monocarbonyls of milk fat increased significantly ($P<0.01$) during spontaneous oxidation in both cow and buffalo milks. The quantities of ketoglycerides in milk fat remained the same during spontaneous oxidation of milk. However, there was no significant correlation between the increase in the contents of carbonyls and the extent of oxidation of milk as expressed by TBA values.

As may be seen from Tables 1 and 2 although the increases in the amounts of total carbonyls and monocarbonyls were significant, they were only marginal. However, the increase in the amounts of n-alkanal class of the monocarbonyls can be considered as substantial and are responsible for the off-flavour observed in the

TABLE 1. CARBONYLS AND KETO GLYCERIDES (μM/g FAT) IN MILK FATS ISOLATED FROM FRESH AND SPONTANEOUSLY OXIDIZED COW MIL

TBA value AX 10^{-3}	Total carbonyls		Monocarbonyls		Ketoglycerides		Monocarbonyl classes* (%)					
							Methyl ketones		Alkanals		Alk-2-enals and Alka-2,4 dienals	
	Fresh milk	Oxidized milk	Fresh milk	Oxidized milk	Fresh milk	Oxidized milk	Fresh	Oxidized	Fresh	Oxidized	Fresh	Oxidized
30	4.30	4.60	0.515	0.600	1.85	1.87						
30	3.00	3.20	0.405	0.430	1.78	1.75	86.2	74.0	9.8	20.7	3.6	5.3
20	4.85	4.90	0.439	0.561	2.23	2.23						
45	4.23	4.70	0.485	0.578	1.85	1.86						
40	4.26	4.60	0.473	0.557	1.92	1.90	87.5	73.2	8.6	21.5	3.9	5.3
35	4.50	4.75	0.485	0.531	1.95	1.98						
't' value:	4.6**		6.74**		2.0							

*2, 4 DNP hydrazones of 3 samples pooled together and separated into classes
**Significant at 1% level.

TABLE 2. CARBONYLS AND KETO GLYCERIDES (μM PER g FAT) IN MILK FATS ISOLATED FROM FRESH AND SPONTANEOUSLY OXIDIZED BUFFALO MILK

TBA value AX 10^{-3}	Total carbonyls		Monccarbonyls		Ketoglycerides		Monocarbonyl classes* (%)					
							Methyl ketones		Alkanals		Alk-2-enals and Alka-2,4 dienals	
	Fresh milk	Oxidized milk	Fresh milk	Oxidized milk	Fresh milk	Oxidized milk	Fresh	Oxidized	Fresh	Oxidized	Fresh	Oxidized
45	4.55	5.26	0.493	0.574	2.03	2.02						
30	3.50	3.81	0.362	0.417	2.00	2.02	80.2	69.6	13.7	22.4	6.1	7.9
35	3.53	3.70	0.430	0.475	1.82	1.86						
35	4.35	4.70	0.370	0.438	1.95	1.98						
25	5.20	5.45	0.510	0.565	2.25	2.20	81.5	71.2	12.3	21.5	6.2	7.3
30	4.75	5.07	0.480	0.545	1.98	1.91						
't' value:	4.85**		11.97**		1.81							

*2, 4 DNP hydrazones of 3 samples pooled together and separated into classes

**Significant at 1 % level.

spontaneously oxidized milk. Hence increase in the quantities of total carbonyl or monocarbonyls, may not indicate the status of the oxidized milk.

The increase in carbonyls in milk fat during spontaneous oxidation of milk seems to differ from that of autoxidation of milk fat in which there is production of ketoglycerides and also increase in methyl ketone concentration[8] which were not observed here. However, to a certain extent the production of aldehyde is typical of classic primary lipid oxidation as observed by Parks *et al*[4]. This difference in the nature of oxidation may be due to the involvement of both phospholipids and triglycerides in spontaneous oxidation but, in the case of auto-oxidation of milk fat, only triglycerides are involved. The milk phospholipids contain large proportions of highly unsaturated fatty acids[9] which are known to get preferentially oxidized spontaneously giving rise to aldehydes.

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CONTROL OF SPOILAGE OF PARBOILED PADDY DURING YARD DRYING

The spoilage of high moisture parboiled paddy during rainy weather was arrested by the uniform application of acetic acid 0.5% (v/w) on the paddy. A single application was found to preserve the parboiled paddy for a period of 6 days. Rice produced from the treated paddy contained only 0.01% residual acetic acid. Commercial application of this method costs only about Rs. 3.50 per quintal of paddy.

Spoilage of parboiled paddy is a common phenomenon during rainy humid weather as yard drying is the only mode of drying in conventional mills. Parboiled paddy can be kept in heaps for 36 hr without drying after which it is to be spread in the yard in spite of downpour to avoid any heat development. When the parboiled paddy gets drenched by rain, leaching loss increases and in humid weather fungi grow profusely on the moist grains resulting in about 30-40 per cent loss in the out turn of rice besides chances for elaboration of mycotoxins. Application of common salt to reduce the moisture content in parboiled paddy was reported earlier.[1] The feasibility of controlling the spoilage loss in parboiled paddy by acetic acid application is reported in this note.

Parboiled 'Co 25' variety of paddy (58 per cent grain opening) taken in 20 kg lots were separately

TABLE 1. EFFECT OF ACETIC ACID TREATMENT ON THE MOISTURE, HEAT DEVELOPMENT, MICROBIAL GROWTH, FORMATION OF SUGARS AND DRY MATTER LOSS

Particulars		Storage period (days) 0	2	4	6
Moisture (%)	Control	33.13	30.25	25.04	23.72
	Treated	33.13	29.83	29.98	30.03
Temperature (°C)	Ambient	—	27.00	29.00	29.00
	Control	—	43.00	56.00	39.00
	Treated	—	27.00	27.00	28.00
Fungi (10^6/g)	Control	1.48	7.71	210.80	649.00
	Treated	1.48	0.77	6.20	9.92
Bacteria (10^5/g)	Control	11.75	56.58	47.28	51.88
	Treated	11.75	6.98	48.20	46.74
Reducing sugars (mg/g)	Control	1.32	—	5.05	4.59
	Treated	1.32	—	1.11	1.71
Total sugars (mg/g)	Control	3.87	—	5.86	5.03
	Treated	3.87	—	4.49	4.64
Dry matter loss (%)	Control	—	0.07	3.10	5.40
	Treated	—	0.07	0.60	1.50
Free fatty acids (%)	Control	2.00	18.48	26.45	28.36
	Treated	2.00	2.22	2.30	2.39

treated with sodium chloride 1 per cent, bleaching powder 0.5 per cent, urea 0.5 per cent and *para* formaldehyde 0.2 per cent (w/w), and formaldehyde 0.2 and 0.3 per cent and acetic acid 0.1, 0.3 and 0.5 per cent (v/w). The chemicals were sprinkled over the paddy and mixed immediately with a winnow and left in heap under gunny cover. The loss in dry matter was determined in weighed 1 kg lots taken in separate cloth bags. Four lots were treated with 0.5 per cent (v/w) acetic acid, while another 4 lots were kept untreated and the bags were embedded in the respective treatments. One lot was dried immediately to determine the initial dry matter content. At 2 days interval one bag from each lot was withdrawn and dried at 105°C for 24 hr and dry weight determined. The loss was calculated and expressed as per cent loss over the initial sample. The milling yield for 200 g of paddy from these lots was determined after shade drying (14 per cent moisture content), shelling in a Laboratory model Satake Sheller and polishing in a McGill miller No.1. The free fatty acid content in the bran oil and the acetic acid residue in the milled rice and bran were determined.[2] The temperature, moisture and general appearance were simultaneously recorded. The fungal and bacterial population were determined employing the standard dilution plate technique.

Application of sodium chloride, bleaching powder, urea, formaldehyde and *para*-formaldehyde delayed the onset of fungal infection by two days but none of them offered a lasting protection. Acetic acid at 0.1 and 0.3 per cent level prevented fungal infection for 2 days only, whereas at 0.5 per cent level it protected the grains for 6 days (Table 1). Under 80-90 per cent R.H. when there is no chance for sundrying, the grains had to be treated again with acetic acid at 0.5 per cent level on 6th day to have continued protection. In general no visible growth of fungi was observed up to 6 days in the acetic acid treated lots, whereas in the untreated

TABLE 2. INCIDENCE (PER CENT) OF DIFFERENT FUNGI IN 0.5 PER CENT ACETIC ACID TREATED SAMPLES

Organism	Storage period (days) Initial	2		4		6	
		C	Tr	C	Tr	C	Tr
Aspergillus flavus	15	74	27	78	55	90	43
A. sydowii	45	—	36	3	32	—	29
A. candidus	20	—	6	—	2	—	—
A. fumigatus	5	—	—	—	—	—	—
A. niger	—	7	—	3	2	—	14
A. tamarii	10	—	—	—	—	—	—
Mucor sp.	—	12	—	6	—	—	—
Rhizopus	—	4	—	6	—	10	14
Others	5	3	—	3	10	—	—

C: Control, Tr: Treated

lots infection commenced on the 2nd day and the grains were fully ramified by fungal growth on 4th day. During the steaming process of parboiling the contaminating fungi are not totally destroyed and fungi like *Aspergillus flavus*, *A. candidus*, *A. sydowi* and *A. fumigatus* were present just after parboiling. *Mucor* sp. contaminates the grains only subsequently. However, these organisms multiply rapidly when conducive climatic conditions prevail. The vegetative growth of the *Aspergillii* was localised and confined to the individual grains and especially in the exposed kernel surface and at the germ end. On the other hand the vegetative growth of *Mucor* spread very fast ramifying the whole lot of grains without penetrating deep into the kernel. Acetic acid treatment prevented fungal growth in general and *A. flavus* in particular (Table 2). In the control the growth of *A. flavus* increased with storage period while the other species of *Aspergillii* declined gradually. Application of acetic acid was found effective in controlling the mould growth on sorghum grains.[3] The efficacy of acetic acid in arresting the development of fungi in high moisture groundnut was also reported.[4] Although acetic acid reduced bacterial multiplication up to 4 th day, the treated and untreated grains exhibited an almost equal extent of contamination after the 4th day.

Superficial infection of fungi observed on second day did not cause appreciable dry matter loss in the parboiled grains. The dry matter loss increased up to 5.4 per cent at the end of sixth day in untreated grains, whereas it was only 1.5 per cent in treated grains. In the untreated control there was an intense heat development raising the temperature up to 56°C on the 4th day which dropped down to 39°C on 6th day. This might be due to the uncontrolled growth of fungi and particularly that of thermophyllic group. On the other hand in the acetic acid treated lots there was no heat development and the temperature was almost equal to the ambient temperature.

The damage caused by fungi showed an appearance of scouring in the grains. Either part or whole of the grain became chalky and thus had a poor strength which broke heavily and got powdered during milling; the quantity of bran also increased (Table 3). On the other hand only slight fungal growth was observed in treated lots and the penetration was not deep into the grains and hence strong enough to withstand milling. The milling test showed that there was higher breakage in infected grains which might be due to the fungal growth than by bacteria since the treated and untreated grains significantly differed only in fungal population.

The free fatty acids content in the bran oil of untreated grains increased from 2.00 to 28.36 per cent in 6 days, whereas in treated grains, it was considerably less (2.32 per cent). Since the acetic acid application prevented fungal invasion the development of free fatty acids was also controlled. The acetic acid residue in the milled rice and bran was only 0.01 and 0.02 per cent respectively and this concentration might not pose any health hazard for human beings or cattle. There is also scope for removal of this residue during drying and cooking processes. The acetic acid treatment also did not show adverse effect on the cooking time and gruel loss. Commercial scale trials were also conducted during the rainy season of 1979 in a few rice mills and the cost of acetic acid treatment was found to be Rs. 3.50 per 100 kg of paddy. The cost of treatment is not high when the loss in out turn is considered.

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TABLE 3. EFFECT OF ACETIC ACID TREATMENT ON THE MILLING QUALITY OF PADDY*

Particulars		Storage period (days) 0	2	4	6
Brown rice (%)	Control	77.5	76.3	75.0	72.7
	Treated	77.5	76.6	76.5	76.0
Milled rice (%)	Control	72.5	70.7	68.0	65.0
	Treated	72.5	71.6	72.0	71.3
Polish (%)	Control	5.5	6.6	9.3	10.3
	Treated	5.5	6.2	5.4	5.5
Head rice	Control	70.5	49.7	45.5	34.5
	Treated	70.5	69.1	69.7	68.8
Brokens in polished rice (%)	Control	2.0	21.0	22.5	30.5
	Treated	2.0	2.5	2.3	2.5

*Sample size 200g paddy

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STUDIES ON THE ASSOCIATION OF WHITE CORE WITH GRAIN DIMENSION IN RICE

Among the 138 varieties of rice (*Oryza sativa* L) examined it was found that the varieties in which the grain breadth was <2 mm all the grains were translucent and when grain breadth was >2.8 mm almost all the grains had white core. The varieties having grains with intermediate breadth consisted both white core and translucent type grains. White core and grain length or L/B ratio appear to be unrelated.

Presence of white opaque core (also called, abdominal white or white belly) in rice grain is an undesirable character since it is liable to break during milling due to its softness and lower acceptability[1,2]. White core occurs usually in the middle portion of the ventral side of the non-waxy rice grain caused by the loose packing of the spherical starch granules. In the translucent grains, polyhedral starch granules are tightly packed[3-6]. Chalky cells of the endosperm are known to contain fewer but larger amyloplasts as compared to the cells found in non-chalky portions[7]. Generally white core is associated with coarseness of the grain[8-10]. Several recently evolved high yielding varieties are coarse grains and possess white belly. Present study was designed to find out whether white belly is related to grain dimensions.

25 randomly selected grains from each of 138 varieties of rice were hand-shelled. 10 healthy kernels in duplicate were arranged carefully side by side (length wise first and breadth wise later) by the side of a centimeter scale and average length, breadth and L/B ratio were determined. Grains were examined under a steriomicroscope for identifying the white core.

Varieties in which all the grains had white core were denoted by + sign, and varieties which had all grains translucent were given – sign, other varieties in which both translucent and white core grains occurred were indicated with ± sign.

Data presented in Table 1 show the frequency of varieties with white core grains in different class intervals of grain length and breadth. It can be seen that

TABLE 1. FREQUENCY OF VARIETIES HAVING WHITE CORE GRAINS IN DIFFERENT CLASS INTERVALS OF GRAIN LENGTH AND BREADTH

Breadth (mm)		Rice var. under the indicated length (mm) range									Total
		4.0 to 4.49	4.5 to 4.99	5.0 to 5.49	5.5 to 5.99	6.0 to 6.49	6.5 to 6.99	7.0 to 7.49	7.5 to 7.99	8.0 to 8.5	
1.8 – 1.99	—	1	0	0	0	1	1	0	1	0	4
	±	0	0	0	0	0	0	0	0	0	0
	+	0	0	0	0	0	0	0	0	0	0
2.0 – 2.19	—	2	2	1	3	9	4	2	0	0	23
	±	0	0	0	1	0	1	0	0	0	2
	+	0	0	0	0	0	0	0	0	0	0
2.2 – 2.39	—	0	0	1	5	9	3	4	1	3	26
	±	0	0	0	0	1	0	1	0	0	2
	+	0	0	0	0	0	0	0	0	0	0
2.4 – 2.59	—	0	0	1	6	4	2	3	3	0	19
	±	1	1	1	0	2	2	3	0	0	10
	+	0	0	1	1	0	2	0	0	0	4
2.6 – 2.79	—	1	0	1	0	1	0	0	0	0	3
	±	1	3	0	2	5	3	1	0	0	15
	+	1	1	0	0	6	3	2	0	0	13
2.8 – 2.99	—	0	0	0	0	0	0	0	0	0	0
	±	0	0	1	0	0	0	0	0	0	1
	+	0	0	3	3	2	0	1	1	0	10
3.0 – 3.2	—	0	0	0	0	0	0	0	0	0	0
	±	0	0	0	0	1	0	0	0	0	1
	+	0	0	1	2	1	1	0	0	0	5

— = Varieties in which all the grains are translucent
+ = Varieties in which all the grains are with white core
± = Varieties which consisted of both + and – type grains.

white core did not occur in varieties in which the breadth of the grains was less than 2 mm. As the breadth of the grains increased beyond 2 mm, the frequency of varieties with white core also increased.

In the breadth range of 2 to 2.39 mm (Table 1), a few varieties had mixture of translucent and white core grains but no variety was found with white core. In the range of 2.4 to 2.59, mm., varieties of all the three groups occurred in which the order of frequency was-group $>\pm$group$>+$group. In the range of 2.6 to 2.79 mm, the order of frequency was changed to-group$<\pm$group $>+$group. When the grain breadth was above 2.8 mm almost all the varieties were of white core except 2 varieties which belong to$\pm$group (Table 1). From this it is clear that to overcome the defect of white core in rice grain, the breadth of the grain has to be considered and it appears that the length of the grain or the L/B ratio have no influence on the expression of white core. Further it is indicated that some of the most popular and high yielding varieties such as 'Jaya', 'IR 8', 'T. 65' have white core in the grain by being coarse in texture (greater than 2.6 mm breadth) (Table 2). It would be useful, therefore, to know whether by genetic or agronomical manipulations, this white core could be eliminated in these coarse varieties also. Blakeney[7] has observed that the low initiation of amyloplasts may be the cause for chalkiness in grain. The inheritance of grain chalkiness in rice was studied by several scientists,[8,11-14] although the genetic make up of this character has not been fully understood. Further investigations would be needed to find out the physiological causes and mode of inheritance of white core in rice varieties.

The authors gratefully acknowledge the assistance of Mr. V. M. Pratape in the anlysis of samples.

TABLE 2. ASSOCIATION OF WHITE CORE WITH GRAIN DIMENSION IN DIFFERENT RICE VARIETIES

Rice variety	White core	Breadth (mm)	Length (mm)	L/B
Jenugoodu	—	1.85	6.10	3.3
Jeerasali	—	1.90	4.35	2.3
Sona	—	1.95	6.70	3.4
Basmati	—	1.95	7.50	3.9
B.S.	—	2.00	6.15	3.1
Vani	—	2.05	6.45	3.2
Gansali	—	2.15	4.30	2.0
IR 20	—	2.15	5.95	2.8
S-701	—	2.15	6.45	3.0
Prakash	—	2.15	6.95	3.2
Gowri Sanna	—	2.20	5.50	2.5
GEB-24	—	2.20	5.95	2.7
Pushpa	—	2.20	7.20	3.3
Madhu	—	2.25	6.05	2.7
SR 26B	—	2.35	8.35	3.6
Halubbulu	—	2.40	6.25	2.6
Intan	—	2.45	6.60	2.7
Peta	±	2.55	6.55	2.6
Mangala	±	2.60	6.20	2.4
Pankaj	±	2.65	6.40	2.4
Ch-45	+	2.65	6.30	2.4
Jaya	+	2.65	6.45	2.4
IR 8	+	2.65	6.75	2.6
GMR 2	+	2.65	7.35	2.8
Sukandi	+	2.85	5.45	1.9
T-65	+	3.05	5.15	1.7

— = Varieties in which all the grains are translucent
+ = Varieties in which all the grains are with white core
± = Varieties which consists both + and - type grains.

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DIETARY EFFECT OF NON STARCH POLYSACCHARIDES OF BLACK GRAM (*PHASEOLUS MUNGO*)

Continuous feeding of non-starch polysaccharides and hemicellulose B of black gram to rats at 10 to 20 per cent and 2 to 4 per cent levels respectively was found to increase the length of small intestine and weight of stomach as well as both intestines.

Legumes, though good source of proteins, are well known for the antinutritional factors present in them[1]. Among them, flatulence producing factors, present in some of the legumes are not yet clearly understood. It has been well established that the oligosaccharides belonging to raffinose family, present in legumes, are probably, responsible for the flatulence[2-4]. It has been observed that the residue after extraction of oligosaccharides also produces gas, indicating participation of some factors other than oligosaccharides in gas production[5-7]. Flatulence has also been attributed to crude fibre contents in food[8]. Thus, in the present work, an attempt was made to study the effect of feeding non-starch polysaccharides of a legume, black gram (*Phaseolus mungo*) to albino rats, to find out the relationship, if any, between these components and gas production.

Non-starch polysaccharides were obtained from black gram dal flour by Southgate's method[9]. For this, 1 kg of flour was defatted by ether extraction and deproteinised by treatment with 0.5 per cent NaOH for 24 hr at room temperature with occasional stirring. The residue was washed and made free from starch by treatment with taka diastase, such that for every 50 g residue 50 ml of 2 M acetate buffer (pH 4-6) and 250 ml of 10 per cent taka diastase were added. The mixture was incubated at 37°C overnight and the residue obtained was the non starch polysaccharide. Hemicellulose separation when carried out according to Kawamura's method[10] under atmosphere of nitrogen yielded only hemicellulose B fraction which was also used in the feeding experiments.

Five groups of rats each containing 6 rats were fed *ad libitum* with 5 diets, A to E, for a period of 28 days. The diets fed to different groups had the same basic composition,[3] but differed only with respect to carbohydrate fractions as follows:—

Diet A 50 g corn starch per 100 g diet. This was the control diet.

Diet B: 40 g corn starch and 10 g non-starch polysaccharides per 100 g diet.

Diet C: 30 g corn starch and 20 g non-starch polysaccharides per 100 g diet.

Diet D: 48 g. corn starch and 2 g hemicellulose B per 100 g diet.

Table 1. *Effect of feeding non-starch polysaccharide and hemicellulose diets eor 28 days on rats

Diet	Body wt. Initial g)	Final g)	Change (%)	Other effects
A	63	85	+34.92	No external or internal changes
B	63	78	+23.81	-do-
C	61	50	- 18.03	Weakness, hair-fall, enlarged cecum and stomach (no gas)
D	62	66	+6.45	No external or internal changes
E	65	55	- 15.39	Weakness, enlarged cecum and stomach (no gas).

*Results are average of 3 determinations.
For description of diets A-E see text.

Diet E: 46 g. corn starch and 4 g hemicellulose B per 100 g. diet.

As shown in Table 1, the feeding of non-starch polysaccharides, as well as hemicellulose did not result in any gross abnormalities over the period of 28 days, except, enlargement of stomach and cecum without any gas, wnich were observed, at 20 per cent level of non-starch polysaccharides and 4 per cent hemicellulose B.

The addition of cellulose to the diet, has been reported to cause a similar enlargement due to the increased quantity of volatile acids.[11] The present observation suggests that neither the non-starch polysaccharides nor the hemicellulose B has a role on gas formation. The general weakness, could be, due to the availability of insufficient calories or reduction in digestibility of other constitutents by presence of unavailable carbohydrates[12]. As shown in Table 1, feeding of diets containing 20 per cent non-starch polysaccharides and 4 per cent hemi-cellulose for 28 days resulted in loss of weight as observed by Fischer[13]. Diets with 10 per cent non-starch polysaccharides showed better gain in weight, than 2 per cent hemi-cellulose diets where the increase in weight was negligible. Thus, hemicellulose B, as such, seems to retard growth, even at 4 per cent level and may just maintain or slightly increase the weight at 2 per cent level, while the combined non-starch polysaccharide fraction showed benificial effect at 10 per cent level but not at 20 per cent level, which is in agreement with the earlier findings[14].

As observed in the Table 2 the different diets containing non-starch polysaccharide or hemicellulose B showed a growth enhancing effect on the small intestine. Both length as well as weight of small intestine were increased in all the rats receiving diets containing hemicellulose B and non-starch polysaccharides. The effect

TABLE 2. *DIETARY EFFECT OF NON-STARCH POLYSACCHARIDES AND HEMICELLULOSE ON STOMACH AND INTESTINES OF RATS

	DIET				
	A	B	C	D	E
Small intestine					
Length (cm)	110.00	115.00	77.40	96.20	99.80
Length/g body wt (mm/g)	11.94	14.75	15.47	13.57	16.18
Moisture (%)	75.68	77.00	79.00	78.33	80.82
Wet wt (g)	2.49	2.56	1.80	2.13	2.24
Wet wt/g body wt (mg/g)	29.28	32.75	36.35	32.23	47.65
Stomach					
Wet wt (g)	1.50	1.49	1.15	1.44	1.27
Wet wt/g body wt (mg/g)	17.60	19.15	2305	21.90	24.25
Large intestine					
Wet wt (g)	1.81	1.93	1.30	1.58	1.41
Wet wt/g body wt (mg/g)	21.35	24.80	26.05	24.00	25.06

*Results are average of three determinations.

increased with levels of the constituents. These observations are in agreement with those of Younoszai *et al.*[14] who have suggested that weight increase is due to an increased water content of the tissues, which is also true in the present case. The type and content of carbohydrates in diet such as corn starch and fibre is also shown to be altering the length and weight of small intestine.[14-16]. However, the factor responsible for increase in length of intestine is not clearly understood. Perhaps it may be due to the extension caused by continuous pressure exerted on the walls of intestine to propell the bulky stools.[13] Although no gross abnormalities were observed with non-starch polysaccharides and 2 per cent hemicellulose B diets, both the stomach and large intestine gained weight, which increased with increasing levels of both non-starch polysaccharides and hemi-cellulose B.

Thus it seems that the non starch polysaccharides also have a dietary effect, because of which probably the mucosal morphology is altered; so as to affect the gastrointestinal tract in particular, as reported earlier with crude fibre.[14]

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BOOK REVIEWS

Food and Health: Science and Technology: Edited by G. G. Birch and K. J. Parker, Applied Science Publishers, London, 1980, pp 532. Price £. 40.

The title of the book tersely outlines the areas covered. There are a group of papers on food as a broad national concept: foods from conception to extreme old age, food control, health foods and healthy eating, and the responsibility of a modern food technologist. Then come papers dealing with health considerations in relation to EEC legislation, to control of salmonellae, clostridia, mycotoxins and other microbiological risks, and to biochemical safety, followed by a group of papers that deal with food additives and contaminants (like pesticides), and to a natural anti-nutrient like erucic acid. After this follow a set of expositions on food processing in its relation to detoxification, interaction between food components, nutritional damage, packaging, and plant performance, with a paper also on the use of ionising radiatiors to process food. Finally come a group of papers on nutrition in relation to health: the adequacy of national diets, optimisation of nutrients, dietary fibre, obesity and anorexia, infant nutrition, and brain development in relation to protein deficiency, to food in general, and to lead in the environment.

A few observations made in these essays may serve to bring out their quality. Babies born small-for-age never catch up later, however well-nourished they are, in contrast to rehabilitation following under-nutrition at older ages. Since the inclusion of materials like immunoglobulins, lactoferrin and lysozyme into baby foods is not easy, infants should have the benefits of colostrum and breast milk, even if only for a very short time. Referring to fortification of chapati flour in England with vitamin D, the opinion is expressed that sunlight is undoubtedly the natural way of getting this vitamin, and if all Asian children living there were to be exposed to sunlight in summer and given supplementary vitamin D in winter, rickets would disappear. The compulsory addition of nutrients to food ingredients, whether fluoride to all drinking water, or iron to wheat, is strongly attacked in another article: ".... we may very properly use a food as a vehicle for nutrients when deficiency is likely but personal liberty must not be infringed, and everyone has a right to a non-medicated water supply". About three and a half pounds of food additives are being consumed by a Britisher annually. In this light, one is asked to view the much-maligned health foods as representing nothing more or less than an attempt to choose high-quality raw materials, and to use in processing no artificial additives whatever, even if these are legally permitted. Nutrition education is meant to convert nutritional knowledge into food on the plate; the question is essentially one of communication, and the best practical approach appears to rest on the basis of meals as eaten. A code of professional conduct drawn up by the Institute of Food Science and Technology of the UK, serves as a guideline to a food technologist regarding both his responsibility for wholesomeness of food, and his integrity towards the profession. It is pointed out that banning of nitrite as a food additive may stimulate home production of ham and bacon, with a significant danger of a much greater incidence of botulism than from supervised industrial processing. There are detailed references to studies in India on the aflatoxin toxicity incident that occurred in a Mysore hospital, and the infested maize consumption episode investigated by the National Institute of Nutrition, Hyderabad. For monitoring manufacturing practices through end-product analysis, properly-devised tests for certain indicator or index organisms like enterobacteriaceae, Lancefield group D streptococci, and particular groups of catalase-positive cocci are considered sufficient. If zero-risk hazard is to be the goal, the difficulties in turn become nearly infinite: risk must balance benefit. Perhaps additives that prevent either food poisoning or food spoilage, for which there is a clear technological and economic need, could be separated from others that improve quality, appearance or nutritive value, the withdrawal of which would increase neither food poisoning nor food spoilage and wastage. Nitrites undoubtedly inhibit clostridium, but whether nitrosamine at a 0.5 ppb level in food is carcinogenic to humans or not is simply not known. In another article, it is pointed that while analytical skills are rapidly becoming more sensitive, the analyst provides only a fragment of the information needed to assess any health risk. Food processing likewise has its pros and cons, eliminating many toxins but creating others. To make baby food, whey or soya appears a more suitable starting material than cow's milk. It is noteworthy that soya protein isolate has a PER of only 1.1-1.2 against 2.3 for the defatted toasted flour. Automation of food processing plants would enhance safety, since this is really built into on-line computer control. The article on the use of ionising radiation for processing food advocates usage of low-dose radiation, below 1 M rad, for control of salmonella. destruction of insects, influence on fruit ripening and

disease, and sprouting-inhibition of root-crops in replacement of ethylene oxide fumigation. The early Maillard reaction can damage food nutritionally; half the lysine is lost in roller drying of milk powder, or even in spray drying of baby foods that contain lactose-hydrolysed milk or glucose. Yet it is the later Maillard reactions that give rise to the attractive flavours of baked bread, roasted coffee, and cooked meat or vegetables. The dye-binding techniques, applied before and after propionylation, is recommended for assessing both early and late Maillard damage. The Ames mutagenicity test is seen as an early warning rather than a final arbiter of animal carcinogenicity. Examples are given to show that calculated protein values are gross underestimates (65 per cent of the analysed value for a total meal), and calculated energy values are gross over estimates (123 percent of actuals), as are also vitamin C estimates in many instances. The article on fibre points out the special value of the fibre in wheat in increasing colonial bacterial mass and faecal bulk, inhibiting bile and dehydroxylation, lowering ammonia levels and diluting colonic contents, effects that are best explained by the promotion by fibre of microbial growth in the large bowel. The metabolic rate varies with individuals, and can explain why everything eaten by some people runs to fat. A little known recent finding is the high content in breast milk of water-soluble vitamin D sulphate, 0.8μg/100 ml, in contrast to just 0.01 μg for vitamin D itself. Dietary components influence brain function through brain neurons that utilise serotonin, acetylcholine or norepinephrine, and certain brain diseases can in turn be cured by massive doses of pure nutrients. While PEM in children is accompanied by evidence of mental and behavioral deficiencies, whether these are a direct effect or through sensory deprivation is not known. The high levels of environmental lead are alarming when viewed against the effect of this metal in disturbing mental function.

There is a great deal in this volume that would interest food and nutrition scientists, processors and technologists, policy makers and controllers, and indeed all those in any way connected with food.

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Glossory on air Pollution: W.H.O. Regional Bulletin No. 9, Copenhagen, 1980, pp. 114. Sw. fr. 12.

Air pollution is brought about by the disposal of organic and inorganic particles into the air. the material originating from living and dead organisms are referred to as biopollutants. The subject requires a knowledge of meteorology, geography, geology, chemistry, physics and biology. It is difficult to have such a wide knowledge in such varying fields when one is specializing in the field of air pollution. The 'glossory on air pollution' is a much needed reference material. The terms included in the book overwhelmingly prove that the problem is a man made one. He excavates various minerals, fractionates them and puts them into air, he breaths and spends billions of dollars to purify air, to reduce 'nuisance threshold'. A good coverage is seen on terms connected with all allied branches except biology and the bulletin would have been more useful if it had included biological terms. I hope, in the second edition the work would be enlarged and W.H.O. would include terms which might be communicated by the users. I have been using the process of 'elutriation' for separation of certain biological material from soils but without the knowledge of the term, and I am sure, users would be benefited by it. The book is available in India from the Regional office of WHO, New Delhi-2, or from Oxford Book Co.

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Advances in Biochemical Engineering-13. *Mass Transfer and Process Control:* by T. K. Ghose, A. Fiechter and N. Blakebrough, Springer—Verlag, Berlin, Heidelberg, N. Y., 1979, pp. Price: $ 48.40.

The book contains four review articles on application of microcomputers in the study of microbial processes, dissolved oxygen electrodes, power consumption in aerated stirred tank reactor systems and loop reactors.

The review on microcomputor applications discusses their use in data requisition, data reduction and process control. It has been shown how on-line data acquisition could be used for the computation of inaccessible variables related to physico-chemical and physiological conditions of culture. The progress made in the microelectronics and microcomputers have been described. Aspects like hardware configuration, interface units, efficacy and limitation of computer aided microbial cultivations, costs involved and future trends have also been dealt briefly.

The article on dissolved oxygen electrodes discusses the recent advances, including the theory and principle of and measurement, design construction methods for different types of electrodes including micro electrodes. Types of construction materials and their selection is discussed for electrodes, electrolytes, membranes. Information on the use of these electrodes for the measurement of volumetric mass, transfer coefficients, respira-

tion rates, permeability measurements in polymer membranes and oxygen solubilities in cultivation media, have been reviewed.

In the article on power consumption in aerated tank reactor system, the functions of stirrers are discussed along with description of mechanisms of energy transfer, fluid flow bubble movement with specific reference to widely used stirrers viz., turbine, paddle, propeller and disk stirrers. The concept of a model for energy transfer has been introduced and developed for paddle and turbine stirrers. Methods have been discussed for energy transfer caculations in simple non-Newtonian fluids based on information from Newtonian fluids.

The fourth chapter presents data on the latest developments on loop reactors. Loop reactors are characterised by their simple construction and operation to meet the exacting demands of bioreactors at comparatively lower costs, energy and maintenance. These have been classified based on mode of flow. Theoritical considerations and data have been presented on fluid dynamics for homogeneous liquid systems and heterogeneous solid liquid systems for the above reactors.

The book is yet another contribution bringing out reviews on topics of current interest thus making the series more valuable.

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Standardization of Analytical Methodology for Feeds: Proceedings of a Workshop held in Ottawa, Canada 12-14 *March* 1979. Editors W. J. Pigden, C. C. Balch and Michael Graham, published by the International Development Research Centre, Box 8500, Ottawa, Canada KIG.

The live stock industry is a multimillion dollar industry which has now been organized in most countries on a highly profit oriented basis. The feed manufacture industry is also consequently highly organized. But the methods of analysis of feed to assess the efficiency of the feeds to meet the nutritional requirements of the live stock are still not fully standardized and differ from laboratory to laboratory. This lacuna was recognised by the commission on animal nutrition set up by the International Union of Nutritional Sciences. To discuss this and if possible correct it, a workshop was held in Ottawa from 12th to 14th March 1979. The proceedings of this Conference form the subject of this monograph.

The topics are discussed under thirteen chapters. It is natural that the major topic in a workshop of this nature should be energy and metabolizable energy for live stock. This has been discussed in five chapters. The analytical methods for assessment are discussed in two chapters. Trade and legal aspects with particular reference to the European Economic Community have been discussed in detail in one chapter. An interesting discussion on the adoption of methods of analysis by AOAC and on the procedures to be followed for collaborative study is included in a chapter.

The summary and recommendations of the workshop are given in the beginning of the monograph. The recommendations are preceded by an introduction, emphasising their importance.

The monograph is of very great interest to analysts and chemists dealing with feeds and fodders and research laboratories.

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BANGALORE.

Human Nutrition: A Comprehensive Treatise–3A Nutrition and the Adult Macronutrients: Roslyn B. Alfin-Stater and David Kritchevsky, (Ed.) Plenum Press, New York, 1980, pp 290+xviii. Price: $ 25.

The Book 'Nutrition and the Adult: Macronutrients' is one of the four volumes on Human Nutrition, A Comprehensive Treatise. The ten chapters in the book may be considered under three categories: (1) nutrients and energy requirements and demands for maintenance, (2) carbohydrates and fats and inter-relationships between them as suppliers of energy and (3) nutrients with special functions like proteins and amino acids, essential fatty acids, cholesterol and dietary fiber.

The opening chapter reviews the techniques used to determine nutritional status, nutrient requirements and the basis for recommendations. Chapters 2 and 6 cover the aspects concerning energy: calorimetry, caloric allowances, energy balance, obesity, under nutrition, basal metabolic rate, etc.

Absorption, digestion and metabolic interrelationships between carbohydrates and fats are discussed in chapters 3, 4 and 5. Two important points that are brought out clearly are: (1) the differences among glucose, fructose and sucrose with reference to their influence on lipid metabolism and (2) the limitations in conclusions arising out of experiments in which carbohydrates and fats are replaced on a quantitative basis but not on an energy basis.

The importance of proteins and amino acids in tissue maintenance; essential fatty acids, their function, effect of excess EFA; cholesterol, dietary fiber--its relationship with intestinal function and disease, relationship with lipid metabolism and the effect of excess fiber are considered in the last four chapters. The chapter on dietary fiber aptly ends thus: 'Dietary fiber is not a panacea, but it may play a role in alleviating

symptoms in some patients. If this is so, the furor over fiber has been worthwhile'.

There is an appendix with three tables: recommended dietary allowances, estimated safe and adequate daily dietary intakes of additional selected vitamins and minerals and mean height and weights and recommended energy intake.

Except for a few minor mistakes: sweetness of fructose given as 110 instead of 160 (Table III, p 99); started instead of stated on page 220; his in place of this on p. 221 and behooves instead of behaves on p. 230, subject coverage and get up are excellent. Written and edited by experienced experts in the area, the volume is a valuable reference work for nutritionists, teachers and research workers.

N. CHANDRASEKHARA
C.F.T.R.I., MYSORE

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FORM IV

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	Nationality	Indian
	Address	CFTRI, Mysore-570 013.

I, Dr. P. Narasimham hereby declare that the particulars given above are true to the best of my knowledge and belief.

P. Narasimham
Signature of the Publisher

ASSOCIATION NEWS

Annual General Body Meeting

The Annual General Body Meeting of the Association of Food Scientists & Technologists (India) was held on May 9 1981 at ESCAP-RECTT, Bangalore, India. Dr. K. T. Achaya, President of the association was in the chair. Dr. K. R. Sreekantiah, Hony. Secretary presented the report on the activities of the association during 1980–81. The salient features of the report are: the increase in the membership of the association from 1354 to 1690; approval for starting of three new chapters in Lucknow, Pantnagar and Parbhani; to hold the First AFST(I) International Food Conference at Bangalore in May 1982; streamlining the procedure for the collection of membership fee by the respective chapters; affiliation of AFST(I) to IUFoST with voting rights, and conferring of Fellowships and Associateship of the AFST(I) to members. The activities of the various chapters of the association were also read out.

The AGBM took note of the publication of the *Journal* of the association in time during the year and appreciated the efforts put by Dr. R. Radhakrishna Murthy, the Editor, the various associate editors and the referees. The CEC, it was pointed out, had recommended for bringing out a new periodical under the title (tentative) *Indian Journal of Food Industries*,, and periodic Critical Reviews in Food Science and Technology.

The recommendation of the CEC to institute an International Industrial Achievement Award on Food Science and Technology named after Dr. H. A. B. Parpia was also announced by the Secretary which was well received by the members. The secretary also informed about the action being taken to get space inside the CFTRI campus to construct an office and library building for the association.

In the discussion that followed, it was accepted by the General Body that the election in future for the different offices of the association will be conducted by the Secretary assisted by 3 non-CEC members. A member's suggestion to send stamped cover for return of the ballot paper to increase the percentage of voting was also agreed.

The inclusion of editors from foreign countries in the editorial board of the *JFST* was also discussed. Accordingly the General Body approved that in the subclause 5.22 "Associate Editors not exceeding six in number", the phrase "not exceeding six in number" may be deleted.

The treasurer presented his report for the year along with the Budget proposals for 1981–82.

This was followed by the announcement of the recepients of the various awards of the association for the current year.

Prof. V. Subrahmanyan Industrial Achievement Award: Presented to Sri C. P. Natarajan, Director, Central Food Technological Research Institute, Mysore, India.

Gardner's Award: Drs. B. M. Mathur and M. R. Sreenivasan of National Dairy Research Institute, Karnal, India, received this award for their paper entitled "Isolation and Utilisation of Proteins from Whey Systems of Buffalo Milk on Pilot Plant Scale" published in *Journal of Food Science and Technology*—Vol. 16(2), 1979.

Young Scientist Award: This was awarded jointly to Dr. B. Ravindranath, Scientist, CFTRI, Mysore, India and Dr. M. Seenappa, Department of Botany. National College, Bangalore (at present in Tanzania),

Suman Food Consultants Travel Award: Mr. B. Srinivasan, student of M.Sc. (Food Tech), CFTRI, Mysore was the recepient of this award for his essay on "Can Modern Food Technology Solve India's Food Problem?"

The Secretary's report and the treasurer's report were unanimously approved by the General Body.

The office-bearers of the Association for the year 1981 82 were announced by the Hony. Secretary.

President	:	Sri M. K. Panduranga Setty
President-Elect.	:	Sri S. K. Majumder
Vice-Presidents		
Headquarters	:	Dr. M. S. Narasinga Rao
Bombay	:	Dr. R. Jayaram
Delhi	:	Dr. J. C. Anand
Ludhiana	:	Prof. B. S. Bhatia
Pantnagar	:	Prof. B. P. N. Singh
Editor, JFST	:	Dr. R. Radhakrishna Murthy
Hony-Treasurer	:	Dr. D. Narasimha Rao
Hony-Secretary	:	Dr. P. Narasimham
Hony-Jt. Secretary	:	Dr. L. V. Venkataraman

The new office bearers were inducted and this was followed by a brief talk by the new President Sri M. K. Panduranga Setty.

Dr. T. N. Ramachandra Rao proposed vote of thanks to the outgoing office-bearers for the able and efficient work and to the Bangalore Chapter for making the meeting a success.

Delhi Chapter

A seminar was organised in March 1981 on 'New Horizons of non-alcoholic drinks in eighties' in New Delhi, in collaboration with the department of small scale industry, government of India. There were two technical sessions, one on "Recent technological advances and future prospects and problems" and the other on "Marketing of non-alcoholic beverages and quality standards". The seminar was attended by nearly 200 delegates representing industry, university, research institutes, government departments, marketing organisations, etc.

BUDAPEST SYMPOSIUM 1982

ON

FOOD INDUSTRY AND THE ENVIRONMENT

The symposium will be held in September 1982. Following topics will be covered.

1. Size and problems of integration of large agro-industrial concerns.
2. Development of waste-free technologies for special branches of the food industry involving environmental pollution problems.
3. Environmental problems of the food industry involving the pollution of water and soil.
4. Environmental problems of the food industry involving air pollution.
5. Acoustic trauma and the elimination of noise in the food industry.

Correspondence may be addressed to Prof. J. Hollo, Central Research Institute for Chemistry, Hungarian Academy of Sciences, H-1025, Budapest Pusztasziri.

Government of India
Ministry of Agriculture
Department of Food
Consultancy Cell

The Consultancy Cell in the Department of Food has been set up to provide consultancy to entrepreneurs interested in setting up of fruits and vegetable processing industries. The Consultancy Cell comprising of experts in the field of engineering, marketing, processing, planning and management of fruit and vegetable processing projects, operates as a Departmental unit of the Ministry of Agriculture, Government of India.

The services offered by the Consultancy Cell include survey for availability of raw materials, market surveys, site selection, cost structure of machinery and buildings, and financial analysis for the economic viability of the projects. In addition, techno-economic feasibility studies can be undertaken and complete project reports can also be prepared. The Consultancy Cell maintains liaison with the leading research institutions of the country and collects information on the performance of existing industries which are analysed for providing consultancy to the entrepreneurs interested in setting up of new industries or for modernisation of the existing industries. The Consultancy Cell has provided consultancy for setting up of Agro Industrial Complexes, pineapple and mango processing industries, fruit juice concentration plants, fruit juice bottling plants and fruit and vegetable canning industry. The Consultancy Cell advises for the size and product mix for such a unit. The Cell also keeps a liaison with the leading nationalised banks and financial corporations for advancing loans against project reports prepared by it as their approved consultant.

Consultancy is provided against a very nominal amount of consultancy fee i.e. 1% of the project cost subject to a minimum and maximum of Rs. 6000 and Rs. 25000 respectively. No consultancy fee is charged in case the project cost is less than Rs. 1.5 lakhs.

For further details, please contact **Joint Director, Consultancy Cell, Department of Food, Ministry of Agriculture, Government of India, 10/118 Jamnagar House, New Delhi, India, 110 011.**

Prof. V. Subrahmanyan Industrial Achievement Award for the year 1981

The Association of Food Scientists and Technologists (I) has instituted this Award. Nominations for this award for the year 1981 are invited. The guidelines for the award are as follows:

1. Indian Nationals engaged in the field of Food Science and Technology will be considered for the award.

2. The Nominee should have contributed to the field of Food Science and Technology, for the development of Agro-based food and allied industries or to basic food science and technology with immediate prospect and/or future potential for industrial application.

3. The nomination should be proposed by a member of the Association; The bio-data of the candidate together with his consent should be given in detail including the work done by him and for which he is to be considered for the award.

4. The Awardee will be selected (from the names thus sponsored) by an Expert panel constituted for the above purpose by the Executive Committee.

Nominations along with bio-data and contributions should be sent by **Registered Post,** *so as to reach Dr. P. NARASIMHAM, Honorary Executive Secretary, Association of Food Scientists and Technologists (India), Central Food Technological Research Institute, Mysore-670 013 before 31 January 1982*

Dr. H. A. B. Parpia International Award

The Association of Food Scientists and Technologists (India) has decided to institute an annual Award from 1981 in the name of Dr. H. A. B. Parpia, former Director of Central Food Technological Research Institute., Mysore, and presently working as senior adviser in F.A.O., Rome.

The Award will be made by the Association of Food Scientists and Technologists (India) for a person or persons who have significantly contributed to alleviate the problem of hunger and malnutrition, particularly in developing countries, and have cut across national borders through food science and technology, especially by way of multidisciplinary activity through research, development, technology transfer, and the building of human resources, institutions and policy.

The Award will include a mounted silver plaque and a citation. It will be made at the Annual General Body Meeting of the Association or at a separate function where the awardee will deliver a special lecture. The award would normally be annual, but if no suitable candidate is available in a particular year, it can be held over.

Young Scientist Award for the Year 1981

Association of Food Scientists and Technologists (India), announces with pleasure the institution of the YOUNG SCIENTIST AWARD for distinguished scientific research and technological contributions to the field of Food Science and Technology.

The award consists of a cash prize of Rs. 1,000, a plaque, and a citation.
Nomination for the Award is open to aspirants fulfilling the following conditions:

1. The candidate should be an Indian National below the age of 35 years on the date of application working in the area of food science and technology.

2. The candidate should furnish evidence of either,
   (*a*) Original scientific research of high quality, primarily by way of published research papers, and (especially if the papers are under joint authorship) the candidates own contribution to the work: *OR*
   (*b*) Technological contributions of a high order, for example in product development, process design etc., substantiated with documentary evidence.

The application along with details of contributions and bio-data (in triplicate) may be sent by *Registered post,* so as to reach Dr. P. Narasimham, Hon. Exec. Secretary, Association of Food Scientists and Technologists (India), CFTRI, Mysore-13 before 31 January 1982.

Best Student Award

The Association of Food Scientists and Technologists (India) has instituted a BEST STUDENT AWARD to be awarded every year to two students with a distinguished academic record and undergoing Final Year Course in Food Science and Technology. The aim of the award is to recognise the best talent in the field and to ensure wider recognition of food science and technology as professional discipline.

There are two awards each comprising a cash award of Rs. 500/- and a certificate:

The candidates to be considered for the awards should fulfill the following conditions:

1. They must be Indian nationals

2. They must be students of one of the following:
   (*a*) M.Sc. (Food Science)/Food Technology
   (*b*) B.Tech.,B.Sc.,Tech., Bsc. Chem. Tech in Food Technology
   (z) B. Tech., in food sciences

3. They should not have completed 25 years of age on 31st December of the year preceding the announcement when their names are sponsored

Heads of Post-graduate Departments in Food Science and Technology may sponsor the name of *one* student from each Institution supported by the candidate's biodata, details starting from High School onwards, including date of birth and his postgraduate performance to date.

Nominations for the year 1981 may be sent by Registered Post so as to reach the Hony. Secretary, AFST(India), Central Food Technological Research Institute, Mysore-570 013 before 31 January 1982.

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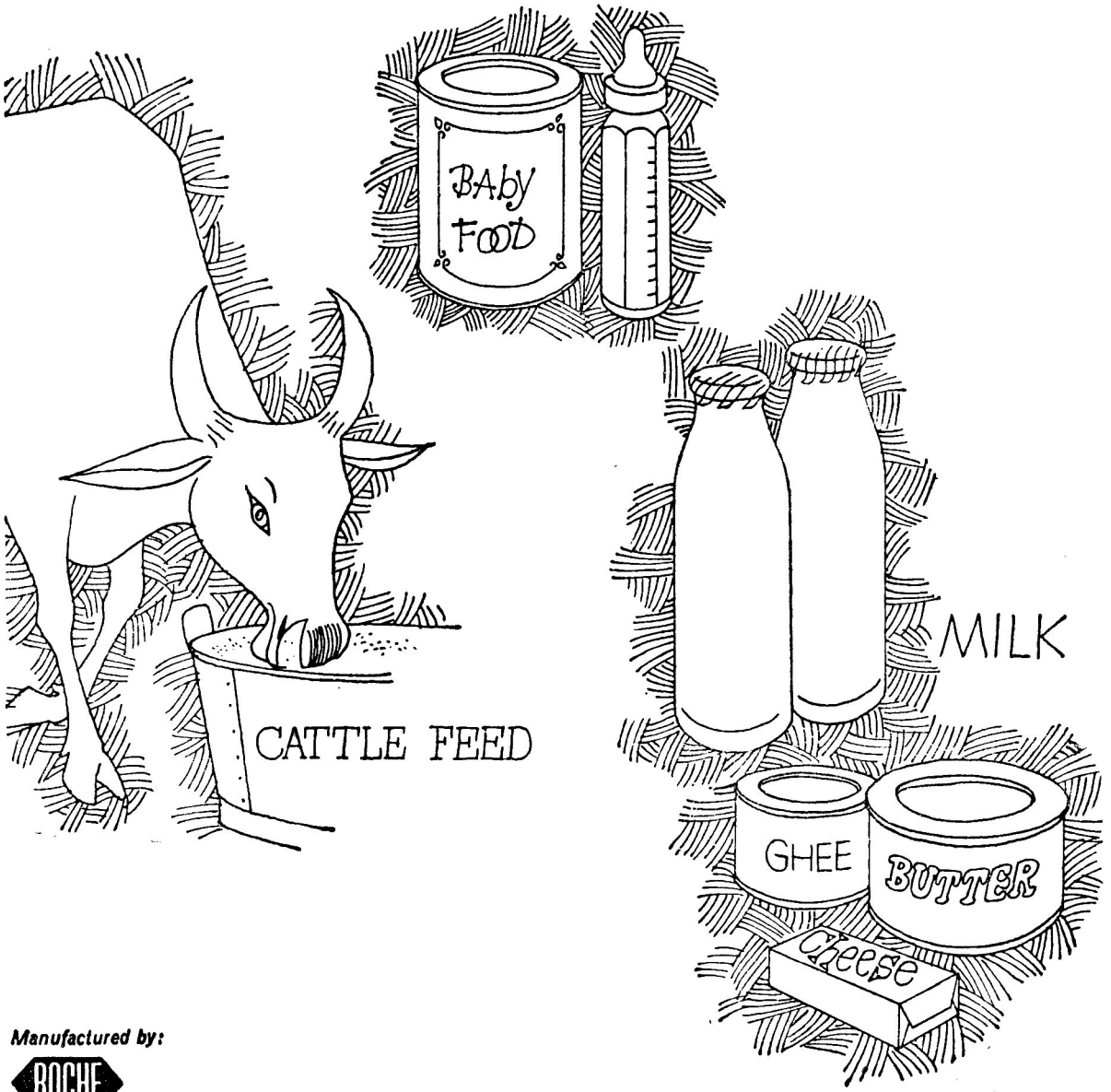

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PUBLICATIONS
OF
THE ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (I)

(Central Food Technological Research Institute Campus, Mysore)

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1. **REVIEWS IN FOOD SCIENCE AND TECHNOLOGY — VOL. IV**
   Royal 8vo, hard bound, P. 255.
   Price: India **Rs. 8/–**
   Abroad: **$5 Surface mail**
   $8 Air mail.

2. **PROCEEDINGS OF THE SYMPOSIUM ON DEVELOPMENT AND PROSPECTS OF SPICE INDUSTRY IN INDIA**
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   Abroad: **$8 Surface mail**
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INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (*in triplicate*) should be typewritten in double space on one side of thick, preferably, bond paper. The manuscripts should be complete and in final form, since only minor corrections are allowed at the proof stage. The paper submitted should not have been published or data communicated for publication anywhere else.

2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.

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

4. **Abstract:** The abstract should indicate the principal findings of the paper. It should be about 200 words. It should be in such a form that abstracting periodicals can readily use it.

5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. They should be typed on separate paper. Nil results should be indicated 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 white drawing paper preferably art paper. The lettering should be in double the size of printed letters. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size 16cms (ox axis) × 20cms (oy axis); photographs must be on glossy paper and must have good contrast; *three copies* should be sent.

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

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

   The list of references should be included at the end of the article in serial order and the respective serial number should be indicated in the text as superscript.

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

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

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

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

   (*d*) *Proceedings, Conferences and Symposia Papers:* As in (*c*), if the article is not published then the title of the article should be mentioned.

   (*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.

9. Consult the latest copy of the *Journal* for guidance.

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Research Papers

Research Notes

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