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RESEARCH PAPERS

Studies on the Suitability of Roller Flour Mill, Hammer Mill and Plate Grinder for Obtaining a Refined Flour from Malted Ragi *(Eleusine coracana)*

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A refined white flour was obtained from malted ragi (*Eleusine coracana*) in 57, 64 and 66 per cent yield after moist conditioning the grain and grinding in roller flour mill, hammer mill and plate grinder respectively. The refined flour was almost free from bran, adequate in calcium (about 250 mg per cent) but slightly low in protein (4.5-5.5 per cent). The bran residues were wet processed to extract an edible fraction in yield of about 5 to 8 per cent. This fraction was fairly rich in protein, minerals, calcium and phosphorus. Flour obtained from roller flour mill was the whitest followed by that from hammer mill and plate grinder. A relative estimate of minute traces of bran in refined flour was made colorimetrically by pigment extraction procedure.

Ragi malt is one of the popular products prepared from ragi[1] and finds application in weaning foods[2,3], beverages[4] and special nutritious foods[5]. In customary preparation of ragi malt flour, either the yield of flour is low or the malt flour has a very high content of coloured bran. A method of obtaining ragi malt flour with negligible content of bran and without sacrifice of yield or nutritional quality of refined flour is, therefore essential. The effectiveness of moist conditioning of ragi with small amount of water followed by grinding to obtain a flour relatively low in bran and its effect on the protein, mineral and fibre content of the different milling fractions were, therefore studied and are reported here.

Materials and Methods

Ragi was germinated for 72 hr and malted grain prepared as described earlier[6]. When the malted grain with moisture content of 8 per cent was pulverised, it was found that the bran also was easily ground along with endosperm. Hence a short period of moist conditioning of the grain with different amounts of moisture was tried and found suitable as a method for minimising the friability of the bran in 3 types of grinding units such as the Laboratory Buhler roller mill, the hammer type Apex Comminuting mill (model-S) and a plate grinder. Preliminary trials indicated that additions of 5, 10 and 7 per cent water to the grain and conditioning for 30 min gave minimum pulverisation and maximum separation of bran with roller, hammer and plate mills respectively and was, therefore, followed in the further studies.

The different break (B_1, B_2, B_3) and reduction (R_1, R_2. R_3) roll fractions as well as shorts and husk fractions were obtained in the roller mill having normal break and reduction roll clearances of 0.13, 0.1 mm for break rolls and 0.07 and 0.03 mm for reduction rolls respectively. In the hammer mill with 0.32 mm screen (4400 r.p.m.) and plate grinding mill (480 r.p.m.), the flour obtained in the first stage grinding was sieved through 200 mesh (B.S.S.) sieve and +200 fraction was reground immediately and again sieved, the process being repeated again. The yield of different milling fractions with the 3 types of mills, their whiteness and content of bran pigments as also the proximate composition were determined. Whiteness was measured by measuring its reflectance in Photovolt Reflectance Meter using tristimulus green filter. Relative bran content was measured by an improvised bran pigment extraction procedure similar to that used in case of rice bran pigments[7], with following modifications. Half a gram sample was dispersed in 50 ml of N KOH, heated on waterbath for 2 hr and the colour was extracted with 50 ml of methanol, the colour in the supernatant after making up the volume to 100 ml with water being measured at 390 mμ. Proximate principles were determined according to standard A. O. A. C. methods.

Nearly 30-35 per cent of the ragi was obtained as highly coloured residue rich in bran containing 5-10 per cent endosperm powder. In order to recover this

TABLE 1. YIELD AND CHEMICAL COMPOSITION OF INDIVIDUAL FRACTIONS OBTAINED BY MILLING MALTED RAGI IN FLOUR MILL

	Control	Break rolls			Reduction rolls			Total flour	Residue		Wet processing of residue	
		B_1	B_2	B_3	R_1	R_2	R_3		Shorts	Husk	Shorts	Husk
Yield (%)		7.5	7.3	1.3	32.3	5.7	2.8	56.9	24.6	18.5	4.9 (20.0)	4.0 (21.5)
Protein (N×6.25) (%)	7.4	2.3	3.8	4.9	3.4	5.9	7.9	4.1	12.5	12.2	8.8	10.0
Ether extractives (%)	1.5	0.3	0.4	0.9	0.3	1.4	2.0	1.0	3.6	3.3	2.7	3.0
Crude fibre (%)	3.6	0.4	0.6	0.8	0.7	0.8	1.0	0.9	6.3	8.1	1.3	1.7
Ash (%)	2.9	0.6	0.7	0.8	1.0	1.7	2.0	1.1	4.4	4.6	1.5	1.8
Calcium (mg %)	430	138	168	205	201	231	280	198	680	750	220	245
Phosphorus (mg %)	250	40	56	119	91	289	366	105	630	460	470	410
Reflectance reading	53	82	79	64	80	71	59	67	12	13	46	40
Bran colour absorbance reading	0.32*	0.14	0.22	0.38	0.19	0.27	0.47	0.30	0.6+	0.59+	0.67	0.74

Values are on dry weight basis.

Figures in parenthesis relate to % yield on the basis of starting material

*Colour reading taken after two fold dilution of extract.

+Colour reading taken after five fold dilution of extract.

edible matter from the residue a wet rendering procedure similar to that adopted by Kurien and Desikachar[8] was used using 100 mesh sieve. The proximate composition of this fraction was also determined.

Results and Discussion

Refining with roller flour mill: The milling trials of malted ragi in the roller flour mill gave a total yield of 57 per cent refined flour with 25 per cent shorts and 18.5 per cent husk (Table 1). Maximum flour was obtained with first reduction roll with an yield of about 32 per cent. The flour from the second and third reduction rolls contained more protein, ash, minerals and phosphorus as compared with the three break roll fractions indicating that the break rolls represent materials from endosperm core while the second and third reduction rolls represent to a greater extent flour from the peripheral part of the endosperm. While the mixed flour from the roller mill was white with a reflectance reading of 67 and bran colour absorption reading of 0.3, it had only 4.1 per cent protein although adequate in respect of calcium and phosporus. The shorts and husk fraction contained nearly 12 per cent protein, 3.5 per cent fat and high proportion of calcium and phosphorus. As could be expected, their crude fibre content was very high (6.3 and 8.1 per cent respectively). When short and husk fractions were subjected to wet rendering extraction procedure, 4.9 and 4 per cent of an edible fraction with 8.8 and 10 per cent protein content respectively were obtained. These were rich in calcium and phosphorus and low in crude fibre content. Addition of this extract from short and husk fraction to the total flour could increase its protein and mineral contents.

Grinding studies with Hammer and Plate mills: The general pattern of results obtained with the hammer and plate mills is almost similar except that fraction 3 of plate mill gave a slightly higher yield than the hammer mill. The total yield of refined flour in the hammer and plate mills was 63.9 and 66.4 per cent respectively. Earlier fractions in both the mills contained lower protein, fat, fibre, ash and minerals than the subsequent fractions. This was due to the later fractions containing minute amounts of pulversied bran and peripheral endosperm. The fraction III in plate and hammer mills had a dull appearance with a lower reflectance reading as compared with fractions I and II. The combined flour (fraction I, II and III) from hammer and plate mills contained 5.2 and 5.4 per cent protein respectively with adequate quantities of calcium and phosphorus and had crude fibre content of 1.3 and 1.5 per cent respectively. The corresponding reflectance values were 62 and 60 respectively. The bran pigment reading was 0.36 and 0.38 respectively indicating very low bran content as compared with the control.

Nearly 1/3 of the grain was obtained in the form of a residue with higher protein, calcium and phosphorus content than original grain but with high bran and crude fibre contents. When these residues were extracted with water, edible fractions rich in protein and minerals and low in fibre content were obtained in yields of 7.2 and 8.4 per cent respectively (Table 2).

TABLE 2. RELATIVE YIELD AND CHEMICAL COMPOSITION OF FLOUR FRACTIONS OBTAINED BY MILLING OF MALTED RAGI IN HAMMER AND PLATE GRINDERS

	Control	Fraction I		Fraction II		Fraction III		Total flour		Residue		Wet processing of residue	
		H	P	H	P	H	P	H	P	H	P	H	P
Yield (%)		47.3	45.0	11.6	12.1	5.0	9.3	63.9	66.4	35.1	33.6	7.2 (20.0)	8.4 (25.0)
Protein (N×6.25) (%)	7.4	4.5	4.2	6.0	6.4	9.2	8.4	5.2	5.4	13.4	11.3	9.5	9.8
Ether extractives (%)	1.5	0.9	0.9	1.3	1.5	2.1	2.4	1.1	1.4	3.1	3.3	3.0	2.7
Crude fibre (%)	3.6	1.2	0.9	2.1	2.0	3.2	3.9	1.3	1.5	6.8	8.8	1.8	2.0
Ash (%)	2.9	1.1	1.0	1.9	2.0	2.0	2.3	1.4	1.6	3.5	3.8	1.6	1.8
Calcium (mg %)	430	220	190	320	270	405	440	245	250	735	870	120	105
Phosphorus (mg %)	250	65	80	155	143	219	225	115	120	600	635	360	365
Reflectance reading	53	74	76	60	58	44	42	62	60	12	13	50	54
Bran colour absorbance reading	0.32*	0.21	0.20	0.34	0.35	0.51	0.53	0.36	0.38	0.58+	0.56+	0.52	0.44

Values are on dry weight basis

H = Hammer mill samples; P = Plate grinder samples.

Figures in parenthesis relate to % yield on the basis of starting material.

*Colour reading taken after two fold dilution of extract.

+Colour reading taken after five fold dilution of extract.

While the roller flour mill normally used for grinding of wheat can be used for obtaining a refined flour from malted ragi for commercial purposes, the less sophisticated and more widely available simple plate or hammer type mills normally used for grinding grains can also be used for obtaining a refined flour nearly free from bran in yields of about 65 per cent. If desired, the residue can be processed further by wet extraction procedure to edible flour in yields of about 7-8 per cent. While this may not be practical in households, it may be feasible and economical for a commercial factory.

The moist conditioning technique facilitates recovery of a white refined flour in yields of about 65 per cent with minimum bran content and is a great improvement over conventional methods. The observation that the simple plate or hammer mill can be adapted for removal of the major portion of the bran is useful and such a technique can be availed of by small establishments and also in rural areas for obtaining malted ragi flour suitable for infant and child feeding. The roller mill can also be used for manufacture of a fully refined flour for sophisticated uses.

The residual fraction rich in bran obtained after milling can be used as a component of feeds and also as a raw material for extraction of natural vegetable pigments needed in the food industry.

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The Chemical Composition of Iraqi Rice and Rice By-Products

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The contents and the distribution of protein, fat, fibre, ash, nitrogen free extract (NFE) and nutrient elements of seven Iraqi rice and rice by-products were determined. Chemical analysis indicated that protein contents of both brown and milled rices have a significant negative correlation with NFE. Bran contained maximum Ca, K, Mg and P, whilist, milled rice contained the lowest levels in all tested varieties.

Although rice is the principal cereal food in Asia, since it is high in caloric value and nitrogen free extract, it is comparatively low in protein. Nutrient distribution in brown rice has been studied in detail by analysis of its sucessive abrasive milling fraction.[1,2] Proximate analysis of brown and milled rices have been compiled by Juliano *et al.*[3] and McCall *et al.*[4] The mineral composition of corn, wheat germ, triticale - wheat and rye have been reported by Czerniejewski *et al.*[5], Zook *et al.*[6] and Toepfer *et al.*[7] The mineral composition of rice is influenced by the soil in which it is grown and the analytical methods employed[4]. The inorganic constituents of brown rice and its fractions have been studied by McCall *et al.*[4].

The objectives of this study were to determine the chemical composition of Iraqi rice and its byproducts, which information is lacking and to determine the changes in nutrients as a result of milling of the grains.

Materials and Methods

Simple identification: Seven varieties of rice-'Anbar' first grade, 'Anbar' second grade, 'Basmati', 'Yareat IR8', 'Khraiba', 'Naima' and 'Hwezwi' grown under irrigation in Iraq during 1976-1977 were obtained from Grain Board of Iraq.

The clean, dried rough rice (paddy) was dehulled with McCill sample sheller, and polished in McCill Miller[8] and cleaned. Samples of brown, milled bran and husks were subjected to detailed proximate analyses. Samples were ground in a Wiley standard laboratory mill with 20 mesh sieve and stored at 0°C in stoppered bottles. The ground samples were divided into two parts. One part was analyzed in duplicate by standard AOAC methods[9]. Moisture was determined by the loss of weight after 5 hr at 98-100°C in a vacuum oven. Crude fat was estimated with a Coldfisch extractor using petroleum ether. Ash was estimated in muffle furnace at 525°C. Crude fiber is the loss on ignition of dried residue remaining after digestion of sample with 1.25% H_2SO_4 and 1.25% NaOH solutions. Protein content was calculated from Kjeldahl nitrogen multiplied by the factor 5.95. Carbohydrate, or nitrogen free extract (NFE) content was calculated by difference. Data were recalculated on the dry basis.

Mineral content detemination: For the analyses of Ca. Zn, Mn, and Fe about 4 g of each of the different fractions were wet ashed and then they were determined by the Atomic Absorption Spectrosocopy (Perkin-Elmer Model 303). The colorimetric molybdenum blue method was adopted for phosphorus estimation. K and Na were determined by AOAC methods[9]. Mg and silicon were determined gravimetrically[9].

Results and Discussion

Milling yields: Highest yield of brown rice (78%) was obtained with 'Anbar' first grade and the lowest (74.4%) was given by 'Kharaiba'. 'Anbar' first grade gave 40.2% head rice (The percentage by weight of the quantity of head rice that are produced from a unit of rough rice). 'Hwezawi' gave the lowest yield (40.8%).

Brown rice: The composition of brown rice is shown in Table 1. The percentage of protein is lower than the percent protein of rice varieties reported by Cagampang *et al.*[11] and Juliano *et al.*[3], whilist the content of crude fat, fibre, ash and NFE is similar to the values reported by Juliano *et al.*[3] A highly significant negative correlation of -0.93 was obtained between crude protein and NFE contents. Crude protein, fat content and fibre contents were significantly postively correlated.

Milled rice: The composition of milled rice is shown in Table 2. These results were similar to those reported by McCall *et al*[4]. A highly significant correlation of -0.82 was obtained between crude protein and NFE contents. Similar correlations for brown and milled rice were previously reported by Juliano *et al*[3]. The fat content of rice was so low, for most of it was lost during the process of milling and is contained in the bran (Table 3).

TABLE 1. COMPOSITION OF BROWN RICE

Variety	Moisture (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	NFE (%)
Anbar first grade	11.13	9.66	2.70	1.17	1.24	85.23
Anbar second grade	10.44	8.01	2.81	1.01	1.45	86.72
Basmati	9.91	7.83	2.17	0.84	1.17	87.99
Yareat IRB	10.10	8.51	3.03	0.93	1.24	86.29
Khraiba	10.14	9.25	3.68	1.58	1.87	83.62
Naima	11.96	8.00	2.15	0.99	1.51	87.35
Hwezawi	11.53	7.68	2.54	1.46	1.46	86.86
Correlation Coff. (Protein ~ NFE, Fat, Fibre and Ash.)			0.61*	0.61*	0.35	-0.93**

Values are on moisture free basis; mean of duplicate samples.
**Highly significant; *Significant.

TABLE 2. COMPOSITION OF MILLED RICE

Variety	Moisture (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	NFE (%)
Anbar first grade	11.26	9.57	.53	.80	.55	88.55
Anbar Second grade	10.38	6.88	.34	.58	.82	91.38
Basmati	10.93	6.79	.48	.81	.48	91.44
Yareat IR8	11.11	6.67	.54	.25	.52	92.02
Khraiba	8.73	8.14	.11	.42	.75	90.58
Naima	10.50	7.06	.24	.38	.25	92.07
Hwezawi	11.42	6.68	.68	.54	.62	91.48
Correlation coeff. Protein ~ NFE, Fat, Fibre and ash			-.19	0.45	0.01	-.82**

Values are on moisture free basis; mean of duplicate samples
**Highly significant

TABLE 3. COMPOSITION OF RICE BRAN

Variety	Moisture (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	NFE (%)
Anbar first grade	10.95	14.45	11.87	8.66	7.05	57.77
Anbar Second grade	8.53	10.20	10.23	9.16	6.30	64.11
Basmati	9.79	11.65	8.79	8.56	6.94	64.06
Yareat IR8	9.91	12.45	8.49	6.91	5.80	66.35
Khraiba	10.32	12.65	8.73	7.93	7.00	63.69
Naima	10.45	10.32	9.79	7.96	6.43	65.50
Hwezawi	10.84	10.20	7.00	6.14	6.02	70.64
Correlation coeff. Protein ~ NFE, Fat, Fibre and Ash			.39	-.37	-.001	-.99**

Values are on moisture free basis; Mean of duplicate samples.
**Highly significant.

Bran polish: The composition of rice bran-polish is shown in Table 3.

Rice bran has a high food value, is generally used in animal feeds, and its efficiency as a feed supplement has been widely tested[12]. The protein content is similar to the results reported by Gad *et al.*[13], whilist crude fat, fibre and ash contents were different from their values.

Husk: Husk from seven varieties form 18.3-28 per cent of paddy. Using 55 samples for analysis, Juliano *et al.*[14] found husk comprise from 16.3 to 26.0%

of paddy. Composition of husks is shown in Table 4. Fibre and ash are the major components of husk and these particularly the latter lower the nutritional value.

Minerals: The ash content of brown rice, milled rice, bran and husk are 5,2,22 and 71 per cent respectively. Mineral composition of these fractions are reported in Tables 5, 6, 7 and 8. A considerable portion of the rice ash is accounted for by P, Mg and K.

Generally, bran contains the highest percentage of P, K and Mg, while milled rice contains the lowest percentage of these minerals. Bran also contains higher concentration of nutritionally important trace elements

TABLE 4. COMPOSITION OF RICE HUSK

Variety	Moisture (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	NFE (%)
Anbar first grade	7.57	2.39	1.24	22.63	20.99	52.75
Anbar second grade	7.46	2.19	.59	27.61	20.45	49.16
Basmati	7.44	2.74	.50	18.61	20.03	58.12
Yareat IR8	7.80	1.69	1.33	25.26	18.59	53.13
Khraiba	5.16	2.19	1.78	38.08	22.00	45.05
Naima	8.00	2.01	1.30	38.52	19.93	38.24
Hwezawi	7.68	1.66	1.27	22.59	27.82	46.66

Moisture free basis; Mean of duplicate samples.

TABLE 5. MINERAL COMPOSITION OF BROWN RICE

Variety	K (%)	Si (%)	Mg (%)	P (%)	Na (μg/g)	Ca (μg/g)	Zn (μg/g)	Mn (μg/g)	Fe (μg/g)
Anbar first grade	0.175	0.103	0.092	0.230	25	130	12	6.0	8.0
Anbar Second grade	0.240	0.116	0.095	0.180	35	190	16	5.4	12.0
Basmati	0.172	0.142	0.107	0.180	40	140	12	6.0	6.0
Yareat IR8	0.128	0.145	0.056	0.140	29	90	8	4.9	7.0
Khraiba	0.192	0.200	0.087	0.200	65	130	12	6.0	8.0
Naima	0.072	0.137	0.166	0.280	60	240	20	64.0	16.0
Hwezawi	0.116	0.170	0.144		55	70	10	21.5	7.0
Correlation coeff. K, Si, Mg, P, and ash	.090	.691*	.361	.003					

Values are on moisture free basis; Mean of duplicate samples; P is by colorimetric method
*Significant.

TABLE 6. MINERAL COMPOSITION OF MILLED RICE

Variety	K (%)	Si (%)	Mg (%)	P[c] (%)	Na (μg/g)	Ca (μg/g)	Zn (μg/g)	Mn (μg/g)	Fe (μg/g)
Anbar first grade	0.050	0.040	0.086	0.140	35	75	9	11.3	2.5
Anbar Second grade	0.099	0.045	0.088	0.060	50	80	12	10.1	2.7
Basmati	0.039	0.034	0.053	0.030	35	125	15	16.0	2.0
Yareat IR8	0.099	0.029	0.046	0.080	25	80	9	10.1	3.0
Khraiba	0.049	0.0384	0.035	0.150	45	120	12	1.0	2.5
Naima	0.055	0.024	0.028	0.100	40	65	12	6.3	4.0
Hwezawi	0.083	0.047	0.062	0.60	35	135	15	19.5	5.0
Correlation coeff. between contents of K, Si, Mg, P and ash	-.044	.432	.333	.185					

Values are on moisture free basis; Mean of duplicate samples; c. By colorimetric method.

TABLE 7. MINERAL COMPOSITION OF BRAN

Variety	K (%)	Si (%)	Mg (%)	Pc (%)	Na (μg/g)	Ca (μg/g)	Zn (μg/g)	Mn (μg/g)	Fe (μg/g)
Anbar first grade	1.575	0.348	0.64	0.95	217	450	60	170	126
Anbar Second grade	1.316	0.313	0.52	0.60	140	280	50	90	84
Basmati	0.616	0.235	0.38	0.81	77	240	40	80	57
Yareat IR8	1.400	0.276	0.64	0.65	168	570	65	135	81
Khraiba	1.225	0.35	0.69	0.80	168	890	55	150	108
Naima	0.966	0.303	0.60	1.30	165	390	55	110	82
Hwezawi	1.400	0.187	0.05	0.75	224	600	75	190	138
Correlation coeff. between the contents of K, Si, Mg, P, and ash	-.016	.788**	.019	.743**					

Values are on moisture free basis; Mean of duplicate samples; c. By colorimetric method.
*Highly significant.

TABLE 8. MINERAL COMPOSITION OF HUSK

Variety	K (%)	Si (%)	Mg (%)	Pc (%)	Na (μg/g)	Ca (μg/g)	Zn (μg/g)	Mn (μg/g)	Fe (μg/g)
Anbar first grade	0.210	36.32	0.110	0.12	1150	1060	8.0	4.0	15.0
Anbar Second grade	0.225	34.68	0.082	0.09	1100	1290	5.0	3.0	17.0
Basmati	0.105	32.92	0.061	0.07	560	450	2.5	2.0	13.0
Yareat IR8	0.375	31.44	0.082	0.09	180	1530	16.0	5.0	15.5
Khraiba	0.150	39.00	0.138	0.231	400	590	2.5	3.1	15.8
Naima	0.210	33.52	0.199	0.20	105	2100	6.4	3.5	8.5
Hwezawi	0.087	44.74	0.108	0.12	470	2860	13.5	8.0	16.0
Correlation coeff. between the contents of K, Si, Mg, P and ash	0.899**	-.125	0.792**	0.99**					

Values are on moisture free basis; Mean of duplicate samples; c. by colorimetric method
**Highly significant.

such as Zn, Mn and Fe, meanwhile, brown and milled rice contain the lowest percentages of the nutritionally important trace elements. Feeds for stocks and poultry are the greatly predominant uses for bran[15]. Husk contains the highest percentage of Si. Therefore, the husk is not useful as feed.

The mineral content of brown rice reported in Table 5 was generally lower than that reported by Grist[16] with the exception of Na. The Ca, Mg and Si content of milled rice, were higher than the values reported in U.S.A. samples by McCall *et al*[4], however, Zn, K and Mn contents were almost similar. With the exception of sodium, mineral composition of bran was found to be much lower than those in the varieties from U.S.A. studied by McCall *et al*[4]. Similarly variations have been noticed in the mineral contents of rice grown in different parts of the world which may be due to the soil and analytical methods employed[17].

Effect of milling on nutritive value: Average loss of nutrients due to milling and polishing of seven varieties are 12.4 per cent of protein, 84.6 per cent of fat, 26.3 per cent of Ca and 59.0 per cent of Fe. Similar loss in protein, fat and Fe were also noted in milling samples of low protein rices, the losses are 9.3 per cent of protein, 80 per cent of fat and 66.6 per cent of Fe[2].

The type of milling and degree of polishing determine the amount of nutrients removed; protein, fat and minerals are present to a greater extent in the germ and other layers than in the starchy endosperm.

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Studies on the Antimicrobial and Stimulatory Factors of Garlic (*Allium sativum* Linn.)

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Storage studies conducted with raw garlic extract at various temperatures, for periods upto 48 hr showed that, both antifungal and antibacterial activities are stable beyond 48 hr in extracts stored at room temperature and 37°C. The antibacterial activity, when stored at 55°C and above was stable upto 36 hr, whereas the antifungal activity was stable only upto 8 hr under these conditions. The components of the broth used for testing the activity have shown an antagonistic effect on the activity of enzyme and on allicin as such. Cooked garlic extract showed a significant growth stimulating activity of bacterial and fungal cultures. It has been demonstrated that the stimulatory activity is not due to alliin, cooked alliin, carbohydrates and organic nitrogen but due to a dialysable ninhydrin positive compound, probably peptide in nature.

Garlic (*Allium sativum* Linn.) is well known for its medicinal, antimicrobial and pesticidal properties. Various extracts of garlic have been known to possess antifungal and antibacterial activitity[1] as well as anticandidal activity[2]. Among water extracts of several spices, antibacterial activity of garlic was the most potent[3]. Garlic extract has been found to be fungistatic at lower concentrations, and fungicidal at higher concentrations[4].

The antibacterial principle (allicin) has been isolated from garlic and its activity has been demonstrated[5]. The existence of allinase, alliin homologues, which are the precursors of allicin as well as allicin homologues and allithiamine in garlic has been indicated[6]. The biochromatograms of an extract of aqueous garlic suggested that allicin was the major anticandidal component and that other thiosulfinates in garlic extract might have some anticandidal activity[2].

In many of the culinary preparations, garlic is used as a flavouring agent where it is cooked along with other ingredients and hence might lose its antimicrobial activity. While testing the cooked garlic extract, it has been found that the extract possesses a microbial growth stimulating factor which has not been reported so far. In this communication, results obtained on antimicrobial as well as growth stimulating factor of garlic have been presented.

Materials and Methods

The garlic obtained from the Mysore market was

dehusked, ground with twice the weight of water in pestle and mortar, allowed to react for 10 min and filtered through cheese cloth. The extract was seitz filtered in E.K. No. 3 filter pad. The filtrate (50 ml) was stored in sterile 250 ml conical flasks at different temperatures. Periodically, 2 ml. were drawn and aseptically added to 10 ml yeast dextrose peptone broth, containing yeast extract (0.5 per cent), dextrose (1 per cent) and peptone (1 per cent) in 17×150 mm test tubes in case of bacterial cultures. Similarly 2 ml of garlic extract was aseptically added to 25 ml Czapeck-Dox broth supplemented with 0.1 per cent each of yeast extract and casamino acids in 250 ml conical flasks for fungal cultures. The microbial cultures used in the studies were obtained from the stock cultures of C.F.T.R.I., Mysore. The tubes were inoculated with 0.1 ml cell suspension of 18 hr old *S. faecalis* and *E. coli* cultures having 6.5×10^7 cells/10 ml of the medium. In case of fungi, 0.2 ml of 3 day-old *A. flavus* and *P. citrinum* cultures having 2×10^7 spores were added to 25 ml medium. The cultures were incubated at 37°C for 24 hr. at stationary conditions in case of bacteria and at room temperature (26-30°C) in shake flask cultures for 48 hr in case of fungi.

The growth in bacterial cultures was measured in terms of acidity titrated against 0.05 N NaOH using bromothymol blue as indicator[8] and expressed as acidity/ml culture. The mycelial weight of fungal cultures was determined by filtering through fluted, preweighed Whatman No. 1 filter paper, washing the mycelial pellets with distilled water and drying in hot air oven at 105°C for 8 hr or until a constant weight was obtained. Difference in weight was calculated and expressed as mg/ml of culture broth.

Storage of garlic extract with YDP broth: Raw garlic extract (2 ml each) was transferred to 10 ml each of YDP broth in 17×150 mm size test tubes and stored at 37°C in incubator for 0, 8, 18, 24, 36 and 48 hr. At the end of each storage period, tubes were inoculated with bacterial cultures and incubated. Acidity was determined as above.

Addition of culture medium components: Seventy grams of cooked garlic bulbs were crushed and suspended in 70 ml distilled water and filtered to obtain the precursor substrate. Twelve milliliter of this substrate was transferred to 12 ml each of 1 per cent peptone, 0.5 per cent yeast extract, 1 per cent dextrose and YDP broth and incubated for 4 hr. Distilled water (12 ml) was kept as control. After 4 hr, 3 ml of freshly prepared raw garlic extract was added to the above solutions as a source of enzyme and incubated for 8 hr and at the end, 2 ml each was transferred to 10 ml of YDP broth tubes. These were inoculated with bacterial cultures, incubated and the growth was measured as described earlier. The above experiment was repeated by changing the sequence of addition i.e. enzyme first and then the precursor substrate.

Preparation and testing of the cooked garlic extract: Cooked garlic bulbs were extracted in distilled water at 1:2 ratio and filtered to obtain the source of alliin. A fresh raw extract of 1:2 level was prepared and added to the former at 4:1 level, as a source of enzyme. These were stored at room temperature (26-30°C) for 8, 18 and 24 hr. At the end of each storage period, 2 ml each of the above solutions were transferred to each of 10 ml YDP broth tubes and bacterial growth was tested. Another set only with 2 ml of cooked garlic extract was maintained.

Preparation of pure alliin: For the extraction and purification of alliin from garlic the procedure of Stoll and Seebeck[7] was followed. Its activity was determined by reacting with allinase and found to be inhibitory to *S. faecalis*.

Fractionation of cooked garlic extract: Twenty milliliter of cooked garlic extract (1:1) was extracted twice with 40 ml of diethyl ether, evaporated to dryness and suspended in 6 ml of distilled water. Its activity was tested by transferring 2 ml of the above solution to 10 ml of YDP broth previously inoculated with *S. faecalis*. The remainder of the aqueous fraction was further extracted with 40 ml of chloroform. After removing chloroform, the activity of the fraction was tested as above. Further, 1 ml of the remaining aqueous phase freed from the traces of solvents was added to 10 ml of YDP broth and the activity was determined by using *S. faecalis*.

Determination of the nitrogen content: The dialysate (5 ml) was used for determination of nitrogen by micro-Kjeldahl method[9].

In all the above experiments, triplicates were maintained and the average values are given.

Results and Discussion

The stability of the antimicrobial principle of garlic extract during storage was examined at various temperatures. The results in Table 1 show that after 24 hr of incubation the extracts developed higher antibacterial activity at 37°C than those kept at room temperature (26-30°C). However, the extract incubated at 55 and 60°C had the same antimicrobial potency after 8 hr. It has been found by Stoll & Seebeck[7] that alliin is completely converted to allicin by allinase in about 5 min after the extraction. The activity of the extract stored at 37, 55 and 60°C increased in a storage period of about 8 hr. This increase in activity attained its maximum at a storage period of 24 hr at 37°C and 8 hr at both 55 and 60°C. This increase in activity may be due to the formation of a new antimicrobial agent

TABLE 1. STORAGE STUDIES OF GARLIC EXTRACT ON BACTERIAL CULTURES, *S. FAECALIS* AND *E. COLI*

Storage period (hr)	Acidity/ml culture at diff. temp.			
	26-30°C	37°C	55°C	60°C
	S. faecalis			
Control	—	0.48	—	—
0	0.25	0.27	0.28	0.28
8	0.26	0.23	0.12	0.13
18	0.26	0.21	0.15	0.18
24	0.27	0.15	0.33	0.47
36	0.28	0.13	0.43	0.47
48	0.25	0.14	0.53	0.60
	E. coli			
Control	—	0.36	—	—
0	0.25	0.25	0.28	0.27
8	0.25	0.21	0.11	0.11
18	0.25	0.22	0.15	0.16
24	0.25	0.14	0.26	0.36
36	0.25	0.13	0.38	0.42
48	0.24	0.14	0.45	0.55

during storage of garlic extract. This supports the view of the probable existence of two or more antimicrobial principles in garlic as obseved by Barone and Tansey[2].

Storage at 37°C not only provides suitable conditions for the synthesis of allicin, but also prolongs the shelf-life of allicin. At higher temperature, the antibacterial principle gets inactivated faster on storage. This is generally followed by stimulation of growth in bacterial cultures.

Since garlic extract is invariably consumed along with other dietary items, it was of interest to know the effect of other organic substances on the antibacterial action. Results tabulated in Table 2 show that the activity of allicin goes down as the storage time increases. This may

TABLE 2. EFFECT OF PRE-STORAGE OF GARLIC EXTRACT IN YDP BROTH TUBES ON ANTIBACTERIAL ACTIVITY

Storage period (hr)	Acidity/ml culture	
	S. faecalis	*E. coli*
Control	0.47	0.33
0	0.27	0.23
8	0.33	0.27
18	0.38	0.32
24	0.45	0.38
36	0.45	0.45
48	0.47	0.47

TABLE 3. ANTAGONISTIC ACTIVITY OF THE MEDIA COMPONENTS WITH ALLICIN AND ALLINASE

Treatment	Acidity/ml culture			
	S. faecalis		*E. coli*	
	A	B	A	B
Control	0.46	—	0.35	—
Water	0.24	0.32	0.24	0.28
Peptone 1%	0.32	0.36	0.30	0.35
Yeast extract 0.5%	0.24	0.32	0.23	0.31
Dextrose 1%	0.29	0.39	0.29	0.34
YDP broth	0.38	0.41	0.33	0.35

A—Enzyme added to precursor after 4 hr.
B—Precursor added to enzyme after 4 hr.

be perhaps due to an interaction between the media components and allicin, thereby bringing down the activity drastically as in the case of *Candida albicans*[2].

Further, the individual components of the broth were tested to find out their interaction with allicin, when stored for a total period of 12 hr. The results presented in Table 3 show that the activity of allicin decreased when it was stored with 1 per cent peptone, 1 per cent dextrose or YDP broth. Whereas, when stored either in distilled water alone or 0.5 per cent yeast extract, there was not much reduction in the activity. In general, it has been found that when enzyme

TABLE 4. STORAGE STUDIES OF GARLIC EXTRACTS ON THE ANTI-FUNGAL ACTIVITY

Storage period (hr)	Mycelial wt. mg/ml (Czapeck broth)			
	26-30°C	37°C	55°C	60°C
	Aspergillus flavus			
Control	—	11.48	—	—
0	1.72	2.08	2.16	2.28
8	1.08	1.48	2.00	2.44
18	0.68	0.72	17.32	14.00
24	0.84	0.96	14.00	11.40
36	2.20	1.20	19.24	18.36
48	1.00	1.28	15.52	16.48
	Penicillium citrinum			
Control	—	9.72	—	—
0	2.24	1.72	1.48	1.48
8	2.32	1.60	1.84	2.68
18	1.92	1.72	11.00	15.44
24	1.44	1.28	13.84	18.60
36	1.60	1.64	15.20	16.92
48	1.24	1.44	12.96	16.68

is added to the precursor along with other individual media components, the activity was found to be better than when the precursor was added to the enzyme after 4 hr of incubation at room temperature. This indicates that the enzyme and allicin get inactivated, when stored along with media components. This corroborates with the observation of Barone and Tansey[2] that the foetal calf serum has an antagonistic effect on the activity of allicin.

Storage studies of the garlic extract were also carried out with fungal cultures such as *A. flavus* and *P. citrinum*. The activity of garlic extract has been found to be quite stable even after 48 hr when the extract was prestored at room temperature (26-30°C) and 37°C as given in Table 4. There appears to be some difference in the activity of the garlic extract against bacterial and fungal cultures. The activity was more in the extract stored at 37°C than that at room temperature in case of bacterial cultures. But no such difference was noticed in case of fungal cultures. The activity of the garlic extract when stored at 55°C and above decreased after 8 hr incubation. The storage of the extract beyond 18 hr at the elevated temperatures lowered antifungal activity. There seems to be a stimulatory effect on the fungal growth as observed in bacterial cultures.

Since garlic extract stored at 60°C for a certain period showed a stimulatory effect which is not observed hitherto by other workers, attempts were made to elucidate the nature of this stimulant. The activity of the extract of the boiled garlic was tested using *E. coli* and *S. faecalis* cultures. Cooked garlic extract showed stimulatory activity in both the cultures It was suspected that the precursor of allicin in the cooked garlic extract was responsible for the stimulatory activity of microbes. Therefore, alliin was prepared in pure form and tested for the stimulatory activity of both cooked and uncooked alliin, with *S. faecalis* culture. The results in Table 5 illustrate that neither alliin as such nor its cooked form stimulates the growth of bacteria. Further, the cooked garlic extract was fractioned into ether soluble fraction, chloroform soluble fraction and residual water phase. Results in the Table 5 show that the stimulatory principle is neither ether soluble nor chloroform soluble and was retained only in the water phase.

TABLE 5. EFFECT OF DIFFERENT FRACTIONS OF COOKED GARLIC EXTRACT ON THE GROWTH OF *S. FAECALIS*

	Acidity/ml culture
Control	0.37
Alliin pure, 25 mg/ml	0.39
Alliin pure boiled, 25 mg/ml	0.41
Cooked garlic extract+fresh enzyme	0.14
Cooked garlic extract as such	0.52
Ether extract	0.38
Chloroform extract	0.36
Residual water extract	0.59

TABLE 6. EQUINITROGEN SUPPLEMENTATION OF CASAMINO ACIDS AND DIALYSATE ON THE GROWTH OF *S. FAECALIS*

Treatment	Acidity/ml culture
Control	0.44
Dialysate	0.53
Casamino acids	0.43

Since garlic is known to contain some amount of carbohydrates which may have the stimulatory activity, glucose was added to the broth at varying concentrations to test the stimulatory activity. Glucose upto 3 per cent did not show any stimulatory activity as compared to the cooked garlic extract.

The stimulatory factors in the cooked garlic extract was found to be water soluble. Hence cooked garlic extract was subjected to dialysis to find out whether the factor was dialysable. The dialysate obtained was concentrated and tested for the stimulatory activity of the growth on *S. faecalis* and it has been found to be stimulatory.

In order to test the nature of this dialysable compound, the nitrogen content of the dialysate was estimated and found to be 0.32 mg/ml of the dialysate. Six ml of the dialysate which was equivalent to 2 mg of nitrogen was used along with 2 mg of nitrogen in the form of casamino acids in a separate treatment to find out whether the dialysate is of amino acids in nature. Casamino acids on equinitrogen basis did not stimulate the growth of *S. faecalis*, whereas the dialysate did stimulate the growth (Table 6).

The UV spectra of the stimulatory factors have been found to conform with those of peptides. These were ninhydrin positive. Further studies are underway to find out the nature of these growth stimulatory compounds in the cooked garlic extract.

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Studies on the Extractability and Chemical Composition of Leaf Proteins from Certain Trees

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Six trees namely, *Cassia fistula*, *Sesbania grandiflora*, *Gliricidia maculata*, *Morus alba*, *Moringa olifera* and *Leucaena leucocephala* were studied for leaf protein extractability and chemical composition of the recovered leaf protein concentrate (LPC). Cassia, Sesbania, Gliricidia and Morus showed fairly good extractability of protein nitrogen (PN), the values being 45.10, 45.91, 37.40 and 33.80 per cent, respectively. They also yielded crude products having good nitrogen content of 6.58, 6.86, 7.00 and 6.44, respectively. Sesbania leaves showed the highest LPC recovery of 9.44 g per 100 g of fresh pulp while Gliricidia LPC showed highest percentage of protein and ether extract of 43.75 and 15.07, respectively, and lowest ash of 3.60 per cent.

Shortage of supply of good quality protein for meeting the requirements of increasing animal and human population has necessitated search for additional sources. Leaf protein has been advocated as a potential source for human consumption[1-4]. All kinds of weeds, cultivated crops and wild plant species have been studied for their leaf protein production potentiality[3,5-8], but the trees have not yet been sufficiently exploited. Moreover, production of edible protein from tree leaves appears to have unique scope as they do not involve recurring cost of cultivation as in the case of crops. Pirie[9] also advocated the potentiality of tree leaves for leaf protein production.

The present study was, therefore, undertaken to find out the extractability and chemical composition of leaf protein from a few promising trees in order to judge their suitability for the bulk production of leaf protein concentrate.

Materials and Methods

Fully developed leaves of *Sesbania grandiflora* (August tree), *Gliricidia maculata* (Gliricidia), *Morus alba* (Mulberry), *Moringa olifera* (Shahjan) and *Leucaena leucocephala* (Ku-babool) and tender leaves of *Cassia fistula* (Amaltash) were obtained from the Horticultural Gardens of C. S. Azad University of Agriculture and Technology, Kanpur. The leaves collected in the morning were processed immediately.

The extraction procedure suggested by Pirie[10] was followed; 500 g of leaves were minced in domestic meat mincer, and squeezed by hand using long cloth. The extraction was repeated four times with a volume of the extractant (distilled water) equal to the weight of pulp. The five extracts were pooled and the total volume and pH of the extract was measured.

Suitable aliquots of extract were taken for estimating protein nitrogen (PN). Protein was precipitated with cold TCA, separated by centrifugation at 3000 r.p.m. for 10 min and washed as described by Singh[6]. Nitrogen in TCA insoluble fraction was determined.

The pooled extract was heated to 80°C to coagulate protein which was centrifuged, washed with distilled water and finally with acidified (pH 4) water.

Analysis: Dry matter of the pulp, extract and LPC was determined by drying in an oven at 100°C for 40 hr. Nitrogen estimations from the dried powdered materials were done by microkjeldahl method. Protein was calculated by multiplying N with 6.25. Ether extract and ash contents in LPCs were determined by the Soxhlet extraction procedure and by incineration in muffle furnace at 560°C to constant weight respectively.

Results and Discussion

Extractability: Data on extractability of protein from the leaves of six trees are presented in Table 1. It is observed that the leaves of Shahjan, Ku-babool, Amaltash, Gliricidia, Mulberry and August contain fairly good protein of 23.63, 27.56, 24.50, 22.75, 22.75 and 31.50 per cent on dry weight basis, respectively. As regards the extractability of total nitrogen as percentage of total pulp nitrogen, *Cassia fistula*, *Sesbania grandiflora*, *Gliricidia maculata* and *Morus alba* gave 74.10, 59.20, 57.40 and 44.7 respectively, while *Moringa olifera* and *Leucaena leucocephala* gave poor extracta-

TABLE 1. EXTRACTABILITY OF PROTEIN FROM SOME TREE LEAVES

Plant name	DM on pulp (%)	N% on DM of pulp	pH of extract	Total extractable N as % of total N in pulp	Extractable protein N as % of total N in pulp	Dry LPC recovery (% of fresh pulp)	% N on dry crude LPC	Protein content (g) in recovered LPC
*Cassia fistula**	26.11	3.92	6.60	74.1	45.1	5.33	6.58	2.19
Sesbania grandiflora	28.00	5.04	6.10	59.2	45.9	9.44	6.86	4.05
Gliricidia maculata	19.90	3.64	6.35	57.4	37.4	3.82	7.00	1.67
Morus alba	38.40	3.64	7.70	44.7	33.8	6.69	6.44	2.69
Moringa olifera	28.43	3.78	5.95	37.4	20.0	3.95	5.39	1.33
Leucaena leucocephala	32.84	4.41	6.20	29.6	17.9	4.27	5.04	1.35

*Tender leaves

bility of 37.40 and 29.60 percent, respectively. The protein nitrogen extractability percentages in the corresponding four tree leaves were 45.10, 45.91, 37.40 and 33.80 while in the other two leaves the values were 20.0 and 17.90 per cent, respectively. Of the four tree leaves, which showed desirable total N and protein N extractability, *Cassia fistula* appeared to be the best. This might be due to the tenderness of Cassia leaves used in the study.

Dry LPC recovery per 100 g of fresh pulp of Cassia, Sesbania, Gliricidia and Morus were 5.33, 9.44, 3.82 and 6.69 g, respectively while nitrogen values of their LPCs were found to be 6.58, 6.86, 7.00 and 6.44 per cent, respectively (Table 1). Thus when the LPC recovery from fresh pulp and the protein content of the LPC are considered, Sesbania, appeared to be the best containing 4.05 g protein in the recovered LPC followed by Morus, Cassia and Gliricidia which contained 2.69, 2.19 and 1.67 g protein in recovered LPC, respectively. Moringa and Leucaena leaves also showed fairly good LPC recovery of 3.95 and 4.27 g per 100 g fresh pulp, respectively. These LPCs, however, showed poor nitrogen of 5.39 and 5.04 per cent, respectively and therefore, the protein contents of the recovered LPCs were also poor, being 1.33 g and 1.35 g, respectively.

Although *Moringa olifera* and *Leucaena leucocephala* leaves had good protein contents they showed poor extractability due to the presence of a lot of mucilage, causing difficulty in squeezing the pulp for extract. If the problem of mucilage is overcome in these leaves, they too apear to be promising sources for LPC. Moringa and Leucaena are fast growing trees of North India having a lot of succulent green foliage. Moreover, Leucaena is getting popular as a fodder tree in Uttar Pradesh.

Chemical composition: Data on chemical composition of the LPCs from the six trees are recorded in Table 2. It is observed that out of the four leaves which showed desirable extractability of protein, Gliricidia LPC contained the highest protein percentage (43.75 per cent) followed by Sesbania (42.88 per cent), Cassia (41.13 per cent) and Morus (40.25 per cent). Gliricidia LPC also contained highest ether extract of 15.07 per cent followed by Sesbania (10.25 per cent), Cassia (7.45 per cent) and Morus (6.55 per cent). Ash content, was minimum in Gliricidia LPC (3.60 per cent), the next being *Cassia fistula* which showed ash percentage of 6.48. *Morus alba* and *Sesbania grandiflora* LPCs contained high ash percentages of 15.46 and 9.50, respectively. High ash content (more than 3 per cent) in LPC is undesirable for human consumption[11]. However, Schwarz[12] opined that higher silica content of ash is beneficial. It is thus suggested that LPCs having high ash contents should be analysed for silica to assess their utilisation.

The LPCs of Moringa and Leucaena contain poor protein percentage of 33.69 and 31.50, respectively, but fairly good amount of ether extract and low ash contents (Table 2). It is, therefore, suggested that beside problem of mucilage, the nature of N-free extract of these tree leaves need further study.

TABLE 2. COMPOSITION OF DRY LPC FROM SOME TREES

Plant name	Protein (%)	Ether extract (%)	Ash (%)	Carbohydrate (%) (by diff.)
Cassia fistula	41.13	7.45	6.48	44.94
Sesbania grandiflora	42.88	10.25	9.50	37.37
Gliricidia maculata	43.75	15.07	3.60	37.58
Morus alba	40.25	6.55	15.46	37.74
Moringa olifera	33.69	12.87	3.55	49.89
Leucaena leucocephala	31.50	14.49	3.42	49.59

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Relationship Between Iron Uptake and Aconitase Activity in *Bacillus cereus* T.

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^{59}Fe uptake studies in *Bacillus cereus* T. revealed that iron uptake is very low during the vegetative growth. Once the growth was complete, the iron uptake was rapid. A peak in iron level in the cells coincided with maximum aconitase activity at five hours. Later a decrease in iron level concomitant with a decrease in aconitase activity continued upto 9½ hr, after which a second flux of iron uptake as well as aconitase activity was observed upto 12 hr. Though the aconitase activity showed a fall afterwards, the iron level continued to rise slowly upto 24 hr. The cells grown in alpha picolinic acid (APA) did not show any appreciable amount of iron uptake which showed APA chelation with iron ions in the medium. The cell free extracts of such cultures exhibited no aconitase activity. In the presence of ethyl picolinate (EP), another inhibitor of sporulation, the pattern of iron uptake was similar to that of normal till first 6 hr, but the second influx of iron uptake was not observed. It is important to note that EP inhibits the later aconitase activity. These results suggest that aconitase synthesis and its activity during the different stages of sporulation of *B. cereus T.* may be regulated by the iron levels in the cells. Possible sites of inhibition of iron uptake by *B. cereus* T in presence of APA and EP have been discussed.

Sporulating bacilli when grown in a medium containing glucose, degrade the sugar to mostly pyruvate and acetate by glycolysis during vegetative growth. Oxidative changes associated with the utilization of these acids were observed after the completion of growth. Alpha picolinic acid (APA), a metal chelator, was found to inhibit sporulation with an accumulation of these acids. It was postulated that APA repressed the synthesis of one of the enzymes required for the oxidation of these acids, by way of metal chelation. This effect could be reversed[1] by heavy metal ions like Zn^{++}, Co^{++} and Ni^{++}. Hanson *et al.*[2] showed the enzyme to be aconitase. Later, it was shown that citric acid cycle (CAC) was a must for the expression of sporulating genes[3]. Fortnagel and Freese[4], working with *Bacillus subtilis* reported that APA inhibits sporulation by inhibiting the aconitase activity but not its synthesis. Washing of the APA grown cells with ferrous sulphate solution did increase the aconitase activity. Results from our laboratory showed that addition of ferrous sulphate solution stimulated aconitase activity in the cell free extract of actively gowing *B. cereus* cells[5]. Ethyl picolinate (EP) an ester of APA, was also found to inhibit sporulation during late stage in *B. megaterium* and *B. subtilis* but when added along with zinc, the inhibition could be reversed[6]. Therefore, a correlation,

if any, between iron uptake and aconitase activity at different levels of growth and sporulation of *B.cereus* T. was sought.

Materials and Methods

B. cereus T obtained from USDA, Washington, DC was cultured in pre-sterilized modified 'G' medium, the percentage composition (w/v) of which is as follows: ferrous slphate, 0.00005; copper sulphate, 0.0005; zinc sulphate, 0.0005; manganese sulphate, 0.005; magnesium sulphate, 0.02; ammonium sulphate, 0.2; calcium chloride, 0.008; dipotassium hydrogen phosphate 0.005; glucose, 0.1; yeast extract, 0.2. The pH of the medium was 7.0±0.1.

The active culture technique[7,8] was used in modified 'G' medium at 30±1°C on New Brunswick gyrotary shaker at 160 r.p.m. Routinely three 10 per cent transfers of cells growing in logarithmic phase were made. Time of the last transfer is referred to as 'zero hour' and susequent hours before harvesting were used to define the age of the culture. For determining pH, optical density (OD) and total viable counts (TVC) at different stages of growth of the culture aliquots of the culture were withdrawn at desired levels of growth and tested. OD was determined by Klett Summerson Colorimeter and pH by Toshniwal pH meter. TVC were made by plating 1 ml of appropriately diluted culture in nutrient agar at two dilutions and in duplicate and incubating at 30°C for 24 hr. The plates were counted after this period in Spencer Colony Counter. For iron uptake study in presence of inhibitors, $^{59}Fe^{2+}$ as ferrous sulphate(10μ c/l) and alpha picolinic acid (APA) or ethyl picolinate (EP) were added simultaneously at 'zero hour', the time of final transfer. A control was also run side-by-side. For iron uptake measurement, 5 ml cultures withdrawn at desired intervals were centrifuged at 5000 rpm at 0-4°C in a Sorvall refrigerate dcentriguge. The supernatant was collected. The separated cells were collected and washed thrice with 0.01 N HCl and centrigued each time at 5000 rpm. The washings were separately collected. The washed cells were suspended uniformly in 5 ml water. $^{59}Fe^{2+}$ activity in cell suspensions, supernatants and washings was measured in a well type Scintillation Counting System (ECIL). Aconitase activity was assayed by the method of Racker[9] described for pig heart muscle and adopted by Hanson *et al.*,[2] for bacterial aconitase. Protein was measured according to the method used by Lowry *et al.*,[10].

Results and Discussion

The relationship between total viable cell count and O D. at different intervals in *B. cereus* T is shown in Table 1. The cell count shows that vegetative growth ceased by 3 hr but the O.D. continued to rise slowly even after this period as shown in Fig 1. The slow rise in O.D. is expected since Rao and Verma[11] have shown that polybeta-hydroxybutyrate (PHB) starts accumulating after two and a half hours age of the culture and reaches the peak value at about 6 hr. The deposition of the polymer has been observed by staining. Whereas, the young cells are uniformly stained by crystal violet, the polymer containing cells appear only incompletely stained and exhibit granular cytoplasm. A slow rise in cell count after 3 hr can be attributed to the observation that as the culture ages, the cells in chains tend to separate and at 12 hr almost all cells exist as single cells. At 24 hr there is once again lowering of viable cell number which may be attributed to the death or lysis of the unsporulated cells. This view is strengthened by our observation that at this stage only free refractile spores are visible under the microscope.

TABLE 1. RELATIONSHIP BETWEEN CELL COUNT AND O.D. AT DIFFERENT LEVELS OF GROWTH IN *BACILLUS CEREUS* T.

Time (hr)	O. D.	Cell count
0.0	0.100	—
1.0	0.186	5.4×10^6
1.5	0.256	9.8×10^6
2.0	0.352	3.6×10^7
2.5	0.396	1.1×10^8
3.0	0.470	1.5×10^8
4.0	0.530	1.7×10^8
6.0	0.590	1.8×10^8
12.0	0.750	1.5×10^8
24.0	0.800	1.5×10^8

The radioactive counts, pH and O.D. at different intervals were plotted against the age of the culture as

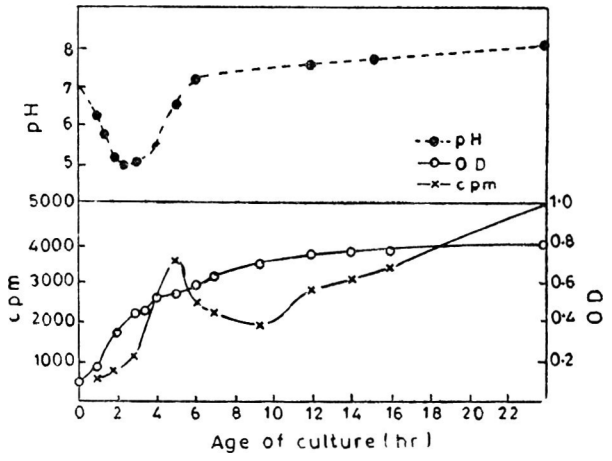

Fig. 1. Relationship between ^{59}Fe uptake, O. D. and pH at different levels of growth in *Bacillus cereus* T.

shown in Fig. 1. O.D. and pH are essentially the same as observed in control culture containing no hot iron as expected. The iron uptake as shown by increased counts per minute by the cells is initially slow showing that the requirement during active growth is small and it acquires peak value at 5 hr when growth has slowed down and polymer, PHB, is accumulating. A perusal of the pH curve indicates that during active growth it continues to fall and recovers as the period of active growth comes to an end. The fall in pH has been shown to be due to accumulation of pyruvate and acetate which are formed from glucose[12]. At this stage Embden Meyerhoof Pathway is operative[13,14].

The rise in pH follows the exhaustion of glucose and induction of citric acid cycle (CAC). This is accompanied by increased oxygen demand after initial fall in it when glucose was exhausted.[8] As already pointed out at this stage PHB also accumulates. The excess energy generated by CAC is at least partially diverted to its formation. This is a useful store-house of energy which is released at the later stages of sporulation in its degradation. An increase in iron uptake parallel to the increase in 0_2 demand indicates that it may be needed for the operation of key enzyme(s). With the assumption that this enzyme may be aconitase, we have studied the activity of aconitase in the cells at different stages of their growth and sporulation. The relationship between iron uptake and acnoitase activity is shown in Fig. 2. At one hour, the aconitase activity could not be detected and at 2 hr, it was still low but from there on an upsurge in the activity could be seen reaching a maximum at 5 hr. The iron uptake followed the same pattern. However, after this a fall could be seen in the aconitase activity as also in the radioactivity upto 9 hr showing a positive correlation between iron uptake and aconitase activity upto this stage. This increase in the aconitase

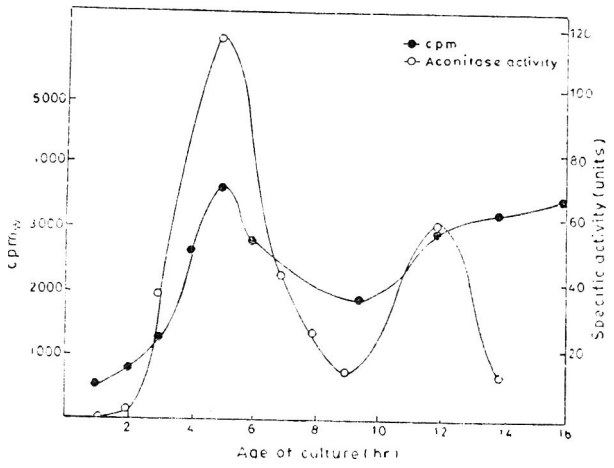

Fig. 2. Aconitase activity and ^{59} Fe uptake at different levels of growth of *Bacillus cereus* T.

TABLE 2. COMPARATIVE STUDY OF ACONOTASE ACTIVITY AND IRON CONTENT IN CONTROL AND INHIBITOR TREATED CULTURES OF *BACILLUS CEREUS* AT 5 AND 12 HOURS

	5 hr		12 hr	
Culture	Fe content (picomole/ml)	Sp. activity of aconitase (units/mg)	Fe content (picomole/ml)	Sp. activity of aconitase (units/ml)
Control	1301.0	120.6	1192.0	60.0
EP treated	1032.4	76.5	539.1	—
APA treated	386.0	—	396.1	—

activity was also related with pH rise demonstrating clearly that operation of CAC, of which aconitase is a key enzyme involves utilization of acidic intermediates as expected (Fig. 1 and 2). With the utilization of the acids, its activity fell and reached a low value.

A second increase in aconitase activity which reached its peak value at 12 hr is also accompanied by increased iron uptake but the fall in aconitase activity after 12 hr was not accompanied by concomitant fall in iron content in the cells. The radioactivity of the cells continued to slightly increase which indicated that iron was being deposited in fore spores. The second rise in the enzyme activity had little effect on the pH as shown by Fig. 1 and 2.

In this case, Acetyl CoA, the substrate for citrate synthatase might be coming from neutral or weakly acidic substrates, such as PHB[15] which were being slowly degraded and the products of their degradation are being simultaneously used up without accumulating acidic intermediates.

To study the relationship between iron uptake and aconitase activity further in *B. cereus* T cells, APA, a known inhibitor of sporulation was added at 0 hr[1]. APA allowed the culture to grow initially (Table 3)

TABLE 3. GROWTH OF *BACILLUS CEREUS* T IN THE NORMAL APA AND EP GROWN CULTURES

Time (hr)	O.D. in normal cultures	O.D. in cultures with APA	O.D. in cultures with EP
0	0.100	0.102	0.102
1	0.186	0.170	0.17
2	0.352	0.360	0.35
3	0.470	0.430	0.40
6	0.590	0.450	0.47
9.5	0.690	0.460	0.58
12	0.750	0.464	0.64
16	0.770	0.464	0.64
24	0.800	0.470	0.64

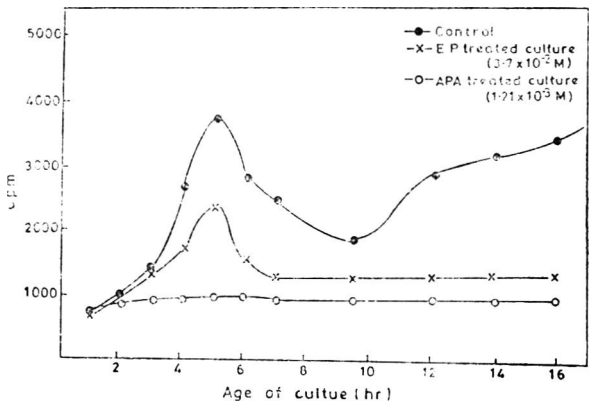

Fig. 3. ^{59}Fe uptake at different times of growth and sporulation in control and inhibitor treated cultures

with concomitant lowering of pH upto 4.9, but as observed by Gollakota and Halvorson[1] it did not allow the utilization of the acidic intermediates indicating that CAC was not operating in its presence. Results given in Table 2 indicate that aconitase activity was absent both at 5 and 12 hr which are the peak hours for the two aconitase activities. At other hours also, this enzymic activity was absent. In cultures grown in presence of APA there was little iron uptake as shown in Fig. 3. The iron content of cells at 5 and 12 hr is also shown in Table 2. The iron content in APA inhibited cells was quite small when compared with that of normal cultures. APA, therefore, did not allow the iron ions to enter the cells by chelating with it outside the cell. This finding is supported by the work of Upreti *et al.*,[6] who showed that Zn^{2+} in low concentration reversed the effect of APA when added at 0 hr along with it. Since Zn^{2+} has high affinity for APA it made iron ions available to the cells. Hanson *et al.*,[2] observed that APA inhibited the synthesis of aconitase in the growing cells. As iron is not allowed to enter the cells by the APA when present in the medium, our results suggest that APA indirectly controls the aconitase synthesis. It is, therefore, concluded that the presence of adequate amounts of Fe^{2+} are responsible for aconitase synthesis.

The change in O.D. of the culture when EP was added at 0 hr is shown in Table 3. EP is another inhibitor of sporulation.[6] A comparison of O.D. under different conditions shows that the trend of increase in O.D. was same as in the case of control but this value in EP was always lower than that in normal culture. In contrast, to this, O.D. of the APA inhibited culture became static after 3 hr of growth. The pattern of iron uptake in EP grown cultures was similar to that of normal upto 7 hr showing a smaller peak at 5 hr. This smaller peak at 5 hr was expected as the growth in EP inhibited cultures was less than that in normal cultures. After 7 hr, the iron level did not increase but remained static at low level in contrast to that in normal culture. The aconitase activity at 5 hr though less than that in normal cultures was significant but at 12 hr no activity was observed. Therefore, EP inhibited the late aconitase activity as also the iron uptake at this stage. Therefore, the mechanism of inhibition of sporulation by APA and EP are different in the sense that EP inhibited only late aconitase activity whereas APA inhibited only early aconitase activity. Although no reports are available it can be logically concluded that since EP also blocks iron uptake at late stages, EP may be blocking the synthesis of late aconitase by checking the entry of iron. APA blocks the iron uptake by the cells by chelating with Fe ions as it is a strong metal ion chelator. However, the mechanism for inhibition of iron uptake by EP does not appear due to metal chelation since APA, its hydrolysis product, when added after 5 hr showed no effect on sporulation whereas EP did inhibit sporulation even when added at this time.[16] Therefore, it may be interfering with iron transport through the membrane.

To understand the mechanism of inhibition of iron uptake by EP an insight into the mechanism of iron transport through the bacterial membrane may be

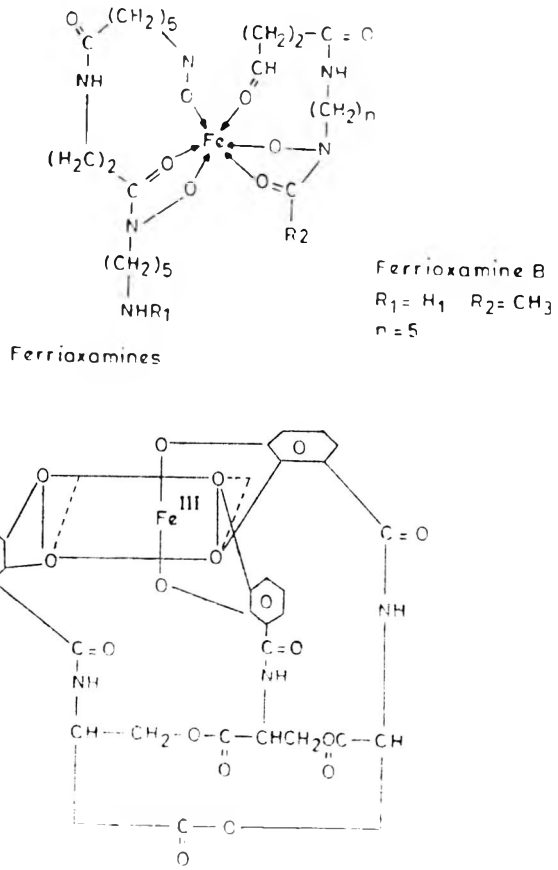

Fig. 4. Structure of Enterobacterin 2, 3-Dihydroxybenzoate Ferrisiderochrome.

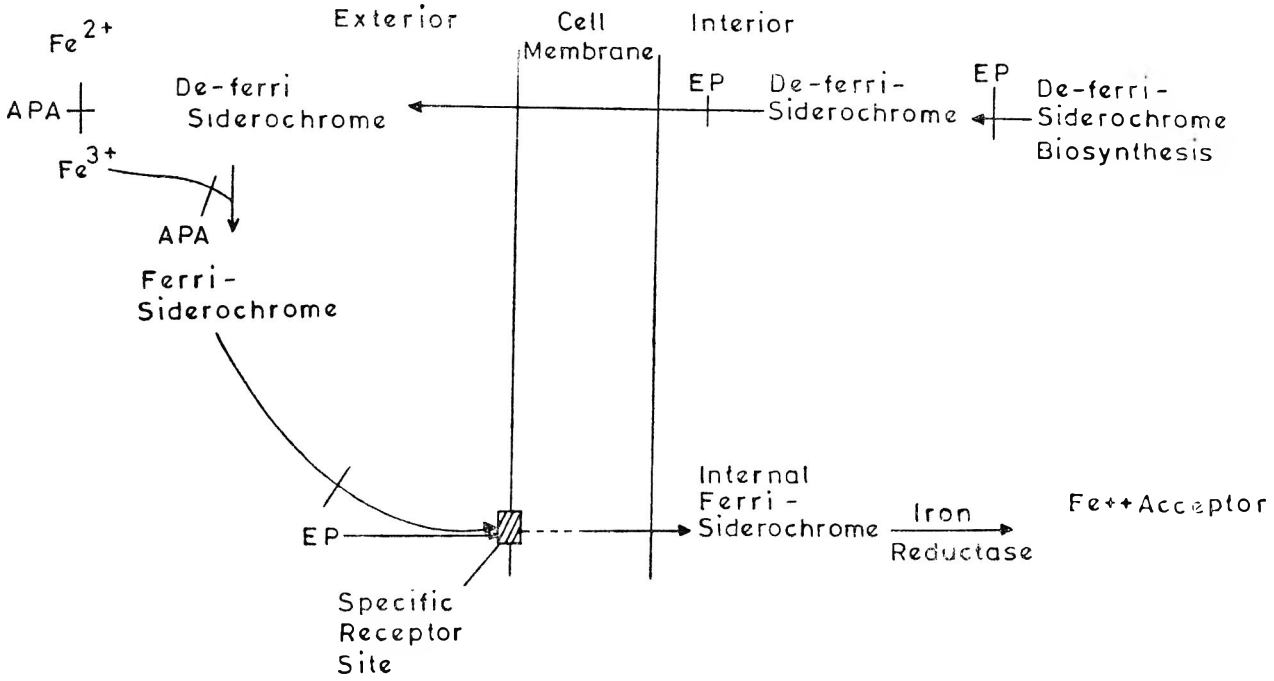

Fig. 5. Postulated mechanism for inhibition of iron transport by APA and EP in Bacillus cereus T.

helpful. There is evidence that secondary hydroxamine acids and the phenolic acids derived from 2,3-dihydroxybenzoic acid participate directly in iron transport in bacilli[17–21]. Neilands[22] proposed the term siderochromes[23]. The structure is given in Fig. 4[23] to represent these iron transport cofactors. Their iron chelates have high stability constants. The initial step in iron transport is chelation of Fe^{3+} by either of the two outside the cell. APA, a chelator itself, may not be permitting this chelation by rendering Fe^{3+} unavailable to these siderochromes. In contrast to this, EP may be attacking the specific receptor site for Fe^{3+} siderochrome on the membrane, thus blocking the iron transport. At 5 hr most of the iron needed to be transported may be present as the chelate of siderochrome which remains unaffected if APA is added at this stage and is transported into the cell. Agrawal *et al.*,[24–26] have collected evidence for existence of 2 isozymic forms of aconitase, the early aconitase having peak activity at 5 hr and late aconitase with peak activity at 12 hr. Similarly, the existence of 2 forms of Fe^{3+} siderochrome receptor, one active at increasing pH and the other at neutral pH can be postulated. The early receptor in that case will be immune to the attack of EP and late receptor sensitive to it. EP may also be interfering with the biosynthesis and release of deferrisiderochrome specifically at late stage. This is possible only when EP enters the cell for which we have no evidence as yet. The proposed model indicating the sites of inhibition of iron uptake are illustrated in Fig. 5.

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Soy-whey Weaning Food. I. Method of Manufacture*

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A low-cost, protein-energy-rich weaning food of acceptable quality was prepared using soybean and cheese whey. The manufacturing method consisted in soaking, blanching and dehulling soybeans, grinding the soy-whey mixture, adding oil and oil-soluble vitamins, homogenizing the mix, spray drying, fortifying with water-soluble vitamins, flavouring, and packaging. The product was free from anti-trypsin activity.

Soybean has been used in various foods to mitigate the shortage of the protein supplies. Its use in weaning food for children can surely be of a great value in tackling the protein—calorie malnourishment prevalent in the developing countries. Although several attempts have been made to combine soybean—a source of protein, (deficient in methionine) with whey—a dairy by-product and source of protein, rich in sulphur-containing amino acids, for the production of milk-like beverages and similar products[1-6], yet no special food for infant and pre-school children has been manufactured. In this paper the development of such a product from whole soybeans and cheese whey is described.

Materials and Methods

Raw materials: Mixed-variety soybean was purchased from J.N.K.V.V., Jabalpur; cheddar cheese whey was obtained from the Experimental Dairy of the Institute. dalda (hydrogenated fat) was purchased from the local market; vitamin premix was purchased from Roche Labs., Bombay and DL-methionine was purchased from Sigma Chemical Co., St. Louis, U.S.A.

Analytical methods: The spray-dried soy-whey weaning food (SWWF) was analysed for protein[7], fat[8], total carbohydrates (by difference), ash and moisture[9], solubility index[8] and free fat[10]. The SWWF was also analysed for soluble nitrogen[11], free fatty acids[12], vitamin A[9], vitamin C[7], methionine[13], anti-trypsin activity[14], colour using Lovibond Tintometer (The Tintometer Ltd., England), wettability[15], dispersibility[16], viscosity (of reconstituted product) using the Hoppler viscometer, and bulk density, average particle density and per cent volume occupied by powder particles[17].

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Manufacturing procedure: Undamaged soybeans were soaked in 5 volumes of water containing 0.5 per cent sodium bicarbonate for about 12 hr at 25±2°C. The soak water was drained and the beans blanched in boiling water for 30 min[18], cooled in tap water and dehulled manually; the cotyledons were separated from plumules by sieving and the hulls were removed by flotation. The soybean cotyledons (containing 44.6 per cent protein, 23.0 per cent fat, 28.3 per cent carbohydrate and 4.1 per cent ash in dry matter) thus obtained were disintegrated with the entire amount of cheese whey in a micropulverizer. The slurry was passed through a muslin cloth, to remove any particles which would clog the homogenizer.

The soy-whey mix was condensed to 35 per cent solids in a vacuum pan. Calculated quantities of fresh hydrogenated oil, fat-soluble vitamins and sodium citrate (0.25 per cent on dry basis) were added to the concentrate (at about 60°C). The blend was homogenized in two stages, first at 3000 psi and second at 500 psi. The blend was fortified with ferrous sulphate (IP) to obtain the desired level of iron before spray drying in an Anhydro (Denmark) pilot-scale unit employing inlet and outlet air temperatures of 190±1°C and 95±1°C respectively.

The powdered product was dry-mixed with water soluble vitamins and DL-methionine using a horizontal mixer. Synthetic Capsoroma flavourings like vanilla, pineapple or strawberry were also added, before packaging.

Sensory evaluation: The SWWF was assessed for its acceptability on the basis of its sensory characteristics by a trained panel using a 9-point hedonic scale. The product was judged as fluid (reconstituted in water to 12 per cent solids and sweetened with sugar at the rate of 7 per cent of the liquid product).

Results and Discussion

Soybean processing: Presoaking, blanching and dehulling resulted in a loss of 24.4 per cent soysolids consisting mainly of soluble carbohydrates, hulls, plumules, chalaja, etc.

Optimization of soy-to-whey-solids ratio: At the soysolids to whey solids ratio of 50:50 and above, the solubility index was too high (more than 20 ml), whereas at lower soysolids level, the index reduced considerably. A ratio of 35:65 gave the minimum solubility index of 11.0 ml. When the ratio was further reduced to 30:70, the product became highly hygroscopic; it had poor free-flowing property, and showed no improvement in solubility index (11.0 ml). Hence the ratio of 35:65 was considered optimum.

Adjustment of the fat level in the soy-whey powder: Since the fat content was only 11.15 per cent, it was raised to 18 per cent or above by including hydrogenated fat (Vanaspathi). Table 1 gives the composition of the soy-whey powder prepared with the addition of vanaspathi. As a result of the addition, the dry mixture showed an increased solubility index of 12.5 ml (from original 11 ml) and increased free-fat content of 11.33 per cent (from 4.67 per cent), with the homogenization pressure of 2000 and 500 psi at the first and second stages, respectively.

TABLE 1. PHYSICO-CHEMICAL PROPERTIES OF SOY-WHEY WEANING FOOD

Characteristics	Mean value
Protein, (g%)	22.9
Fat, (g%)	21.2
Carbohydrate, (g%)	50.0
Ash, (g%)	4.6
Moisture, (g%)	1.3
Soluble nitrogen, (% of total N)	43.3
Free fat, (g%)	4.8
Free fatty acids (g% of total fat)	0.9
Methionine (g/16 g N)	2.8
Anti-trypsin activity	Nil
Colour, (Y)	0.75
Solubility index, (ml)	7.3
Wettability, (sec at 40°C)	76.6
Dispersibility, (%)	68.0
Viscosity (10% TS in water), cp at 30°C	2.029
Bulk density, (g/ml)	0.656
Av. particle density, (g/ml)	1.138
% volume occupied by powder particles	57.3

SWWF (100 g) also contained: 1700, 300 and 1.5 IU of Vitamins A, D & E respectively; 1.0, 0.9, 0.3, 5.3, 42.0 and 4.0 mg each of Vitamins B_1, B_2, B_6, Niacinamide, C and Iron respectively and 0.9 and 95 μ g of B_{12} and Folic acid respectively.

Effect of the homogenization pressure: With the increasing pressure of homogenization (from 2000 to 3000 psi in the first stage, keeping the second stage constant at 500 psi), both the solubility index and free-fat content of the soy-whey powder decreased from 12.5 to 8.0 ml and from 11.3 to 4.3 per cent, respectively. Hence homogenization at 3000 psi in the first stage and 500 psi in the second stage was adopted (Fig. 1).

The average proximate composition as well as other physico-chemical properties of 3 batches are presented in Table 1.

Sensory quality of SWWF: The SWWF without flavouring, and with vanilla, strawberry and pineapple flavours scored 5.8, 6.7, 7.5 and 6.4 respectively. This indicated that while the unflavoured product was fairly acceptable (liked slightly), flavouring significantly improved its palatability (liked moderately to liked very much), with strawberry flavour being the most acceptable one.

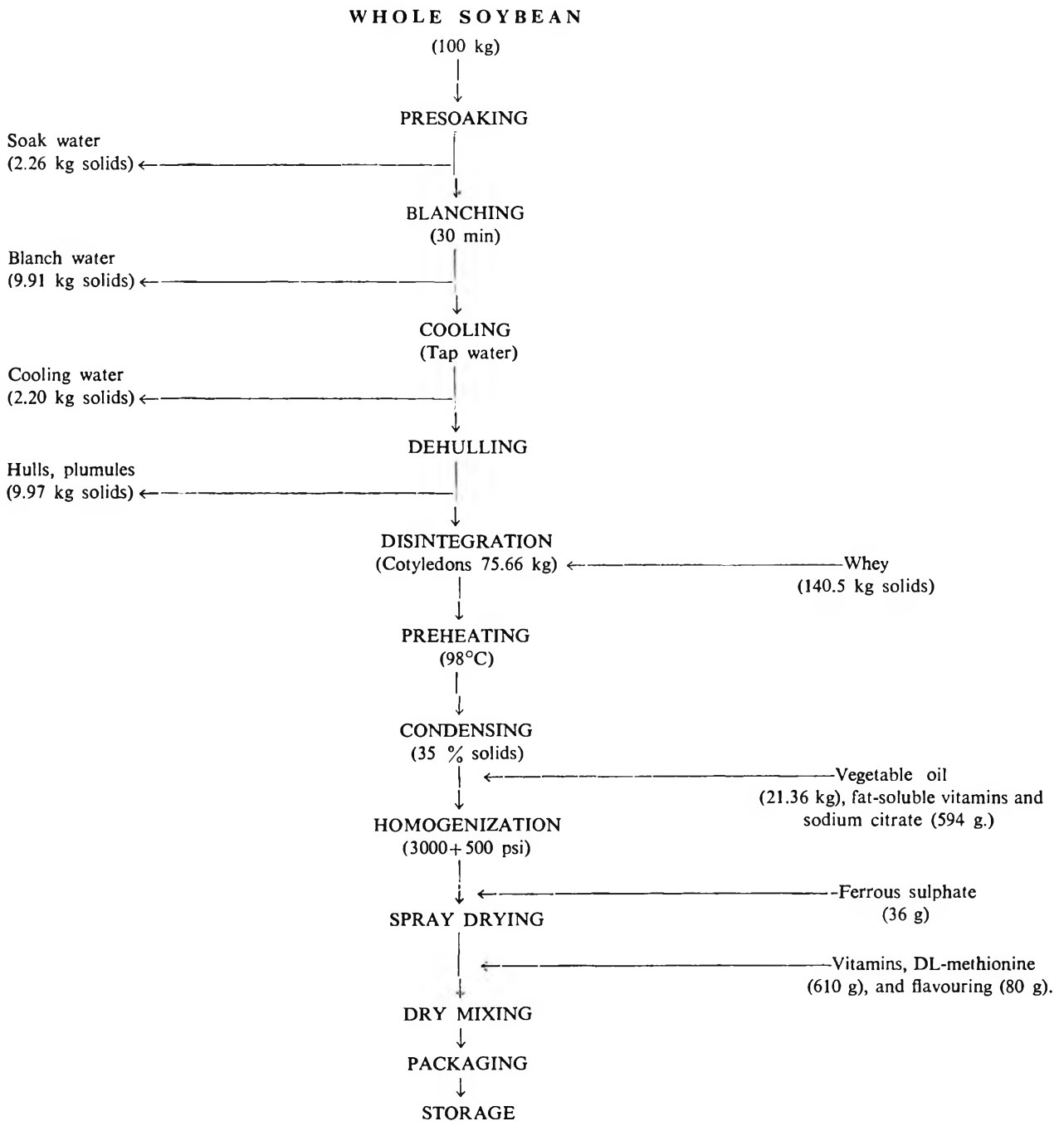

Fig. 1. Flow sheet for the manufacture of soy-whey weaning food.

Cost of SWWF production: The cost of manufacturing the weaning food estimated[19,20] with certain assumptions and modifications, was Rs. 4.86 per kg (excluding the packaging cost), it included Rs. 2.98 for raw materials and Rs. 1.88 for processing. This cost was apparently much lower than that of the commercially available cereal-based and milk-based formula even if 100 per cent more is added towards packaging, profits and marketing.

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Soy-whey weaning Food. II. Storage Studies*

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The soy-whey weaning food (SWWF) developed by the authors was stored for 12 months under different conditions and examined for changes in its physico-chemical and sensory characteristics. The product packed in polyethylene bags showed much faster deterioration than that in tin cans with or without nitrogen at 5±2°C as well as 30±1°C, the higher temperature being more conducive to spoilage. The product could be stored without showing significant deterioration for 7 and 4 months in polyethylene bags and for 10 and 6 months in tin cans at 5±2°C and 30±1°C respectively. The nitrogen packed product kept well all through the storage at both the temperatures.

Soy-whey products in powder form have been found to store well for fairly long time. Guy *et al.*[1,2] observed no oxidized flavour in soy-whey powder after 7 months storage in polyethylene bags at room temperature, although the product was slightly lacking in freshness. Increase in free fatty acids and peroxide value[3,4] and decrease in available lysine content[5-7] during storage have been reported in soy-based powders. In the present study soy-whey weaning food (SWWF) formulated earlier[7] is assessed for storage stability in tin cans and polyethylene bags at 30±1°C or room temperature and at 5±2°C or at refrigeration temperature.

Materials and Methods

Storage conditions: The product manufactured in 3 different lots was packed in 300 gauge polyethylene bags (P), lacquered tin cans with nitrogen (TN) and without nitrogen (T). All the samples were stored at 5±2°C (C) and 30±1°C (R) with approximately 80 per cent relative humidity. Samples were analysed for physico-chemical and sensory properties at monthly intervals.

Analytical methods: The SWWF was analysed for moisture, soluble nitrogen, free fat, free fatty acids, colour, solubility index, wettability, dispersibility, viscosity (after reconstitution), bulk density, average particle density and per cent volume occupied by powder particles as done for the fresh powder[7]. Non-protein nitrogen[8], peroxide value[9] and available lysine were also determined following a combination of methods of Booth[10] and Baliga[11].

Sensory evaluation: The SWWF (in the reconstituted

*Data taken from the thesis by the first author for Ph.D. degree, Punjab University, Chandigarh, 1979.

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form) was assessed for its acceptability on a 9-point hedonic scale. The product was flavoured with strawberry, vanilla and pineapple.

Results and Discussion

Effect on physical properties: Data presented in Table 1 on the changes in the physical properties of the SWWF during storage indicate that the product in PR deteriorated substantially with respect to its colour, solubility index, wettability, dispersibility, viscosity and bulk density unlike the product stored under other conditions. All these characteristics showed statistically significant changes at 6 months. However, the deterioration–colourwise in PC and dispersibility-wise in TR was also appreciable though less than that in PR after 9 and 7 months respectively. No significant change was observed in average particle density or in per cent volume occupied by powder particles of the product during the entire period of storage.

Effect on chemical properties: Spoilage of the SWWF

TABLE 1. PHYSICAL PROPERTIES OF SOY-WHEY WEANING FOOD DURING STORAGE

Characteristics	Packaging and storage condition	Period of storage (months)			
		0	4	8	12
Bulk density, (g/ml)	PC	0.655	0.647	0.648	0.616
	PR	0.655	0.632	0.568	0.505
	TC	0.655	0.655	0.652	0.649
	TR	0.655	0.652	0.652	0.645
Av particle density (g/ml)	PC	1.138	1.134	1.121	1.103
	PR	1.138	1.133	1.092	1.041
	TC	1.138	1.136	1.138	1.140
	TR	1.138	1.136	1.137	1.136
% volume occupied by powder	PC	57.53	57.44	57.07	56.61
	PR	57.53	56.85	49.02	46.63
	TC	57.53	57.49	57.47	57.07
	TR	57.53	57.50	57.25	56.86
Colour, (Y)	PC	0.75	0.75	0.88	1.07
	PR	0.75	0.85	1.20	1.58
	TC	0.75	0.75	0.75	0.75
	TR	0.75	0.75	0.80	0.90
Solubility index, (ml)	PC	7.3	7.3	7.3	7.8
	PR	7.3	7.3	8.8	10.7
	TC	7.3	7.3	7.3	7.7
	TR	7.3	7.3	7.4	7.9
Wettability, (sec)	PC	76.6	77.0	77.9	80.3
	PR	76.6	73.3	44.5	25.8
	TC	76.6	77.0	77.6	78.5
	TR	76.6	77.1	77.9	79.9
Dispersibility, (%)	PC	67.9	67.0	63.7	59.1
	PR	67.9	55.0	38.9	24.9
	TC	67.9	66.8	64.2	61.4
	TR	67.9	65.2	58.9	53.6
Viscosity, (cP)	PC	2.029	2.044	2.057	2.098
	PR	2.029	2.057	2.233	2.397
	TC	2.029	2.043	2.066	2.096
	TR	2.029	2.047	2.065	2.121

PC: Polyethylene bag at 5±2°C; PR: Polyethylene bag at 30±1°C; TC: Tin cans at 5±2°C; TR: Tin cans at 30±1°C.

TABLE 2. CHEMICAL PROPERTIES OF SOY-WHEY WEANING FOOD DURING STORAGE

Characteristics	Packaging and storage condition	Period of storage (months)			
		0	4	8	12
Moisture, (%)	PC	1.36	1.66	2.66	4.78
	PR	1.36	2.94	5.58	8.13
	TC	1.36	1.37	1.42	1.48
	TR	1.36	1.38	1.57	1.68
Non-protein N (mg/100 g)	PC	280.0	280.0	352.0	448.0
	PR	280.0	298.0	476.0	690.0
	TC	230.0	280.0	336.0	439.0
	TR	280.0	280.0	443.0	573.0
Soluble nitrogen, (%)	PC	43.3	43.0	41.5	39.6
	PR	43.3	39.8	26.2	16.9
	TC	43.3	43.1	42.8	41.6
	TR	43.3	42.5	40.3	38.0
Free fat, (%)	PC	4.8	4.9	5.0	5.4
	PR	4.8	5.1	6.2	7.6
	TC	4.8	4.9	5.0	5.2
	TR	4.8	4.9	5.2	5.5
Free fatty acids, (%)	PC	0.88	0.93	1.44	2.36
	PR	0.88	1.94	4.85	8.01
	TC	0.88	0.88	1.02	1.74
	TR	0.88	1.17	1.90	3.15
Available lysine, (g/16 g N)	PC	7.66	7.66	7.29	6.48
	PR	7.66	7.37	5.32	4.49
	TC	7.66	7.66	7.44	6.82
	TR	7.66	7.58	6.83	5.76
	TNC	7.66	7.66	7.66	7.53
	TNR	7.66	7.66	7.47	7.12
Peroxide value, (meq/kg fat)	PC	0.0	2.04	7.28	13.10
	PR	0.0	6.09	19.47	42.17
	TC	0.0	1.17	2.11	7.89
	TR	0.0	2.11	8.33	22.95

TNR: Tin cans under nitrogen at 30±1°C. Other legend as under Table 1.

was fastest in PR with regard to its chemical parameters (Table 2) and the changes were found to be statistically significant at 6 months. While soluble nitrogen, free fat and free fatty acids under the other conditions did not show considerable change during storage, the increase in moisture content was appreciable in PC also. The changes in available lysine and peroxide value was greater in TR and statistically significant as compared to PR or TC after 8 months storage. The non-protein nitrogen content of SWWF packaged in P and T rose considerably at both the temperatures, the rise being faster at 30±1°C than at 5±2°C.

The increase in the moisture content of the SWWF was comparatively less than that observed in corn-soymilk by Bookwalter and co-workers[4]. However, the present findings were similar with regard to free fatty acid[3,4] available lysine[4-6] and peroxide value[5] for soy-based powdered products. As per the PAG guidelines[12] the minimum level required of available lysine in such a food is 5 g per 16 g nitrogen. Accordingly, the SWWF stored well for 8 months in PR and throughout the storage period under all the other conditions, during which the lysine content did not fall below the required level.

Effect of storage on the sensory quality: The overall acceptability score of the unflavoured and flavoured SWWF dropped during storage, the drop being faster at 30±1°C than at 5±1°C. If a score of 6 (like slightly) is taken as the minimum desirable, then the product could be stored well at 30±1°C for 6,5 and 1 months with stawberry, vanilla and pineapple flavours respectively. The samples stored at 5±1°C were acceptable

for longer time i.e. for 12, 10 and 4 months for the above flavourings.

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RESEARCH NOTES

EFFECT OF SORBIC ACID ON THE CHEMICAL AND RHEOLOGICAL CHANGES DURING MIXING AND CHAPATI BAKING

Sorbic acid addition to the dough was found to have no effect on the rate of amylolysis of polysaccharides, changes in protein extractability in water, alcohol (70%) and acetic acid during dough mixing, extensibility, resistance to extension and gelatinization viscosity of wheat flour. Sorbic acid decreases dough development time and mixing tolerance of dough, but does not effect change in sulphydryl groups.

Sorbic acid has been successfully used for the preservation of chapaties[1,2]. In aqueous systems, sorbic acid has been shown to degrade into volatile aldehydes. When used in higher concentrations this may impart off flavour during long storage of chapaties[3]. In isolated systems, sorbic acid has been shown to react with sulphydryl groups of cysteine[4]. Sorbic acid has also been reported to inhibit proteolytic enzymes[5,6]. Hence the effect of sorbic acid on chemical and rheological changes taking place during dough mixing and chapati baking were investigated to understand its role on the organoleptic qualities of chapaties.

'Sharbati' wheat was procured from local market and milled in 2 kg lots. The whole wheat meal was sieved through a 30 mesh sieve to remove bran (6.5 per cent). Dough was prepared in a mechanical mixer with sorbic acid (0.2 per cent on wheat flour basis) suspended in water. Chapaties were prepared according to the procedure described earlier[2]. Farinograms and extensograms were drawn using Brabender farinograph and extensograph respectively according to AACC procedures[7]. Amylograph curves were drawn according to Meredith[8]. Reducing and non-reducing sugars were analysed by AACC method[7]. The changes in proteins were followed by determining their solubilities in water, acetic acid and alcohol (70 per cent). The concentration of proteins were measured by Lowry's method[9]. Sulphydryl groups were determined colorimetrically using Ellman's reagent[10].

Changes in reducing, non-reducing and total sugars during dough mixing are shown in Table 1. It is seen that dough mixing resulted in a considerable increase in the concentration of reducing and total sugars indicating hydrolysis of polysaccharides into smaller fragments by native wheat amylases. Major changes occurred in the first four minutes of mixing. Further mixing and keeping the dough at room temperature for one hour resulted in only slight increase in their concentrations. Incorporation of sorbic acid in the dough (0.2 per cent sorbic acid on *atta* basis) did not influence the changes in sugars either during mixing or during resting period (one hour) and during subsequent chapati baking. Apparently sorbic acid did not significantly affect the rate of hydrolysis of polysaccharides by native wheat amylases and concomitant sugar levels. This is further supported by the fact that addition of sorbic acid was found to have no effect on the gelatinziation behaviour and peak gelatinization viscosity (590 BU) of wheat flour.

Changes in proteins during dough mixing and baking mostly arise from the changes in the degree of association and dissociations between polypeptide chains

TABLE 1. EFFECT OF SORBIC ACID (SA) ON SUGARS AND SOLUBILITY OF PROTEINS DURING DOUGH MIXING AND CHAPATI BAKING

	Reducing sugars (mg maltose/ 10 g flour)		Sugars (mg sucrose/10 g flour) Non-reducing		Total		Protein (%) Water soluble		Alcohol soluble		Acetic acid soluble proteins(%)	
	Control	With SA	Control	With SA	Control	With SA	Control	With SA	Control	With SA	Control	With SA
Wheat flour (*atta*)	55	—	295	—	350	—	2.1	—	6.2	—	5.1	—
Dough (4 min)*	320	321	253	254	573	575	2.2	2.1	6.6	6.6	7.1	7.0
Dough (60 min)**	382	380	215	214	597	594	2.4	2.4	6.7	6.7	7.3	7.3
Chapaties	357	360	206	205	562	565	1.9	2.0	4.1	4.2	4.1	4.1

*Mixing time in farinograph mixer.

**Resting period of dough at room temp after 4 min of dough mixing.

All data are on moisture free basis.

Sorbic acid at 0.2 % level.

TABLE 2. FARINOGRAPHIC CHARACTERISTICS OF DOUGH WITH SORBIC ACID

	Sorbic acid level (%)			
	Control	0.15	0.3	0.48
Peak time (min)	6.5	6.0	6.0	6.0
Stability time (min)	5.5	3.5	2.5	2.0
Tolerance index (BU)	40	120	120	140

TABLE 3. EFFECT OF SORBIC ACID ON THE SULPHYDRYL GROUPS

Treatment	Sulphydryl (μ M/g dry wt.)
Dough + 34 ml water	1.6
Dough + 34 ml of 0.15% sorbic acid	1.6
Dough + 5 ml of 0.0125% cysteine hydrochloride + 29 ml water	2.5
Dough + 5 ml of 0.0125% cysteine hydrochloride + 29 ml of 0.15% sorbic acid	2.5

with concomitant changes in their solubility. During dough mixing extractability of proteins in water did not change to a significant extent (2.1-2.2 per cent) but acetic acid soluble proteins increased considerably (5.1-7.1 per cent) whereas alcohol soluble proteins increased slightly (6.2-6.6 per cent). Keeping dough at room temperature for one hour did not result in any further changes in acetic acid and alcohol soluble proteins. But water soluble proteins increased very slightly. Chapati baking resulted in a decrease in the extractability of proteins in all the three solvents.

Addition of sorbic acid had practically no effect on changes in protein extractability resulting from dough mixing and chapati baking. Tanaka and Bushuk[11] have reported increases in alcohol (70 per cent) and acetic acid (0.1N) soluble proteins as a result of dough mixing from 70 per cent extraction flour; but water and salt soluble proteins did not change significantly.

Effect of sorbic acid on the farinographic characteristics of wheat flour is shown in Table 2. It is seen that significant modifications of dough properties are caused by sorbic acid. Sorbic acid lowered the time to reach maximum consistency and also the consistency of the dough decreased faster than the control dough. But sorbic acid, did not either significantly influence the farinographic water absorption or extensibility and resistance to extension of the dough. Since the changes in farinographic characteristics of flour may be brought about by the reaction of sulphydryl groups with sorbic acid, sulphydryl contents of dough were, therefore determined. Sulphydryl content of dough containing sorbic acid was not significantly different from that of the control dough (Table 3). Apparently effect of sorbic acid on the farinographic characteristics of dough is not due to blocking of sulphydryl groups. Further evidence to this was obtained by incubating solutions of sorbic acid (0.15 per cent w/v) and cysteine hydrochloride (0.125 per cent w/v) at pH 6.5 for one hour at room temperature (28-30°C) and at 37°C. No significant decrease in sulphydryl group was observed in cysteine-sorbic acid reaction mixture compared to control solution containing only cysteine. Also sulphydryl blocking reagents are known to increase the extractability of proteins in dilute acids on mixing[12] but no such increase was observed by the addition of sorbic acid. This further supports that changes in farinographic characteristics are not due to blocking of sulphydryl groups in the dough.

It is evident that incorporation of sorbic acid for preservation of chapaties does not cause significant changes in the biochemical reactions taking place during dough mixing and baking of chapaties. Sorbic acid does not react with sulphydryl groups in dough and its effect on visco-elastic behaviour results from some mechanism other than blocking of sulphydryl groups during dough mixing.

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PRODUCTION OF FUNGAL PROTEIN ON CELLULOSIC SUBSTRATE

Five celluloytic fungi were isolated from soil and decaying plant materials and characterized as *Aspergillus terreus* GN1, *Aspergillus sydowi* GN2, GN3, GN4 and *Fusarium* sp. GN5. These cultures and other cultures collected from different laboratories were screened for protein production on cellulosic substrate. The crude protein content of the biomass produced by these cultures using untreated bagasse and alkali-treated bagasse, as sole carbon source was in the range of 2.41-5.40 and 5.39-19.88 per cent, respectively. Maximum crude protein was present in the biomass produced by *Aspergillus terreus* GN1. After pretreatment of bagasse with 4.0% NaOH and autoclaving for 30 min. there was considerable increase in cellulose utilization and increase in crude protein content.

Attempts have been made to produce microbial protein (single cell protein) (SCP) using various substrates[1,2]. Recently attention has been given to the use of cellulosic wastes for the use of SCP production as they form not only an inexhaustible source of organic carbon but also a major constituent of municipal and agricultural wastes. Of the cellulolytic microorganisms, fungi have been the subject of most intensive study because of their high cellulolytic activity, ease with which they can be cultured and the large amount of biomass produced[3,4]. The present paper describes the isolation and screening of molds for their ability to utilize sugarcane bagasse for the production of SCP.

Fungi were isolated from decaying leaves, wood, straw and soil. These were added to enrichment mineral medium having alkali-treated bagasse as a substrate and incubated for 7 days at 28±1°C. The isolates were maintained on the Czapek's medium containing filter paper as carbon source.

The isolated cultures were identified by their cultural, morphological and biochemical characteristics[5,6].

Celluloytic fungal cultures of *Aspergillus niger*, *Alternaria tenius* and *Myrothecium verrucaria* were obtained from I.A.R.I., New Delhi and *Trichoderma viride* TVL and *T. viride* 6AQM were obtained from N.D.R.I., Karnal. *Aspergillus terreus* 1 and *Fusarium moniliforme* QM 1224 were obtained from Punjab University, Chandigarh. The other five isolates used were characterized as *Aspergillus terreus* GN1, *Aspergillus sydowi* GN2, GN3, GN4 and *Fusarium* sp. GN5.

All the fungal cultures were grown in Czapek's mineral medium with 1.0 per cent bagasse (untreated and treated) as sole source of carbon at a pH of 5.4 and 28±1°C for 7 days on rotary shaker. The cultures were then harvested by filtering the biomass with repeated washing. After drying at 60°C, the crude protein content was determined.[7]

Table 1 presents the biomass yield and crude protein contents of the molds grown on untreated and alkali-treated bagasse substrate. Evidently all the cellulolytic molds produced more crude protein with treated bagasse substrate than the untreated ones. The crude protein content of 5.40 and 19.88 per cent obtained from *Aspergillus terreus* GN1 was maximum with untreated and treated bagasse, respectively. The alkali pretreatment of bagasse considerably increased the fungal growth rate and cell density and also increased the biodegradability of bagasse. The utilization of the substrate was improved due to the fact that there are some easily degradable parts in the cellulose fibre. A highly crystalline and

TABLE 1. PRODUCTION OF SINGLE CELL PROTEIN FROM UNTREATED AND ALKALI-TREATED SUGARCANE BAGASSE

Organism	Untreated bagasse		Alkali-treated bagasse	
	Biomass dry matter* (mg/g)	Crude protein (%)	Biomass dry matter* (mg/g)	Crude protein (%)
Aspergillus terreus 1	757	5.10	538	18.17
A. terreus GN1	750	5.40	555	19.88
A. niger	727	4.18	688	9.54
Trichoderma viride TVL	770	3.84	702	8.75
T. viride 6AQM	699	4.54	725	7.98
Aspergillus sydowi GN2	749	3.70	687	8.75
A. sydowi GN3	930	2.41	786	7.09
A. sydowi GN4	763	3.00	830	5.39
Fusarium sp. GN5	903	3.04	701	7.85
Myrothecium verrucaria	702	4.47	681	10.93
Fusarium moniliforme QM1224	901	2.45	771	6.23
Alternaria tenius	890	2.84	797	7.00

*Fungal mycelium + unutilized bagasse.

lignin containing lignocellulosic waste was more difficult to utlilize and yielded a biomass with a lower crude protein content[8].

The biomass crude protein content ranged from 2.41 to 5.40 and 5.39 to 19.88 per cent as compared to initial crude protein content of 2.01 and 3.04 per cent in untreated and treated bagasse respectively. Crude protein content in the biomass was minimum in *Aspergillus sydowi* GN4 and GN3 with treated and untreated bagasse substrate.

The biomass dry matter yield was less with higher crude protein content of biomass and conversely, with higher dry matter yield, the crude protein content was low. For the production of more crude protein, more energy is required for the breakdown of cellulosic substrate. Hence, efficient cellulose utilizers like *Aspergillus terreus* GN1 produced a biomass yield of 555 mg/g alkali-treated bagasse with a high crude protein content of 19.88 per cent, as compared to the more biomass yield of 830 mg/g alkali-treated bagasse with a lower crude protein content of 5.39 per cent.

The SCP recovered ranged from 13 to 21.26 per cent by using various cultures and substrates[9,10]. When *Chetomium globosum* was grown on bagasse substrate, 12.0 per cent crude protein was obtained which could be increased upto 17.5-23.75 per cent by various treatments[11]. It could be concluded that by growing *Aspergillus terreus* GN1 in alkali-treated bagasse and untreated bagasse the biomass crude protein content was increased from the initial 3.04 to 19.88 per cent and 2.01 to 5.40 per cent respectively.

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STAINING TECHNIQUE FOR THE DIFFERENTIATION OF PAPAYA SEED FROM BLACK PEPPER

Adulteration of black pepper with papaya seeds can easily be detected by treatment with 1% iodine in 1% aqueous potassium iodide solution. While cut pepper gave deep blue colour, cut papaya seeds gave pale red colour.

Black pepper being a costly spice is liable to be adulterated with cheaper plant materials of similar size, shape and colour. Papaya seed being round and black is one of the possible adulterants[1]. Others are *Mirabilis jalapa* L (family Nyctaginaceae) and *Lantana camara* (family Verbenaceae). Microscopic examination is a good method for detection of adulteration[2]. Another useful method of detection of papaya seeds used as an adulterant in black pepper is the flotation technique in alcohol, when pepper seeds are liable to sink[2]. A third method for detection is by chromatographic separation and examination under UV light after spraying with aluminium chloride when papaya shows fluorescing spot of low R_f and pepper greenish yellow to yellow fluorescing spot of high R_f[3]. We have now been able to suggest a simple reliable method based on the colouration with iodine.

It was seen that cut sections of black pepper are stained blue with 0.5 to 2 per cent iodine in acqueous or alcoholic solution of potassium iodide. While the pepper gives dark blue colour, papaya seed sections gave only pale red colour.

Black or white pepper and papaya seeds were cut into half and treated with solutions as given in Table 1. After 5 min, the staining liquid was decanted, cut sections washed and dried by pressing between filter paper.

The bluish colour formed in the case of pepper berries is due to starch, forming starch iodide. It is also known that dextrins of 6 unit chain length give no colouration while those of 8 to 12 units give a pale red colour[4]. The reddish colour obtained in the case of papaya seeds indicates the presence of medium length carbohydrate rather than starch. It may be noted that pepper section on hydrolysis with hydrochloric acid gives pale red colour.

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TABLE 1. STAINING CHARACTERISTICS OF PAPAYA SEEDS AND BLACK PEPPER

Stain	Papaya seed (halves)	Pepper black (halves)	White pepper (halves)
Acetocarmine	Immediate reddish colour	Immediate reddish colour	Immediate reddish colour
Ninhydrin 5% in water	No discolouration	No discolouration	No discolouration
Ninhydrin 5% in butanol	No discolouration	No discolouration	No discolouration
1% solution of iodine in aqueous potassium iodide	Reddish	Bluish	Bluish
1% alcohol solution of iodine	Reddish	Bluish	Bluish
Acetocarmine-iodine mixture 9:1 ratio	Reddish	Bluish	Bluish

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EFFECT OF ATMOSPHERIC HUMIDITY ON DRYING OF PAPADS

Blackgram (*Phaseolus mungo*) papads were dried under (*a*) widely varying atmospheric humidity (38-82 per cent RH at 24-25°C) and also (*b*) controlled conditions (64-66 per cent RH at 31°C) in a humidity cabinet. Drying at 38-43 per cent RH resulted in an over-dried, warped and brittle product in just 4 hr, while drying for 6 hr at higher humidity (78-82 per cent RH) gave an incompletely dried product, susceptible to fungal spoilage. When dried for 6 hr at atmospheric humidity of 66-70 per cent RH at 25°C, the papad was pliable with a desirable moisture content of 14.5 per cent. Drying under controlled humidity (64-66 per cent RH) and temperature (31°C) for about 5 hr also gave a pliable papad of desired moisture content.

Sample papads collected in a survey from different states have shown that papads made in the traditional way have widely varying moisture content (9-17 per cent)[1], which is the main factor in determining their shelf-life. Papad has two levels of critical moisture content[2]: (*i*) at higher than 17.5 per cent, papad is susceptible to the onset of fungal spoilage, and (*ii*) at less than 12 per cent, its pliability is affected leading to warping and breakage.

Traditionally, papad is air-dried under shade. In such drying wide fluctuation in atmospheric humidity and non-uniformity in thickness of papads affect their drying characteristics. The results of a comparative study of drying of papads by the traditional method vis-a-vis under controlled conditions of humidity are presented in this note.

Atmospheric drying: Blackgram (*Phaseolus mungo*) papads prepared according to Shurpalekar *et al.*[3] were air dried on different days with varying atmospheric conditions: (*i*) low humidity (38-43 per cent RH at 24.5°C), (*ii*) optimum humidity comparable with controlled condition (66-70 per cent RH at 25°C), and (*iii*) moderately high humidity (78-82 per cent RH at 24.5°C). During drying, papad samples were drawn every hour and the moisture content was estimated according to AOAC method[4]. In the light of the two critical moisture levels, the optimum level was fixed at about 14.5 per cent, facilitating thereby adequate allowance for the ingress or loss of moisture due to the vagaries of atmospheric conditions during storage. The results of the changes in the moisture contents of the papads during drying under different conditions are presented in Fig. 1.

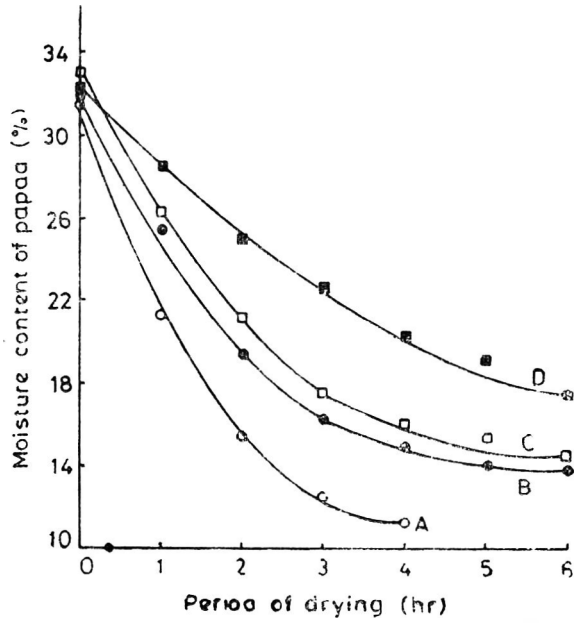

Fig. 1. Effect of relative humidity on drying of papads A, Atmospheric drying at 38-43% RH and 24.5°C; B, Cabinet drying at 64-66% RH and 31.0°C; C, Atmospheric drying at 66-70% RH and 25.0°C; D. Atmospheric drying at 78-82% RH and 24.5°C

On days with low humidity (38-43 per cent RH), papad could be dried from an initial moisture content of 32 to a final moisture content of 14.5 per cent in about 2.5 hr (Fig 1, curve (a), with a relatively high rate of drying in the initial stages. Such fast removal of moisture resulted in an over-dried and warped product. When the drying was continued for 4 hr, the papad became brittle, with a final moisture content of less than 12 per cent.

As seen in Fig. 1 curve C, drying of papads under desirable atmospheric conditions with a RH of 66-70 per cent was somewhat comparable to that in the humidity cabinet. The steady decrease in the rate of drying prevented warping and a good pliable product was obtained at the end of 6 hr.

At moderately high RH (78-82 per cent) of the atmosphere on a somewhat humid day, the papad dried at a comparatively slower rate and even at the end of 6 hr, it had a moisture content of 17.9 per cent (Fig. 1 curve D), which was above the upper critical limit of 17.5 per cent. Such a product is susceptible to fungal attack, more so, when packed in polythene bags, due to build up of high humidity within the package. It may be inferred from the above that the desirable humidity for atmospheric drying is 66-70 per cent RH at 25°C.

Drying under controlled conditions: Papads were also dried under controlled conditions (64-66 per cent RH at 31°C) in a humidity cabinet for comparison. Salt solution of required concentration was used for maintaining the desired RH in the cabinet. With a can rotating at 2800 rpm in the cabinet, the air velocity was maintained at (ft. sec): 40 at the entrance, 16 over the saturated solution, and 2.5 over the cabinet shelves. Papad samples were drawn every hour and the moisture content was estimated according to AOAC method.[4] The results are presented in Fig. 1, curve B.

When the RH in the humidity cabinet was 64-66 per cent, the papad could be dried at 31°C to the desired moisture level of 14.5 per cent in about 5 hr (Fig. 1 curve B). The rate of drying in the first 2 hr was almost the same and subsequent steady removal of moisture at slower rate prevented warping and resulted in a good pliable product at the end of 5 hr.

The duration for lowering the moisture to the desired level was less (about 5 hr) under the controlled conditions than that of atmospheric drying (about 6 hr) because the temperature under controlled conditions was higher (31°C) than that of atmospheric conditions (25°C).

The above findings indicate that drying of papads under atmospheric humidity, which fluctuates widely on different days resulted in an over-dried or an infompletely dried product, leading to either warping and breakage or fungal spoilage respectively. At 25-31°C the range of RH for drying of papads to the desired moisture content of 14.5 per cent in 5-6 hr was found to be 64-70 per cent.

In recent years, papad making has grown into a cottage scale industry. As such, the dependence on atmospheric drying is of great disadvantage, because it restricts the papad making to a particular season in a year. Based on these considerations it was felt desirable to adopt artificial drying of papads under controlled conditions of temperature (25-31°C) and humidity (64-70 per cent RH) which could be worked out using simple technique, such as the use of electric heaters in combination with spraying of the required quantity of water inside the room.

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NUTRITIONAL EVALUATION OF NDRI SOY-WHEY WEANING FOOD

The soy-whey weaning food developed at the National Dairy Research Institute, Karnal, was evaluated for PER at various stages of manufacture as well as after one-year storage. Blanching of soybean, admixing with whey and fortification with methionine appreciably improved the PER. The PER considerably decreased during storage, the decrease being greater at 30°C than at 5°C.

Soybean has been well known for its protein value. Its essential amino acid profile makes it valuable for protein nutrition[1]. Since sulphur amino acids are limiting in soyprotein, its fortification with materials containing these amino acids or with the amino acids as such can enhance its nutritive value[2]. Whey, a cheese industry by-product is a good source of protein rich in sulphur amino acids, and therefore, it can form

a nutritionally superior combination with soybean. Guy *et al.*[3] reported a PER of 2.42 for a whey-soy flour blend as compared to 2.50 for casein. The NDRI soy-whey weaning food (SWWF) developed by Kapoor and Gupta[4] was made from whole soybean cotyledons and Cheddar cheese whey. In this paper, have been presented the changes in its PER at different stages of manufacture and after storage for one year.

Raw soybean, blanched soybean[5], spray-dried blend of blanched soybean and whey (35:65 on dry matter basis) and SWWF (the soy-whey blend fortified with 1.12 g DL-methionine per 16 g nitrogen) were fed to weaning albino rats to find out their PER as per the AOAC method[6]. The SWWF was also examined for its PER after 12 months storage in polyethylene (PE) bags and in tin cans at 5±2°C and 30±1°C.

Blanching of raw soybean resulted in a substantial increase in the PER (from 0.14 to 2.1) as seen from Table 1. Similar improvement in the PER as a result of heat treatment of soybean has been reported.[7-8] Such enhancement of the protein quality has invariably been attributed to the destruction of certain inhibitory substances present in raw soybean. In the present study blanched soybean did not show any trypsin inhibitor activity.[9]

The PER of the soy-whey blend (2.3) was significantly higher than that of soybean alone, apparently because of the supplementation of the sulphur amino acids through the whey protein[10]. The fortification of the soy-whey mixture with methionine further raised the PER to 3.24. This value was very close to that reported by Hansra[11] for such a product. This was interesting in the light of the fact that the soy-whey blend contained as high as 65 per cent whey solids.

It may be noted that when the SWWF was used as a supplementary source of protein at 1 and 2 per cent level in wheat flour, by Hansra[11], the PER increased from 2.09 to 2.16 and 2.46 respectively. The true digestibility of SWWF was reported to be 86.0 per cent as compared to 95.0 per cent for skim milk powder. In the feeding trials on pre-school children from low-income families an increase in anthropometric measurements, blood haemoglobin and improved clinical picture were also observed[11].

Table 1 indicates that the PER of the SWWF considerably decreased after 12-month storage. The decrease was maximum in PE bags at 30°C followed by that in cans at 30°C, and PE bags and tins at 5°C. The decline in the protein quality during storage could be ascribed to certain essential amino acids becoming partially unavailable due to their chemical reaction with other constituents of the product. The available lysine content[12-13] of the SWWF at 5° and 30°C was found to decrease from 7.7 to 6.5 and 4.5 g/16 g nitrogen in PE bags and to 6.8 and 5.8 g/16 g nitrogen in tins, respectively. It may however, be seen that the PER and available lysine content did not drop below 2.1 and 5.0 per cent respectively (the minimum requirements as per PAG[14] guidelines) under all the storage conditions except in PE bags at 30°C. Thus, the weaning food showed good storage stability.

TABLE 1. PER AT DIFFERENT STAGES OF MANUFACTURE OF THE WEANING FOOD BEFORE AND AFTER ONE YEAR STORAGE*

Diet**	PER	PER corrected to 2.5 (casein)
Before Storage		
Casein	3.1	—
Raw soybean	0.2	0.14
Blanched soybean	2.6	2.1
Soy-whey powder	2.9	2.3
Soy-whey powder + methionine (SWWF)	4.1	3.2
After Storage		
Casein	2.6	—
SWWF in PE bags at 5±2°C	2.5	2.4
SWWF in PE bags at 30±1°C	1.9	1.8
SWWF in tins at 5±2°C	2.5	2.4
SWWF in tins at 30±1°C	2.2	2.1

*Average values for 12 male weaning albino rats per group, duration 4 weeks.

**10% level of protein in diet (on moisture free basis).

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REVIEW PAPER

Ghee: A Resume of Recent Researches

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The various aspects covered in the review are: chemical composition of ghee including saponifiable and unsaponifiable constituents; flavour components; physico-chemical properties; thermal oxidation effect on saponifiable and unsaponifiable constituents, physico-chemical constants and carbonyl compounds and flavour; keeping quality of ghee influenced by feeding, season, method of preparation and packaging material, moisture, trace elements, amino acids, phospholipids, synthetic antioxidants, betel and curry leaves; improvements made in analytical/test methods; advances made in ghee making techniques and the future line of work needed.

Introduction

The principles of ghee production entail (*i*) fermentation of milk or cream, (*ii*) collection of milk fat in concentrated form, and (*iii*) heating to remove moisture and to induce interaction of concentrated milk fat with fermented residue of solids-not-fat of serum. The characteristic aroma and taste of ghee depend on the first and the third process[1]. The clarified milk fat obtained is almost anhydrous and is known as ghee in India. Srinivasan and Anantakrishnan[2], and Rangappa and Achaya[3] have dealt exhaustively with earlier work. Ganguli and Jain[4] have reviewed the chemistry, processing and technology of ghee in 1973, and it is mostly the period since then that is covered in this article. The role of phospholipids (PL) on the keeping quality of ghee has also been covered.

Chemical composition

Recent researches on the quantitative aspects of various major and minor constituents of buffalo and cow milk fat and/or ghee are summarised in Table 1.

TABLE 1. THE MAJOR AND MINOR CONSTITUENTS OF BUFFALO AND COW MILK FAT AND/OR GHEE

Constituent	Buffalo	Cow	Reference No.
Saponifiable constituents			
Triglycerides*			
Short chain (%)	45.3	37.6	5,6
Long chain (%)	54.7	62.4	
Trisaturated (%)	40.7	39.0	
High melting (%)	8.7	4.9	
Partial glycerides*			9
Diglycerides (%)	4.5	4.3	
Monoglycerides (%)	0.6	0.7	
Phospholipids (mg %)	42.5	38.0	15
Unsaponifiable constituents			
Total cholesterol (mg %)	275.0	330.0	27
Lanosterol (mg %)	8.27	9.32	30
Lutein (μ g/g)	3.1	4.2	29
Squalene (μ g/g)	62.4	59.2	29
Carotene (μ g/g)	0.0	7.2	52
Vitamin A (μ g/g)	9.5	9.2	52
Vitamin E (μ g/g)	26.4	30.5	52
Ubiquinone (μ g/g)	6.5	5.0	29
Flavour components			
Total carbonyls (μ M/g)	8.64	7.2	33
Volatile carbonyls (μ M/g)	0.26	0.33	34
Head space carbonyls (μ M/g) (Gas-stripped carbonyls)	0.027	0.035	35

*Based on the percentage of total glycerides.

(*a*) *Saponifiable constituents*

The glycerides represent by far the major bulk of milk fat. Buffalo and cow milk fats contain as percentages: short chain triglycerides 43-49 and 36-40, trisaturated glycerides 32-47 and 32-43, and high melting triglycerides 6-12 and 3-7 respectively. The 4:0 and 6:0 acids occurred exclusively in the short chain triglyceride fraction while 16:0 was largely concentrated in the long chain triglycerides. Fatty acids in trisaturated glycerides and whole milk fat were similar, indicating an apparent random distribution of fatty acids in milk fat. The high melting triglyceride fraction of buffalo milk fat contained a higher amount of 18:0 than that of cow milk fat. Significantly higher amounts of short chain and high melting triglycerides in buffalo milk fat were due to higher amounts of short chain and long chain fatty acids respectively[5,6]. Clarified milk fat from mastitic

and late lactation milk contained a lower concentration of triglycerides[7]. Feeding cottonseed or groundnut cake increased the unsaturation of milk fat and decreased the lower chain fatty acids whereas feeding concentrates increased lower chain but decreased unsaturated fatty acids. Thyroprotein supplementation increased the concentration of unsaturated fatty acids[8].

Amount of di-and monoglycerides were 4.5 and 0.6 per cent in buffalo and 4.3 and 0.7 per cent in cow milk fat: in both species these partial glycerides were poorer in 4:0, 6:0, 8:0 and 8:1 acids and richer in 14:0 and 16:0 fatty acids than the respective whole fats. Comparatively, monoglycerides contained much lower quantities of 4:0, 6:0, 18:0 and 18:1 fatty acids but higher quantities of 14:0 and 16:0 fatty acids than diglycerides. By thin layer chromatography (TLC), the diglyceride fraction of milk fat was resolved into A, B and C, fractions, but those of the cow milk fat into only two fractions, A and B. Fraction B constituted 73-74 per cent of the total diglycerides in both the species. Fraction A was rich in long chain fatty acids but contained virtually no 4:0 or 6:0 fatty acids; on the other hand fraction C was very rich in short chain fatty acids. TLC resolution of the diglyceride fraction was not merely on the basis of positional isomers but also on the degree of polarity and molecular weight of the components[9]. Higher amounts of partial glycerides were found in mastitic and late lactation milk fat.[7] Presence of β-ketoglycerides has been demonstrated in buffalo milk fat[10]. Winter ghee contained higher amounts of 12:0, 14:0 and total saturated fatty acids, whereas in summer, higher amounts of 14:1, 18:1 and total unsaturated fatty acids were recorded[11]. High concentrations of polyenoic acids were observed in clarified fats from mastitic and late lactation milk[12].

Method of preparation did not effect the free fatty acid (FFA) composition[13] but their concentration was higher[14] in the ghee prepared from ripened (0.34-0.40 per cent) than from unripened (0.23—0.28 per cent) cream or butter.

The PL content of ghee varied considerably. Buffalo ghee on an average contained slightly higher[15] amounts (42.5 mg/100g) of PL than cow ghee (38.0 mg/100g). Narayanan *et al.*[16] confirmed that the PL content of ghee was dependent on its method of preparation: ghee prepared from butter had more PL than that prepared from cream, and ghee filtered at 110°C contained more PL than when filtered at 60°C. Pruthi *et al.*[17] reported that ghee prepared by direct heating of butter at 120°C contained less PL than the ghee prepared by prestratification and subsequent heating of the fat and serum at 120°C. Thus a higher ghee PL content arises from a higher temperature of clarification and greater removal of moisture. When butter was heated to 120°C for 0, 10, 20, 30, 40, 50, 60 and 90 min, the average PL contents were 10.2, 50.1, 70.5, 104.4, 132.0, 94.2, 84.9 and 71.8 mg/100g of ghee respectively. Decrease in PL on continued heating was attributed to thermal oxidation resulting in dephosphorylation of PL.[18]

Ghee prepared from ripened cream or stored butter contained more PL than when prepared from fresh material. The acidity appeared to facilitate removal of moisture from the ghee residue, forcing more PL into the oil phase[19]. Ghee prepared from fresh cream (cow) at 120°C contained 34.3 mg PL/100g fat whereas the one prepared from the corresponding ripened cream contained 45.3 mg[20]. Winter ghee contained a higher amount of PL (28.94 mg/100 g) than summer ghee (16.91 mg) with monsoon ghee an intermediate level (22.82 mg) showing the highly significant effect of season[21]. Mastitic and colostrum fats also contained higher PL[7].

Pruthi *et al.*[22] reported the presence in ghee of phosphatidyl ethanolamine (PE), phosphatidyl choline (PC) and sphingomyelin (SM) with other minor components identified as phosphatidyl inositol (PI), phosphatidyl serine (PS), lysophosphatidyl ethanolamine (LPE) and lysolecithin (LL). Studies on the composition and distribution[18] revealed that the major PL types present in ghee prepared at 120°C contained as percentages: PC, 27.9; PE, 24.6; and SM, 32.4. The other species present were PI, 4; PS, 3; LPE, 5; and lysophosphatidyl choline, 3. There was decrease in the percentages of PC, PE, PS and PI and an increase in lysophospholipids as the period of heating was increased. The brown colour progressively increased and heating for 40 min and more the ghee became deep dark brown and had a fishy odour. Interaction of amino groups of PE and PS with aldehydes were held responsible.

Fatty acid compositional studies revealed that cephalins exhibited the maximum unsaturation, with oleic acid accounting for almost 50 per cent of the total fatty acids. Stearic was the principal saturated fatty acid followed by palmitic. Lecithins showed lesser unsaturation than cephalins. Cerebrosides and SM had a similar fatty acid composition and a high degree of saturation. A characteristic feature of their fatty acid was the presence of 22:0, 23:0 and 24:0 fatty acids in appreciable amounts. Oleic acid occurred only as a minor component comprising less than 5 per cent of the total fatty acids both in the cerebrosides and SM[23]. The PL content in both cow and buffalo milk, and their fatty acid composition have been found to vary with the stage of lactation[24,25].

(*b*) *Unsaponifiable constituents*

The unsaponifiable matter in buffalo ghee ranged from 391 in autumn to 410 mg/100g in spring (average,

398 mg/100g) and in cow ghee from 428.4 in summer to 465.0 mg/100 g in spring (average, 449.7 mg/100g). Differences were significant between species ($P < 0.01$) and between seasons within each species ($P < 0.05$)[26]. The cholesterol content was lower in buffalo ghee ($P < 0.01$) (275 mg/100g) than in cows' ghee (330 mg) and was significantly influenced by season ($P < 0.01$). Significant differences in total cholesterol content were found in desi ghee (292 mg/100g), direct-cream ghee (306 mg/100g) and butter fat (318 mg/100g) made from cow milk, but not in desi ghee (263 mg/100g) and direct cream ghee (275 mg/100g) made from buffalo milk. Mastitic milk fat contained much more (cow 729 and buffalo 625 mg/100g) cholesterol, and the latter was significantly higher in fore milk than in strippings. Clarification temperature had no effect on cholesterol[27]. Free cholesterol was significantly higher in cow than in buffalo (283 mg/100g against 212 mg/100g) ghee, whereas esterified cholesterol as also observed earlier[28], was higher in buffalo ghee.

Buffalo ghee contained (per g of fat) lutein: 3.1 μg, vitamin A 39.83 I.U., squalene 62.4 μg, and ubiquinone 6.51 μg as against 4.2 μg, 28.3 I.U., 59.2 μg, and 5.03 μg respectively, in cow ghee. The concentrations of vitamin A ($P < 0.05$), squalene and ubiquinone ($P < 0.01$) were significantly higher in buffalo than in cow ghee, whereas the concentration of lutein was significantly ($P < 0.05$) higher in cow's ghee. Season had a significant ($P < 0.01$) effect on lutein but not on vitamin A[29]. Lanosterol content[30] in cow ghee ranged between 5.8 and 13.0 mg/100g and in buffalo ghee between 4.8 and 12.1 mg/100g[30]. Higher concentrations of carotene, vitamins A and E and cholesterol in mastitic and other abnormal milk fats have been reported[31]. Thyroprotein supplementation increased cholesterol content in both species[8]. Cholesterol esters in buffalo ghee contained lower levels of 16:0, 18:0 and 18:1 acids but those in cow ghee contained relatively higher levels of 18:2 and 18:3 acids. The concentration of 18-carbon acids in general and 18:1 in particular were higher in buffalo ghee[32].

(c) Flavour components

A variety of factors and possible components responsible for ghee flavour have been identified. Total carbonyls in fresh desi buffalo ghee were higher (8.64 μM/g fat) than that in cow (7.2 μM/g fat). These carbonyls consisted of alkan-2-ones (about 90 per cent), alkanals (about 6 per cent), alk-2-enals (about 2 per cent and alka-2, 4-dienals (about 2 per cent). Out of 38 monocarbonyls detected, 34 were identified. On storage and change in flavour, the level of total alkan-2-ones declined significantly with concomitant increase in level of other monocarbonyl classes[33].

The volatile carbonyls of fresh desi ghee from buffalo and cow constituted 0.26 and 0.33 μM/g fat respectively. After 200 days storage at 37°C, these increased to 1.41 μM in buffalo and to 1.25 μM in cow ghee. There was a marked increase in alka-2, 4-dienals which could have been the reason for deterioration in ghee flavour[34]. In the gas-stripped material, carbonyls in cow fresh ghee were significantly higher (0.035 μM/g fat) than in buffalo ghee (0.027 μM/g fat) and in each case constituted about one-tenth of the volatile carbonyls. On storage for 100 days at 37°C, off-flavour developed with a significant 4-5 fold rise in gas-stripped carbonyls. After 200 days' storage, all ghee samples developed pronounced off-flavours and levels of such carbonyls increased 9-10 fold[35]. The rate of increase was almost double the rate of increase of total[33] and volatile[34] carbonyls.

Total carbonyl content of fresh cow ghee prepared from ripened butter was significantly ($P < 0.01$) higher (9.66 μM/g fat) than that prepared from fresh butter (7.26 μM). For the buffalo products these were identical (ripened butter 8.62 μM and fresh butter 8.60 μM/g fat). The volatile carbonyls in cow ghee prepared from fresh butter (0.33 μM/g fat) and ripened butter (0.42 μM/g fat) were significantly higher ($P < 0.01$) than for corresponding buffalo products, the difference being significant ($P < 0.01$) for both species and type of butter used for ghee making. On storage the volatile carbonyls showed a 2- and 3- fold rise in cow and buffalo ghee respectively prepared from fresh butter, whereas the rise was about 4- and 6.5-fold in ghee made from the ripened butter of the cow and buffalo respectively. Organoleptic evaluation and chemical tests revealed that the flavour score of fresh ghee prepared from ripened butter was slightly higher than that from fresh butter for both species. On storage, off-flavour in cow and buffalo ghee became perceptible at 100 and 80 days respectively suggesting that cow ghee has a better shelf-life than buffalo ghee[36]. This was consistent with an earlier report[37]. Also, irrespective of species, ghee prepared from ripened butter or cream deteriorates faster than that from unripened butter or cream, probably due to the higher concentration of free fatty acids in ghee prepared from the ripened materials[14]. Ghee prepared from buffalo milk deteriorated faster than that from cow milk due to the higher concentration of poly-unsaturated fatty acids and variations in levels of tocopherol and β-carotene[37].

Singh and Ram[20] reported that ripening did not affect the concentration of carbonyls as observed earlier by Gaba and Jain[36], and concluded that the total carbonyl content of ghee was unrelated either to flavour quality of fresh ghee or deterioration during storage. However, Malhan *et al*[38] also observed that ripening of cream and

increase in temperature of clarification increased significantly the total carbonyls in goat ghee; out of 38 carbonyls, 37 were identified.

Carbonyls isolated at various stages of desi ghee manufacture revealed that total carbonyls (μM/g fat) contents were : raw milk fat 8.04, boiled milk fat 7.87, dahi fat 8.03, butter fat 7.91 and desi ghee 8.00. Total carbonyls of raw milk fat, dahi fat and ghee were significantly higher ($P < 0.01$) than those of boiled milk fat and butter fat. Boiling of milk decreased the carbonyls by about 2 per cent possibly through volatilization of low molecular weight carbonyls, while conversion of boiled milk into dahi increased them by about 2 per cent perhaps as a result of fermentation; churning of dahi into butter caused a decrease in carbonyls possibly through loss in butter milk. Heating of butter to prepare ghee increased the level of carbonyls by about one per cent. Clarification of butter into ghee led to a tremendous increase in the proportion of alkan-2-ones with a concomitant decrease in the proportion of aldehydes. The flavour differed in the fat isolated at different stages involved in ghee making, possibly due to differences in the distribution of carbonyl components. Thus, a definite blend of carbonyls in quantitative and qualitative terms appears to be critical as far as the characteristic ghee flavour is concerned[39]. The lactone profiles varied significantly with the method and temperature of clarification used in preparation of ghee. Quantitative differences in lactone levels, and the ratio of gamma-to delta-lactones, are important in assessing the flavour attributes of ghee prepared by different methods and at different temperatures[40].

In attempting to simulate ghee flavour, it was observed that while the heating of butter oil alone did cause changes in relative proportions of various carbonyls, it failed to induce a ghee-like flavour. However, when molten butter was heated with butter oil serum, butter serum and sediments, a ghee-like flavour was perceptible[41]. A flavour almost like that of desi ghee was induced in butter oil when it was treated with good stirring with skim milk dahi (20 per cent w/w) or spray-dried skim milk dahi (5 per cent w/w) at 120°C for 3 min[42].

Physico-chemical properties

When ghee was stored at room temperature, it crystallized into three distinct fractions or layers namely (*a*) oily, (b) granular semi-solid-sitting, and (*c*) hard flaky portion floating on the top and sticking to the sides of container. The melting point of the solid and liquid fractions differed by 10.2-16.6°C, while the butyrorefractometer index and the iodine, Reichert, Polenske and saponification values were higher in the liquid fraction. Layer formation in ghee could be prevented by storing it at 20°C or below immediately after preparation. Ghee thus solidified could be subsequently stored at higher temperature without formation of layers[43].

Winter ghee showed a higher acidity, melting point and grain size. In summer, the iodine value was higher while in the monsoon season the saponfication value was higher[11]. The liquid portion of ghee varied according to storage temperature. At 24°C, ghee was small-grained but of compact texture while at 34°C it was in completely liquid state. Good texture and granulation were obtained by storing ghee between 28-29°C for 72 hr[44]. Granulation of ghee at 29°C was complete in 3 days after melting at 80°C. The granules of buffalo ghee were irregular clusters whereas those of cow ghee were smaller and made up of fine divergent monocrystals. These variations were attributed to differences in the glycerides and fatty acid composition[45].

The opacity of ghee increased with cotton seed feeding whereas the melting point decreased by 1.5°C for cow and 2.5°C for buffalo ghee. On storage, the least increase in acid value was found in ghee prepared from the milk of animals fed a high level of cotton seed[46]. Hydrolysis of buffalo ghee by pancreatic lipase was significantly lower than that of cow ghee, and was attributed to the presence of higher amounts of long chain saturated fatty acids like 16:0 and 18:0, and high-melting triglycerides[37,45]. Liberation of FFA was faster in clarified fats from colostrum, mastitic and late lactation milk than that from normal milk[47]. This was attributed to the differences in fatty acid composition of these abnormal milk fats[7,12].

Thermal oxidation

In India, 80 per cent of the ghee produced is used for culinary purposes and the remainder for confectionery[48]. Changes during heating at different temperatures are, therefore, of great interest. Studies on these aspects are delineated below.

(*a*) *Effect on saponifiable matter*

Heating of ghee at temperatures ranging from 150 to 225°C caused a gradual decrease in triglycerides and an increase in di-and monoglycerides, perhaps as a result of hydrolysis[49]. In high-acid ghee, the hydrolytic changes in triglycerides were more pronounced than in ghee with a lower acidity[50]. Heating of ghee upto 225°C had no appreciable effect on saturated fatty acids from 4:0 to 12:0 but caused a decrease in 10:1, 14:1, 16:1, 18:1, 18:2 and 18:3. Generally the higher the temperature, greater was the degree of unsaturation in fatty acids, and greater was the effect[51].

(*b*) *Effect on unsaponifiable constituents*

Cholesterol content of buffalo ghee (0.282 per cent)

decreased by about 12, 22 and 27 per cent when heated at 150, 200 and 225°C respectively, for upto 2 hr[52]. The initial levels of cholesterol in cow ghee (0.325 per cent) also showed similar changes. The average percentage losses in carotene in cow ghee at 150°C for heating periods of 15, 30, 45 and 60 min were about 38.5, 64.2, 90.0 and 100 per cent; that of vitamin A were 28.4, 50.3, 75.5 and 100 per cent and that of vitamin E 18.3, 40.3, 64.5 and 100 per cent respectively. At 200°C and 225°C of heating, carotene and vitamin A were destroyed completely within 15 min but the losses in vitamin E were 65.5 and 71.0 per cent respectively. Vitamin E was completely destroyed at the end of a 30-min heating period. Vitamin A and E losses in buffalo ghee were almost similar to those in cow ghee for all heating temperatures.

(*c*) *Effect on physico-chemical Constants*

No appreciable change in Reichert, Polenske and saponification values were observed but there was slight increase in FFA and butyro-refractometer reading and drop in iodine value of ghee when heated at 150, 200 and 225°C upto 2 hr. The higher the temperature and greater the period of heating, the greater was the effect[53]. While studying the effect of FFA on thermal oxidation similar results were reported, except that there was a slight reduction of FFA in high-acid ghee perhaps through distillation of volatiles[49,50].

(*d*) *Effect on carbonyl compounds and flavour*

On heating fresh ghee at 150°C, peroxides and epoxides increased with heating period. Thermally-oxidized ghee contained more peroxides of lower polarity than of higher polarity. There was a great increase in the ratio of monocarbonyls to total carbonyls and the main mono-carbonyls formed as a result of thermal oxidation were saturated aldehydes, unlike in fresh ghee where methyl ketones formed the greatest part[54]. In high-acid ghee, saturated and unsaturated carbonyls increased with increase in temperature, but the ratio of saturated to total carbonyls at 150°C was much less in high acid ghee because of faster development of unsaturated carbonyls. This anomalous behaviour of ghee especially at low temperature may be due to the greater susceptibility to oxidation of FFA compared to glycerides; at high temperatures this difference was not marked possibly because of rapid rates of reaction[50]. Presence of PL in butter fat modified the levels of long-chain carbonyls during oxidation. The concentration of 14-C carbonyl content was observed to be high in PL-free butter fat, whereas 16-C carbonyls were higher in butter fat carrying added PL. Carbonyls of 17-C and 18-C were absent in PL-free butter fat but were present in low levels in butter fat with 0.6 per cent PL[55].

Keeping quality

Several factors have been found to affect the keeping quality of ghee. Recent studies on these aspects will now be summarised.

(*a*) *Effects of feeding, season, method of preparation and packaging material*

Ghee prepared from cotton seed fed animal's fat showed better keeping quality presumably because of the antioxidant properties of gossypol, a phenolic substance occurring in cotton seed[46]. Ghee produced and packed in winter had a longer shelf-life (10-11 months) than that packed in summer (6 months) and the rainy season (3 months)[56]. Ghee prepared by the desi method initially had a slightly better flavour[34] but the curd ripening process involved, enchanced its subsequent storage deterioration[19,20]. Higher temperatures, or longer periods of heating at a particular temperature, have been shown to impart better oxidative stability[57] because of greater liberation of PL from phospholipid-protein complexes[16]. Greater liberation of PL was observed in the ripened cream ghee, but did not lead to an improvement in storage quality[19,20]. With respect to methods of preparation, the order of shelf-life of ghee was: direct cream method > desi method > creamery butter method. The total reducing capacity of the system, which involves in particular, certain sulphur compounds responsible for a cooked flavour, appear to play a dominant role in the flavour and keeping quality of ghee[14]. Flavour-induced butter oil could be stored at room temperature for 70 days as against just 30 days for butter oil[58]. Polyethylene plastic containers were found to be as good as brown glass bottles for ghee storage[20].

(*b*) *Effect of moisture, trace elements and amino acids*

Water was found to act as an antioxidant when present at levels of 2.5-5 per cent. This effect was further enhanced in the presence of phenolic compounds but sodium thioglycolate had a negative effect[59]. Addition of 0.1-1.0 ppm of copper showed considerable adverse effect on the shelf-life of ghee[60]. Migration into cream of added copper and iron was found to depend on the method of preparation; use of the direct cream process resulted in ghee with higher levels of copper and iron, both of which enhanced oxidative rancidity[61]. Glycine and alanine were found to be potent antioxidants. Among aromatic amino acids, tryptophan was most potent and of the sulphur-containing ones, cystine was best. Acidic amino acids were slightly better antioxidants than basic ones. In general the antioxidant properties of amino acids were attributed to their ability to chelate with prooxidative metals, and hence the structure decided antioxidant properties[62].

(*e*) *Effect of phospholipids*

Ghee containing lower levels of PL exhibited faster development of peroxides[57]. During ghee preparation, only small amounts of total PL present in cream or butter were transferred to the fat phase, and the rest remained with the GR[16]. Addition of GR to ghee enhanced shelf-life considerably[63]. The antioxidant effect was found to depend on the temperature and method of clarification of GR; the potency decreased in the sequence: cream butter GR > desi butter GR > cream GR. This was in accordance with their PL content[64], which may be the factor responsible[57]. This was established by addition of isolated PL to ghee[65]. The main fraction of PL which exerted antioxidant property was found to be cephalin[66]. This fraction also showed maximum browning which presumably was correlated with antioxidant properties[67]. It was demonstrated that PL acts synergistically with α-tocopherol and it has also a metal-inactivating action with copper[60]. Among the lipid constituents of GR, PL had the maximum antioxidant property followed by α-tocopherol and vitamin A. Among non-lipid constituents, addition of amino acids, lactose, glucose, galactose and their interaction products to ghee along with protein and PL increased oxidative stability. It was concluded that the antioxidant property of GR was due not only to PL but to other constituents[68].

Instant mixing of ghee with an equal amount of GR at 120°C yielded free fat carrying about 2 per cent PL. This fat, when added to ghee at a 5 per cent level brought the PL concentration to about 0.1 per cent.[69] This concentration of PL in ghee more than doubled the induction period[65], and was suggested as a commercial method of increasing the shelf-life of ghee.

Addition of PL from other sources has also been tried. Gram seed lecithin was found to be less effective than cephalin[70]. The antioxidative effectiveness of whole PL from seeds was in the order: sunflower > groundnut > soyabean > cottonseed. This was also the order of decreasing PE content, and of various PL classes, studied, PE had the best effect on oxidative rancidity in ghee[71].

(*d*) *Effect of synthetic antioxidants*

Addition of synthetic antioxidants individually (0.005-0.02 per cent) and also in combinations of two (mixture not exceeding 0.02 per cent) with or without PL to ghee revealed that the efficiency decreased in the sequence: propyl gallate (PG) > cetyl gallate (CG) > dodecyl gallate (DOG) > butylated hydroxy toluene (BHT) > butylated hydroxy anisole (BHA). On addition of mixtures of two (0.01 per cent each) the protection factor decreased in the sequence: BHA+PG > BHT+PG > BHA+GHT > BHT+OG > BHA+OG > BHT+DOG > BHA+DOG. Among the mixtures, only, BHA+PG and BHA+BHT gave a synergistic effect. Addition of PL either to an individual or mixed antioxidant increased the protection[72]. In an another study also BHT was found to be more effective than BHA and the two showed a synergistic effect[73]. Under commercial conditions BHA could improve the aroma, flavour and shelf-life of ghee[56].

The antioxidant potentialities of certain other compounds tested were in the order: hydroquinone > catechol > resorcinol, and again: palmitoyl ascorbate > PG > OG > BHA. A phenolic group was found necessary; when a second group was present the compound proved a better antioxidant, the order being para > ortho > meta. The strong effect of hydroquinone could be due to the formation of a stable quinone which can terminate the chain reaction[70].

(*e*) *Effect of betel and curry leaves*

Betel or curry leaves at one per cent concentration may be used instead of BHA and BHT for extending the shelf-life of ghee, since their addition during clarification yielded products with a higher resistance of oxidation and higher sensory score than those carrying synthetic antioxidants[74]. In betel leaves the active principle has been found to be hydroxy chavicol[75].

(*f*) *Effect of abnormal milk fat*

Clarified butter fat from mastitic and other abnormal milks was reported to be more prone to oxidative spoilage than normal milk fat because of a high concentration of PUFA and FFA[47].

Analytical/Test methods

Methods for estimation of lanosterol[30], iodine value through refractometry[76,77] carbonyl content[78] and thiobarbituric acid value[79] have been reported, as have procedures to determine moisture instantly[80], distinguish cotton tract ghee through methylene blue reduction[81] and detect vegetable oils and other fats[82].

Advances in ghee making techniques

A positive correlation (0.64) between solids-not-fat of cream and fat losses was established and hence use of fat-rich cream in manufacture of ghee was recommended[83]. After large-sacle trials, De *et al.*[84] also recommended that either butter or cream of the highest fat content should be used as raw material to obtain high fat recovery in ghee. Significant increases in yield and decreases in fat-losses during ghee making have been observed when cream was washed with 0.05 per cent lactic acid water or ripened to 0.15 per cent acidity before use[85].

Singh[86] developed a continuous ghee-making plant which was found suitable for manufacturing ghee both

from butter and cream. The quality could be controlled according to Agmark or any other specific standard. Abichandani *et al*[87]. designed a plant on the falling film principle, whose operation, performance and advantages have been described. A ghee-making machine that employs a direct-contact heat exchanger was devised which could be used in both batch and continuous operations[88]. A semi-automatic machine to fill ghee in 1 to 4 kg tins was designed; its capacity of about 100 kg/hr could be increased by changing the pump[89]. Methods using a centrifuge, hand screw-press-and hydraulic press for recovery of ghee trapped in ghee residues were described[90].

Conclusions and future research

Work done during the last seven years on ghee provides a wealth of both basic and applied knowledge. Many problems like simulation of desi ghee flavour in dairy made ghee; simple, quick and efficient methods for the detection of adulteration; acceptable methods for preservation, crystallization and granulation; refining spoiled ghee; packaging materials; industrially feasible continuous methods for ghee manufacture; and preparation of low cholesterol high PUFA ghee need further investigation.

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BOOK REVIEWS

Advances in Biochemical Engineering Vol. 14: Edited by A. Fiechter, Springer-Verlag, Berlin, Heidelberg, New York, Vol. 14; 1980; Pp. 162.

This volume on microbial metabolism contains five articles, four of them concerned with the microbial metabolism while the fifth one is on the cultivation of animal cells in monolayers.

The first article gives a lucid account on the subject of microbial conversion of nitriles to cyanhydrins, amides, acids, etc. with well documented literature coverage upto 1979. Besides the authors briefly summarise their own work on nitrilase and amidase activity in various strains isolated by them from soil and discuss their results with respect to technological applications for the production of economically important componds. The bridge between the microbial metabolism of nitriles and its possible industrial applications is well built and highlights the favourable points for the bioconversion over the chemical processes. The article should prove useful to those who wish to know more about nitrile metabolism and to apply such knowledge for commercial exploitation.

In the second article on ergot alkaloids the author discusses with admirable clarity the chemical, physiological, biochemical and genetic aspects of these alkaloids. The author has done a commendable job in assembling a large number of references on recent advances in the biosynthesis of ergoline ring system and peptide moiety of ergot alkaloids. The role of tryptophan in the biosynthesis and the regulations of these alkaloids have been discussed both at the enzymatic and genetic level by providing adequate references.

The location, components, characteristics and induction of cytochrome P 450 dependent monooxygenase of mammalian and microbial systems have been briefly reviewed in the third article. Since this is a rapidly growing subject, the authors seem to have got confronted in deciding to include from a large amount of latest references. It is surprising that the role of cytochrome b_2 and its reductase in the cytochrome P 450 mediated monooxygenase of mammalian system has not been mentioned. Among the extrahepatic tissues that contain P 450 monooxygenases, placenta, brain and skin have not been included in the list provided. The latter part of the article provides significant information on the nature and induction cytochrome P450 monooxygenase in fungi, yeasts and bacteria. The mammalian and microbial hydroxylations of various compounds have been compared. The minor criticisms raised cannot detract from the considerable merit of this article.

The fourth article is brief but contains significant amount of valuable information with up-to-date references on the chemical nature and spatial representation of cellulose and its physical as well as mechanical properties. The various structural features of this insoluble and complex material has been discussed in the light of its susceptibility to enzymatic degradation. The article provides a great deal of interesting material in a lucid fashion.

The last article is devoted to the recent developments in the large scale cultivation of animal cells in monolayers. A comprehensive source of cell culture literature can be found in this article. The usefulness of animal cells in the production of great variety of products such as hormones, enzymes, vaccines etc. have been briefly discussed. The influence of medium composition, cell quality, and the interaction between cell and its supporting substratum in the propagation of animal cells and the different approaches currently used for the large scale cultivation of animal cells have been critically evaluated.

One of the salient features of the book is that each article finishes with a set of "conclusions" and points out areas where further work needs to be done. The text is liberally supplied with illustrations, line diagrams and tables which provide useful summaries of important information. The book achieves its objective very well and all the articles are valuable additions to the literature. The book will be definitely useful to research workers in microbiology, biochemistry and medicine.

K. M. MADYASTHA
INDIAN INSTITUTE OF SCIENCE, BANGALORE

Consumer Behaviour—Theory and Applications: by Barbara J. Redman, Avi Publishing Company, Inc. Westport, Connecticut; 1979; pp. 225.

The book is an outcome of several years of teaching experience of the author in the field. It is primarily developed for undergraduate course in consumer behaviour which has prerequisite in introductory social science courses. It is also intended for professional economists, with little knowledge of other social sciences, as an introduction to behavioural science.

The book argues for the unity of various social science disciplines. It focusses on consumer behaviour

as an aspect of human behaviour. It envisages that general theoretical pattern of all social sciences dealing with areas of behaviour are the same. Also, it considers how the most general and formal theories developed by economics could be simplified further by contributions from other disciplines. Next, the two major factors influencing the attitudes, viz. the individuals' personality and the social influence on him, are considered. Several views on personality and the major theories and evidence to link them to consumption behaviour are presented. Finally, exchange theory is described as an example of economic and social psycological theory. A few major ideas are enunciated in all the theories considered, viz. persons' need for organisation and consistency as stressed by perception, attitude and personality theories. Secondly, a principle of diminishing return wellknown in the field of economic production appears to be operative psychologically as well. A third, frequently occurring subject on these theories, is based on the structure concerning desires versus restraints on the same. The final universal theme concerns with persons' motivation—all these themes could form major general laws of human behaviour. The book integrates various theories without resorting to systems approach.

The theoretical approaches have been made by the author and the practical applications have been contributed by the former students of the author.

The contents are covered in nine chapters and selected references are given at the end of each chapter to stimulate further reading.

The book is a paper back edition. The get up is good and it contains a large number of illustrative diagrams and an index.

S. RANGANNA
C.F.T.R.I., MYSORE

Recent Developments and Trends in Refrigerated Transport: International Institute of Refrigeration 177, Boulevard, Malesherbes-750 17, Paris, Franco 1978; Pp. 199.

This book is the proceedings of a conference held jointly by the Commission D_2 of the IIR and the office for Refrigeration and Experiments of the International Rail Union (UIC) at Vienna, during September 18-21, 1978. It contains 16 reports presented by experts from countries—8 European countries, South Africa and United States. The papers are both in English (10 papers) and French (6 papers). Each paper carries an abstract in the other language and the text of the paper is followed by a summary and conclusion together with a discussion on the paper by the experts.

For the benefit of those engaged in research work in this area, the english titles of the papers presented under each section are:

Section one deals with the present situation and future of refrigerated transport. It contains 3 papers—(*a*) The self-propelled refrigerator car powered by LNG or liquid hydrogen (in English) by R. L. Whitelaw of USA., (*b*) Maintaining quality of perishable produce in coldchains by proper time—temperature conditions (in English) by H.F.Th. Meffert of Netherlands and (*c*) Experience gained during checking of special transport vehicles according to ATP regulation (in French) by J. Kriha of Austria.

Section two is on developments in the testing of refrigerated vehicles. It contains 4 papers—(*a*) The influence of heat flow bridges and thickness of walls on overall co-efficient of heat transfer values determined during various test conditions for controlled temperature. Transport vehicles (in French) by E. Biogt *et al*, France, (*b*) Rapid methods for overall heat transfer coefficient for controlled temperature transport vehicles, (in French) by Bigot and Bennahamias, France, (*c*) The testing of individual refrigerating units at the government test station at Antony (in French) by Zimmermann, France and (*d*) Simplified on site testing (quick-check) of refrigerated containers (in English) by Baum Gartner, Austria.

Section three on Test equipment, new rail wagons, container inspection covers 5 papers—(*a*) Testing chambers for simulation of environmental conditions (in English) by H. Plapper, Fed. Rep. of Germany, (*b*) New test car of the German Federal Railways (DB) (in French) by J. Kausckke, FDR, (*c*) Refrigerated railwagons of German Federal Railways (DB) (in French) by H. Munske of FDR, (*d*) Developments in refrigerated rail transport in South Africa. 1963-1978 (in English) by E. C. Mardon of South Africa and (*e*) Container Inspections, (in English) by J. Kompe of FDR).

The fourth section on transport experiments and simulations contains 4 papers—(*a*) Interactions between the refrigeration system, air distribution and perishable foodstuffs in refrigerated rail-borne transport (in English), by S. Metz of GDR), (*b*) Refrigerated transport: distribution of temperature in the load (in English) by U. Amadio of Italy, (*c*) Comparative tests to evaluate the influence of the direction of airflow inside refrigerated vehicles: tests on a 40' containers (in English) by G. H. Van Nieuwenhuizen, Netherlands and J. Minder (Switzerland) and (*d*) Experiments with simulated loads transported in refrigerated vehicles (in English) by J. Potynski of Poland.

Each research paper is well written giving ample details with tables and figures by the research scientist

directly involved in the programme of work, followed by a useful discussion by the experts assembled.

The information furnished in this book will be very useful for any research scientist or technologist directly involved in the design and fabrication of refrigerated transport vehicles for perishable produce.

P. NARASIMHAM
C.F.T.R.I., MYSORE

Food Systems: by R. S. Forrest, W. Edwardson, S. Vogel and G. Yaciuk, International Development Research Centre, Ottawa, Canada, 1979, pp. 72.

This elegant booklet is a compendium of post production research programmes supported by the AFNS Division of IDRC. It provides a brief account of on-farm technology followed by post-harvest operations like drying, storage, primary processing (examples given are grain milling, icing of fish on page 10), transportation, marketing and utilisation.

A total of 50 projects covering the post-harvest technology of cereals, millets, legumes, fish, onion and tubers has been listed with brief information on finance, name of scientist, objectives, background and progress. This information should prove useful to research management as well as those engaged in laboratory and field level work.

J. V. SHANKAR
C.F.T.R.I., MYSORE

Chemical Carcinogenesis: Vol. 36, Number 1, January 1980: British Medical Bulletin, Published by the Medical Department, The British Council, 65, Davies Street, London W1Y 2AA.

This number devoted to Chemical Carcinogenesis gives an indication of the major contributions of British laboratories in this area of research during the past fifteen years. The fifteen papers covering a wide range of topics could be considered as dealing with: (1) Metabolic activation of carcinogens and carcinogenesis, (2) Interaction between carcinogens and macromolecules, (3) Short-term tests for carcinogenicity and (4) Epidemiology of chemical carcinogenesis.

Papers under the first category bring out the fact that despite the great variety of chemical structures of carcinogens, there are many common features in the ultimate reactive species and the cellular receptor is probably the same in most instances. The evidence for a 'promotion stage' in the induction of bladder tumor along with available information that asbestos and alcohol act as promoters in human cancers support the theory that in general, tumorigenisis is a multistage phenomenon in man as well as in experimental animals. An important approach to control bladder cancer would be in exploring the possibilities of delaying or reversing 'Promotion' rather than attempting prevention of 'Initiation'...

The second category of papers show that the DNA of target organs is specifically modified by chemical carcinogens and miscoding rather than deletions of genetic material is responsible for tumorigenesis. These promutagenic lesions are less easily removed from target DNA than cytotoxic lesions. Two intracellular proteins: ligandin and fatty acid binding protein have been found to enhance the transport of lipophilic carcinogens in the cell and increase the efficiency of their metabolites.

To the third group belong papers on bacterial mutation, carcinogenic synergism and antigenic changes in chemical carcinogenesis. The usefulness of short-term tests now available and the need for developing similar tests for promotion and inhibition of DNA repair as also the possibility of immuno-diagnosis and therapy are discussed.

The last paper has dealt with innovations in the epidemiological evaluation of carcinogenesis by chemicals.

This publication serves as a valuable reference to the current status of research on carcinogenesis.

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

TRIVANDRUM CHAPTER

Seminar on the prevention of Food Adulteration

A seminar on *Prevention of Food Adulteration* jointly sponsored by the Trivandrum Chapter, Regional Research Laboratory, Trivandrum, and Corporation of Trivandrum was held on 20th September 1980 at Corporation Buildings, Trivandrum. The seminar was inaugurated by Sri E. Chandrasekharan Nair, Hon'ble Minister for Food and Civil Supplies, Kerala, and presided over by Sri M. P. Padmanabhan, Mayor of Trivandrum Corporation. Dr. P. K. Rohatgi, Director, RRL, and Dr. C. P. Madhavankutty, Head, Department of Preventive Medicine, Medical College, Trivandrum, addressed the gathering on the occasion. Sri H. Sreemula Nathan, President, AFST Trivandrum Chapter, welcomed the delegates. Dr. C. Balagopal, Vice-President of the AFST Trivandrum Chapter proposed a vote of thanks.

About 90 delegates attended the technical session of the seminar. The technical session was chaired by Sri P. Janardhana Iyer, Retired Chief Govt. Analyst. Following recommendations were made in the Seminar.

(1) The enforcement of PFA Act and Rules shall be tightened.

(2) The Act and Rules should be suitably amended wherever necessary to ensure the easy enforcement of food laws.

(3) The existing food standards shall be examined to see whether they are realistic and necessary corrections made wherever necessary.

(4) All packaged foods shall be tested and certified at the manufacturer's level to ensure that they conform to the standards claimed on the labels.

(5) Proper labelling should be done to give correct description of the contents.

(6) More articles of commonly used foods like rice, oils, etc. and food products be brought compulsorily under Ag-mark and ISI certification schemes.

(7) Simple tests for detecting food adulteration should be evolved and popularised by providing necessary kits.

(8) Sanitation and quality of foods in hotels should be improved.

(9) All manufacturers, retailers, hoteliers and vendors of food should be properly educated about the prevalance of health hazards of food adulteration so that they could take positive steps to prevent food adulteration.

(10) Voluntary organisations should be encouraged to involve in a big way in public distribution of food articles to help distribution of genuine foods.

(11) Proper modifications should be made in the syllabus for various training programmes in the field of education.

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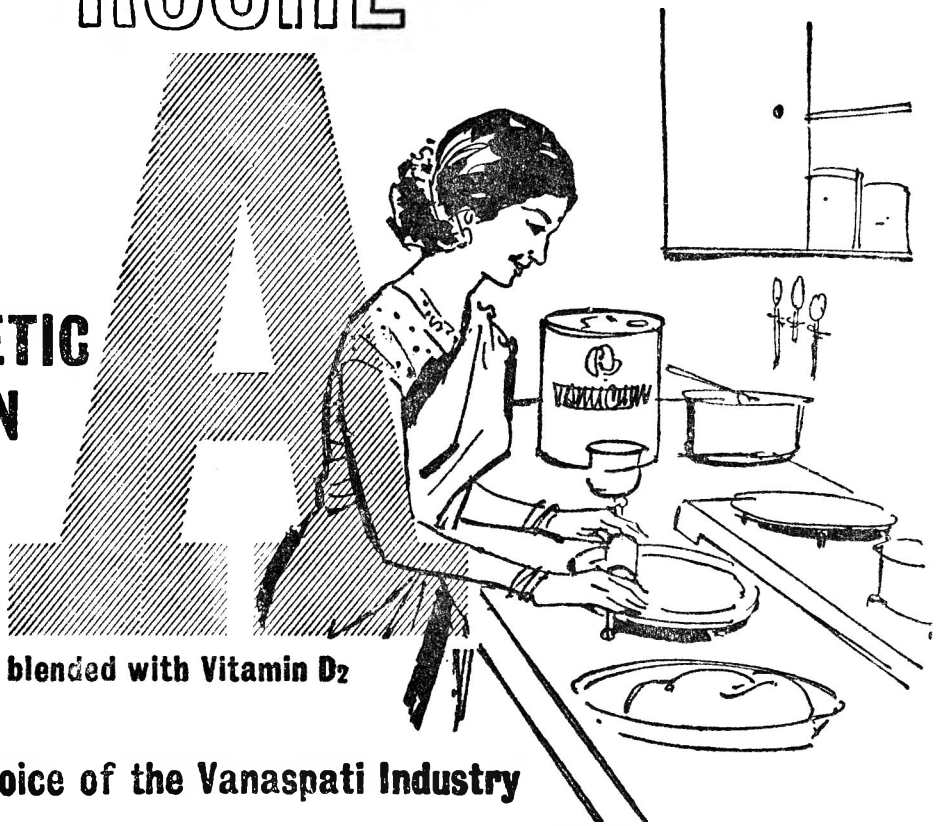

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2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.

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.

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   (*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.

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

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