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ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS

(INDIA)

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Affiltiaed to the Institute of Food Technologists, USA

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2. To provide a forum for the exchange, discussion and dissemination of current developments in the field of Food Science and Technology.
3. To promote the profession of Food Science and Technology.

The ultimate object is to serve humanity through better food.

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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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EDITORIAL

At the beginning of this year, I briefly mentioned about the progress made by the Journal during the 24 years of its service. Now, at the end of the SILVER JUBILEE YEAR, it would be appropriate to present some data regarding the journal (Figure overleaf). without further elaborating on this point, I wish to record that annually about 200 research papers/notes are received for consideration and about 100 get accepted; the journal size is 300-350 pages which includes Book Reviews also. Out of the 100 odd papers which are accepted for publication, 65-70 are communicated from institutions other than CFTRI and about 10 are from other countries.

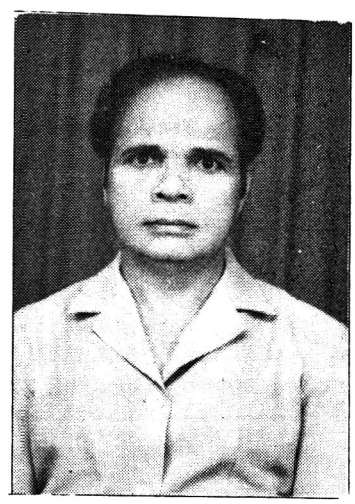
Since the beginning of this year, the quality of printing has been improved with the introduction of computerised phototypesetting and offset printing. Efforts have also been made to cut down the time lag between receipt of a manuscript and its publication by establishing as prompt a refereeing-revision-editorial improvement cycle as possible. At present, this time lag is about 12-15 months.

As my editorship will be ending with this *number*, I would once again like to thank everyone who has contributed to the publication of the *Journal*. I would also like to wish the Incoming Editor, Dr. J.R. Rangaswamy, the best of luck in running the journal.

N. CHANDRASEKHARA

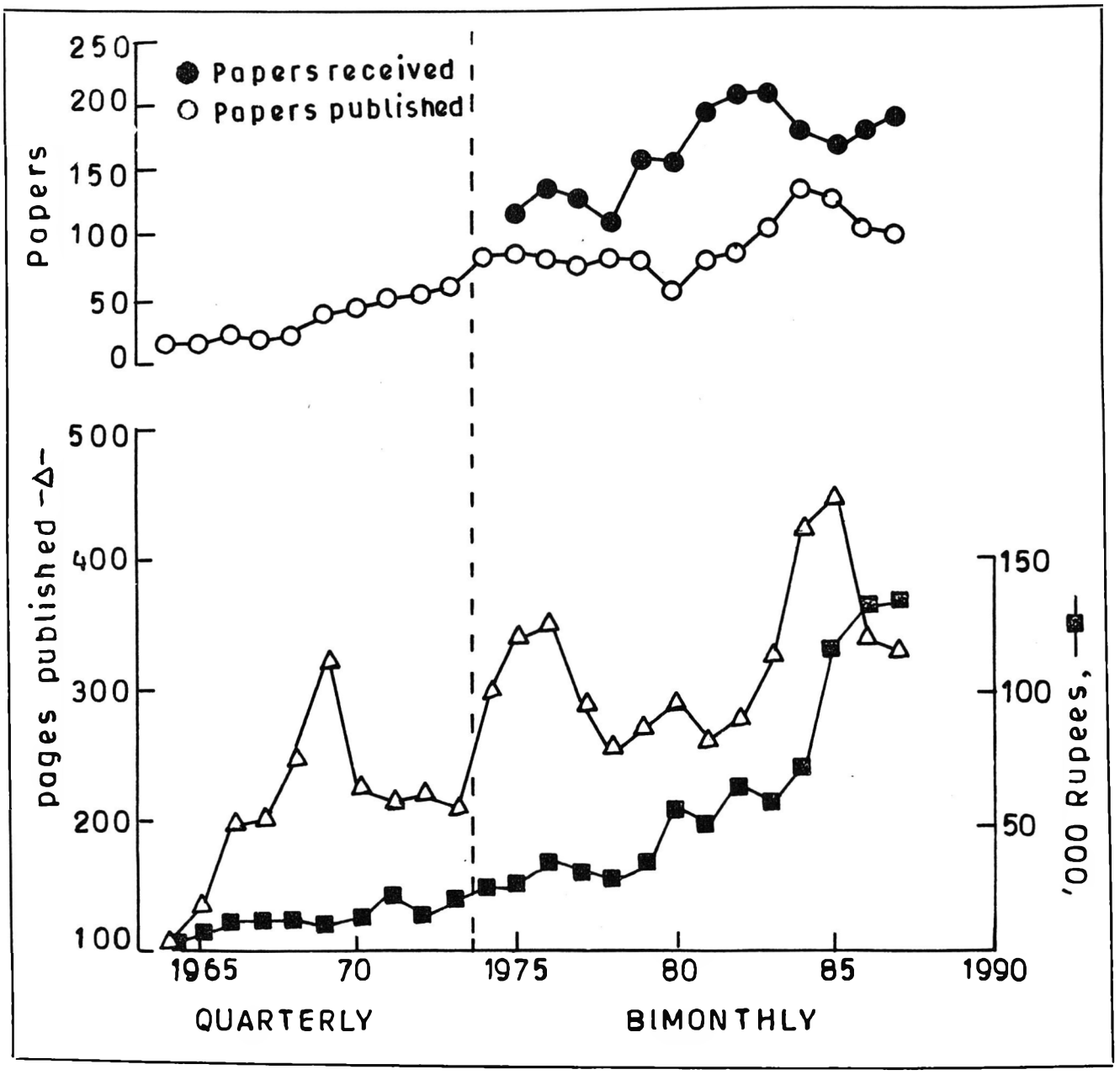
OUR NEW EDITOR

Dr. J.R. Rangaswamy will be taking over as the editor of the Journal of Food Science & Technology from January 1989 until the end of 1991. Dr. Rangaswamy is presently working as a scientist in the ICP Discipline at Central Food Technological Research Institute, Mysore. After a brillant career Mr. Rangaswamy obtained his M.Sc. Degree in Organic Chemistry from Karnataka University in 1961. He joined CFTRI in 1962. In 1971, he was awarded the Ph.D. Degree of the University of Mysore for his work on fish, meat and poultry in which he demonstrated the presence of an alternate catabolic pathway for AMP. Later, he worked on Organic fungicides, carbamate insecticides and other fumigants. He has developed methods for determination of EDB and phosphine residues in foods. Recently, he has successfully synthesised Locust Adipokinetic Hormone and the Potent Aggregation Pheromone of red flour beetle (*Tribolium castaneum*). Further, he has also developed the technology for the use of the pheromone in flour mills. He was the recipient of a WHO fellowship in 1978 during which time he worked in England on phosphine residues in cereals. Later, he was also awarded a Post-doctoral fellowship during 1979-80 and he worked in the Department of Chemistry at Exeter University, England.



He has published more than 50 research papers on various aspects of food technology and organic chemistry in National and International journals. He has also authored a host of popular scientific articles and two books in Kannada. He has been an active Member of the Association of Food Scientists and Technologists (India) Mysore for more than 20 years and he was also an Editorial Board member during 1986-88.

Progress of the Journal of Food Science and Technology during 1964–1987



J. Fd. Sci. Technol., 1988, Vol. 25, No. 6, 327–329

Effects of Malting on Proximate Composition and *in vitro* Protein and Starch Digestibilities of Grain Sorghum

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Received 6 November 1987; revised 29 February 1988

The changes in dry matter, nutrient composition and *in vitro* protein and starch digestibilities were studied during malting of grain sorghum. The steeping of grains in water for 10 to 30 hr and germination at 30°C upto 72 hr significantly decreased the dry weight and starch while significantly increasing the water soluble proteins, free amino acids and reducing sugars in malted meal. The steeping of grains for 10 hr and subsequent germination for 24 hr significantly improved *in vitro* protein and starch digestibilities of the malted meal. However, prolonged steeping or germination of grains exhibited adverse effects on the digestibility of starch. The results indicated that 10 hr steeping followed by 24 hr germination is optimum to produce a sorghum malt for traditional food uses.

Grain sorghum is a staple human food for more than 700 million people of the arid and semi-arid tropics. Almost entire produce of sorghum is consumed in the form of traditional products in India and Africa. However, the nutritional quality of sorghum products is poor due to the deficiency in lysine, threonine and tryptophan, low starch availability, low protein digestibility and presence of certain antinutritional factors[1] while the sensory properties are poor due to coarse nature of grains[2].

Malting of grain sorghum has been reported to increase the water soluble proteins, lysine, methionine, soluble sugars and diastatic activity in the malt.[3,4] Most of the reports on sorghum malting are related to its possible utilization in brewing industry. However, Desikachar[2] had reported that sorghum may not be suitable for brewing due to low amylase activity of the malt. The biochemical modifications in the grain during malting may be, however, advantageous to produce a malt with improved nutritional quality with the result that it can be used in various traditional foods. The malting of sorghum grains for prolonged period results in significant loss of dry weight[5]. This is undesirable when malting is intended for traditional food uses. Hence, it is essential to standardize the malting conditions to obtain nutritionally improved meal with minimum loss in dry matter. The purpose of the present investigation was to study the changes in dry weight, proximate composition, and storage proteins and starch during malting and to optimize the malting conditions for sorghum intended for traditional food uses.

Materials and Methods

Malting of grains: The sorghum grains (cv 'M 35-1') were obtained from the Sorghum Breeder, Mahatma Phule Agricultural University, Rahuri. One hundred gram lots of grain were steeped in running tap water for 10, 20 and 30 hr and germinated in petri dishes with double layer of moistened filter paper for 24, 48 and 72 hr at 30°C in triplicate. Water was sprinkled on the grains every 12 hr to avoid drying. The germinated grains were dried to constant weight at 60°C. The root portions were manually removed and weighed. The difference in weight of grains before steeping and after drying of germinated grains was taken as malting loss. The malted grains of each treatment were pooled together and milled to 80 mesh for analyses.

Nutrient composition: The meals were analysed for proximate composition[6], water soluble proteins[7], free amino acids[8], reducing sugars[9] and starch[10].

Protein and starch digestibilities: The *in vitro* protein digestbility (IVPD) was determined by pepsin hydrolysis of meal proteins[6]. The *in vitro* starch digestibility (IVSD) was determined using α-amylase[11]. All analyses were performed in triplicate and data analysed for statistical significance.

Correspondence: Dr. J.K. Chavan, Department of Biochemistry, Mahatma Phule Agril. University, Rahuri – 413 722. India.

Results and Discussion

Malting loss and nutrient composition: Malting losses were directly proportional to steeping and germination periods (Table 1). However, the losses in dry weight were more predominant due to germination as compared to steeping. Similar results have been reported by Pathirana *et al*[5]. The malting loss in 48 hr germinated sorghum grain was found to increase from 3 to 9.2 per cent when the steeping period was increased from 8 to 18 hr. The losses due to germination can be attributed to respiratory activity of grains while the increased losses, due to prolonged steeping may be due to faster rate of germination[5]. However, such losses need to be minimum to avoid the loss of available nutrients. The changes in protein, fat, fibre and ash due to malting were not of significant order (Table 1), although in general, prolonged steeping and germination caused a slight decrease in these nutrients. This can be attributed to the leaching losses. Similar results have been reported earlier[3,4,12].

Soluble proteins, free amino acids and IVPD: The soluble proteins and free amino acids were found to increase continuously and significantly during 3-day malting (Table 2). The IVPD increased significantly when the grains were steeped for 10 hr followed by germination upto 3 days (Table 2). An increase in the steeping period to 20 or 30 hr seemed to nullify this effect. The possible reasons for such behaviour needs further investigation. The protein digestion is a multi-enzyme process while only pepsin has been used in the present study to digest the proteins. An increase in soluble proteins and free amino acids can be attributed to the partial hydrolysis of storage proteins by endogenous proteases produced during malting[4]. Such partially hydrolysed storage proteins may be more easily available for pepsin attack.

Starch, sugars and IVSD: The starch content was found to decrease continuously and significantly with concomitant increase in the reducing sugars on progressive malting of grains (Table 2). The IVSD increased significantly when the grains were germinated for 24 hr after 10 hr steeping. However, the susceptibility of malt starch to α-amylase decreased when both steeping and germination periods were extended. The starch is hydrolyzed by endogenous α-and β-amylases during germination. For IVSD determination, only α-

TABLE 1. EFFECT OF MALTING ON MALTING LOSS (%) AND PROXIMATE COMPOSITION OF SORGHUM

Compositon	Steeping period (hr)	Malting period (hr) 24	48	72
Malting loss (%)	10	3.2	11.2	19.5
	20	6.2	13.6	20.9
	30	9.5	17.5	25.6
Crude protein (%)	10	11.6	11.3	11.2
(11.6)	20	10.6	10.5	10.1
	30	10.2	10.2	9.9
Crude fat (%)	10	4.0	3.0	3.0
(4)	20	4.1	3.2	2.8
	30	4.2	3.2	2.6
Crude fibre (%)	10	2.0	1.8	1.7
(1.8)	20	1.9	1.8	1.8
	30	1.8	1.7	1.6
Ash (%)	10	1.0	1.0	1.0
(1.7)	20	1.0	0.9	0.9
	30	1.2	1.0	1.0

LSD at 5%
Malting loss = 0.05;
Protein = 0.3;
fat, fibre and ash = 0.2.

Figures in parantheses represent the composition of unmalted dry grain.

TABLE 2. EFFECT OF MALTING ON SOLUBLE PROTEINS, FREE AMINO ACIDS, STARCH, REDUCING SUGARS AND *IN VITRO* PROTEIN AND STARCH DIGESTIBILITIES IN SORGHUM

Constituent	Steeping period (hr)	Malting period (hr) 24	48	72
Soluble proteins (%)	10	1.2	1.6	1.9
(1.2)	20	1.3	1.9	2.4
	30	1.9	2.3	2.8
Free amino acids (mg/g)	10	3.3	5.9	7.1
(2.2)	20	3.5	6.5	8.1
	30	5.8	7.8	10.2
Starch (%)	10	68.0	60.5	57.6
(69.5)	20	64.3	55.1	53.1
	30	62.3	50.5	49.1
Reducing sugars (%)	10	1.4	2.7	4.8
(0.2)	20	1.7	3.7	6.4
	30	2.6	4.7	7.3
IVPD (%)	10	82.7	85.3	90.2
(80.8)	20	79.5	80.9	83.4
	30	78.4	79.4	83.8
IVSD (mg maltose/g/2 hr)	10	204.0	125.7	97.1
(137.1)	20	136.4	124.7	76.0
	30	121.4	78.5	60.7

LSD at 5 %

Soluble proteins	– 0.2;	Free amino acids	– 3.2
Starch	– 2.8;	Reducing sugars	– 0.2
IVPD	– 2.5;	IVSD	– 2.8

Figures in parenthesis represent the values for unmalted dry grains.

amylase was used. Prolonged steeping accelerates the rate of germination[5], thus hydrolyzing the starch at a faster rate. It is likely that the residual starch in the malt obtained after prolonged steeping or germination is resistant to hydrolysis by α-amylase alone. This may result in lower values of IVSD. The decrease in starch content and an increase in sugars during malting can be attributed to starch hydrolysis by endogenous amylases[5]. However, the decrease in a large amount of starch is undesirable when malting is meant for the preparation of traditional products. This would result in significant loss of dry matter.

The soluble sugars and water soluble proteins in the meal of sorghum have been positively correlated with improved *bhakri* making quality[13]. Hence, an increase in both soluble proteins and sugars during malting may be advantageous to improve the sensory properties of traditional sorghum products. However, the optimum concentrations of water soluble proteins and sugars in the meal for improved *bhakri* quality need to be standardized to avoid the possible browning reactions during baking. The results indicated that steeping of grains for 10 hr followed by germination for 24 hr at 30°C was optimum for malting of sorghum grain. Such treatment would yield a malted meal with higher protein and starch digestibilities with minimum losses in dry matter. Further investigations on the utilization of meal as such or as supplement to prepare traditional products commonly consumed by sorghum eaters are essential.

Acknowledgement

The research work was supported by the Indian Council of Agricultural Research, New Delhi under an *ad-hoc* project on "Sorghum malting for improved nutritional and *bhakri* making quality" (No. F.1-21/82-F.C.I.).

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Effect of Blending Laboratory Sprouted Grains on Milling and Baking Properties of Wheat

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Two commercial bread wheats ('WL-711' and 'WL-1562') were germinated in the laboratory for 24 and 48 hr and blended with sound ungerminated wheat in requisite proportions (0 to 100%) to see its effect on milling and baking properties. Sprouting did not alter the crude protein, ash and crude fat of grains. However, significant changes were observed in starch and total sugars. Blending of 1-2 day sprouted wheat upto 30% level did not produce any change in flour recoveries of the blends, rather yielded low ash flour. The flours from the blends (10-30 per cent) had significantly lower protein and starch, with concomitant increase in free amino acids and sugars. Sprouting, its duration and blending increased amylolytic and proteolytic activities significantly. Damaged starch decreased in the 24 hr sprouted flours whereas increased values were recorded with 48 hr sprouting in both the cultivars. Blending of 10-30 per cent sprouted grains with sound wheat produced substantial improvement in loaf volume of bread and cake but cookie spread was not affected with the blending. Upto 20 per cent of 2-day sprouted wheat in the grist produced non-sticky doughs and *chapatis* puffed full.

The problem of grain sprouting in the field is not common to one specific area in the world. Latest mechanized technology for post harvest processes of wheat has been adopted by farmers but damage to wheat by rains has not been eliminated completely. This natural calamity brings about pronounced changes in enzymatic activity leading to undue hydrolysis of starch and protein during dough mixing and fermentation imparting stickiness and gumminess to the crumb[1-2]. The use of 100 per cent flour from extensively sprouted wheat yields breads with poor characteristics having darker crumb and inferior grain quality and texture[3].

Punjab which is known to be the wheat bowl of India produces about one fourth of the total wheat produced in the country and contributes more than 6.7 million tonnes to the Central Food Grains Pool. This year, the rains played havoc with the standing ripe crop and the share of central pool reduced to 4.7 million tonnes. Various treatments have been suggested to use the sprouted wheat flour, which though feasible, could not be adopted commercially for a number of reasons. Blending of sprouted wheat in requisite proportions with sound ungerminated wheat is simple and can be adopted by millers without any extra expenditure. Results of such a study conducted in the laboratory are described in this paper.

Material and Methods

Two commercial cultivars of bread wheat, 'WL-711' and 'WL-1562' were obtained from the University Farm located at Ludhiana.

Pre-treatments: Cleaned samples from each variety (10 kg) were soaked in tap water for 8 hr at 30°C. After requisite soaking, the sample was divided into two lots and sprouted for 24 and 48 hr in a germinating chamber at 30°C/80 per cent RH and dried in a forced air circulation drier at 32°C to about 14 per cent moisture. The dried samples along with control were stored in bins for use in the study.

Blending of wheat and milling: The sound and sprouted wheat were tempered separately to 16 and 15 per cent moisture respectively and rested for 48 hr. The sprouted wheat was then mixed with sound ungerminated counterparts at levels 0, 10, 20, 30 and 100 per cent just before milling. The samples were milled in a Buhler Pneumatic Laboratory Mill (MLU-202) and the yields of straight grade flour computed on recovered product basis.

Physico-chemical characteristics of grains and flour: Grain weight, Hectolitre weight, pearling index, protein, ash, crude fat, starch, free sugars, diastatic activity and damaged starch were determined using AACC[4] standard methods. Free amino acids were determined using the method of Lie[5]. Proteolytic activity was determined using modified Ayre Anderson method[6].

Baking studies: For bread making, straight dough method of AACC with remixing procedure of Irvine and McMullan[7] was followed. AACC[4] procedures

TABLE 1. EFFECT OF LABORATORY SPROUTING ON PHYSICO CHEMICAL GRAIN CHARACTERISTICS

Sprouting duration (hr)	1000 kernel wt (g)	Hecto-litre wt (kg)	Pearling index (% overs)	Protein (N × 5.7) (%)	Ash (%)	Crude fat (%)	Starch (%)	Free sugars (%)
				WL – 711				
0	40.18	80.76	60.00	9.00	1.63	1.66	63.24	4.73
24	39.20	60.27	53.60	9.10	1.52	1.73	61.25	5.71
48	37.66	63.90	48.70	9.10	1.54	1.45	60.03	6.66
				WL – 1562				
0	44.42	82.95	58.70	10.20	1.93	1.51	63.81	4.10
24	44.10	73.32	57.20	10.30	1.48	1.52	62.85	4.35
48	42.79	70.37	46.40	10.40	1.43	1.45	60.16	5.73
				LSD (0.05)				
Var. Sprouting	N.S.	N.S.	N.S.	0.016	N.S.	N.S.	N.S.	0.91
	N.S.	8.1	N.S.	N.S.	N.S.	N.S.	3.3	1.12

were applied to bake cookies and cakes from blended samples and *chapati* was made using the procedure of Austin and Ram[8].

Statistical analysis: Values reported are an average of duplicate tests. Analysis of variance and least significant range (LSR) were computed at 5 per cent level of significance and tested with Duncan's multiple range test[9].

Results and Discussion

Grain characteristics: Both the cultivars, 'WL-711' and 'WL-1562' did not differ significantly with respect to physical grain characteristics. The pearling index values revealed that grains of 'WL-711' were harder than 'WL-1562'. The sprouting of grains significantly decreased the hectolitre weight of the grains in both the cultivars (Table 1).

Neither the sprouting nor its duration made any impact on the protein, ash and crude fat of the grains; however, significant changes were recorded in starch and total sugars in both the cultivars.

Milling characteristics: Table 2 depicts the effect of sprouting and blending the sprouted grains with sound wheat on flour yield and ash content. Both the cultivars differed significantly with respect to flour recoveries but sprouting upto 48 hr did not affect the flour yield in both the cultivars. The blending of 1-2 day sprouted wheat upto 30 per cent level with sound unsprouted wheat did not affect the straight grade flour yield in 'WL-711' and 'WL-1562' wheats. There was no significant difference in the ash values of flour in the cultivars. Sprouting produced significant decrease in the flour ash of these wheats. The levels of blending had a pronounced effect in lowering the ash

TABLE 2. EFFECT OF SPROUTING AND BLENDING THE SPROUTED GRAIN WITH SOUND WHEAT ON MILLING PROPERTIES

Milling characteristics (%)	Var.	Sprouting period (hr)	Values at indicated % blending levels				
			0	10	20	30	100
Flour yield	WL – 711	24	73.58	73.35	72.04	70.85	71.42
		48	73.58	73.24	73.17	72.80	72.75
	WL – 1562	24	75.19	74.63	74.39	74.66	73.79
		48	75.19	74.28	74.60	75.59	71.50
Flour ash	WL – 711	24	0.51	0.50	0.49	0.47	0.41
		48	0.51	0.52	0.51	0.50	0.45
	WL – 1562	24	0.54	0.53	0.52	0.51	0.48
		48	0.54	0.51	0.52	0.49	0.47
LSD (0.05)	Var	Sprouting	Sprouting period		Blending level		
Flour yield	1.19	N.S.	N.S.		N.S.		
Flour ash	N.S.	0.038	N.S.		0.035		

TABLE 3. EFFECT OF SPROUTING AND BLENDING THE SPROUTED GRAINS WITH SOUND WHEAT ON PROXIMATE COMPOSITION OF FLOUR

	Variety	Sprouting period (hr)	Values at indicated % blending levels				
			0	10	20	30	100
Crude protein (%)	WL – 711	24	8.30	7.85	7.55	7.49	7.00
		48	8.30	7.62	7.53	7.40	6.65
	WL – 1562	24	9.14	8.84	8.68	8.47	8.32
		48	9.14	8.83	8.61	8.45	7.92
Crude fat (%)	WL – 711	24	0.71	0.70	0.66	0.63	0.58
		48	0.71	0.68	0.64	0.60	0.56
	WL – 1562	24	0.83	0.82	0.80	0.79	0.79
		48	0.83	0.81	0.80	0.75	0.72
Starch (%)	WL – 711	24	68.18	67.19	66.12	63.84	62.93
		48	68.18	64.51	63.61	62.71	61.30
	WL – 1562	24	63.85	62.90	62.20	61.25	60.44
		48	63.85	62.55	61.92	60.58	59.58
Free sugars (%)	WL – 711	24	4.58	5.07	5.32	5.60	5.83
		48	4.58	5.24	5.56	5.92	6.54
	WL – 1562	24	3.44	3.84	3.90	4.53	4.79
		48	3.44	4.06	4.39	4.80	5.42
Minerals (%)	WL – 711	24	0.51	0.50	0.49	0.47	0.41
		48	0.51	0.52	0.51	0.50	0.45
	WL – 1562	24	0.54	0.53	0.52	0.51	0.48
		48	0.54	0.51	0.52	0.49	0.47

LSD (0.05)	Varieties	Sprouting	Sprouting period	Blending level
Crude protein	0.15	0.26	0.11	0.24
Crude fat	0.013	0.023	0.004	0.020
Starch	1.05	1.80	0.75	1.67
Free sugars	0.24	0.42	0.17	0.38
Minerals	N.S.	0.038	N.S.	0.035

values. Both the varieties behaved similarly and there was no interaction between variety and blending level in influencing the flour ash. The decrease in ash values may be the result of leaching the ash yielding components and the use of inorganic ions during respiration. Some of the earlier findings also revealed the low ash flours sprouted grains[1, 10].

Flour characteristics

Proximate composition: Proximate composition as affected by sprouting and level of blending is given in Table 3. The cultivars 'WL-711' and WL-1562' differed significantly with respect to crude protein, crude fat, starch and free sugars. Sprouting of grains decreased the protein content of flour significantly in both the cultivars. Blending the sprouted grains with sound wheat also affected the protein content considerably. The decrease in protein may be ascribed to the milling of sprouted wheats to lesser recoveries as compared to control samples.

Blending with 10-30 per cent levels of sprouted wheat brought about marginal changes in crude fat of the blends. Beyond 30 per cent level, the decrease was sharp and the interaction between variety and blending level was found to be significant. Sprouting of grains decreased the starch with concomitant increase in the free sugars. With the increase in the blending levels, free-sugars increased proportionately indicating progressive hydrolysis of starch by amylases. The interaction between variety and blending levels with respect to free sugars was significant.

Amylolytic and proteolytic properties: The data for amylolytic and proteolytic activities are given in Table 4. Diastatic activity registered significant increase with sprouting in both the cultivars. The blending level of 10-30 per cent with sprouted wheat used in the investigation also affected the activity. The increased activity of blended samples may be due to the fact that alpha-amylase developed during the germination of grains. 'WL-711' and 'WL-1562' differed a lot with respect to diastatic activity.

TABLE 4. EFFECT OF SPROUTING AND BLENDING THE SPROUTED GRAINS WITH UNSPROUTED SOUND WHEAT ON AMYLOLYTIC AND PROTEOLYTIC CHARACTERISTICS OF FLOUR

	Variety	Sprouting period (hr)	Values at indicated % blending levels				
			0	10	20	30	100
Diastatic activity (mg maltose/10g)	WL – 711	24	260	422	514	524	546
		48	260	640	681	720	768
	WL – 1562	24	404	418	445	474	575
		48	404	503	528	662	772
Damaged starch (%)	WL – 711	24	7.31	7.14	6.90	6.73	6.64
		48	7.31	8.78	9.04	9.24	9.60
	WL – 1562	24	7.13	6.83	6.73	6.71	6.40
		48	7.13	7.28	7.43	7.51	7.98
Proteolytic activity (Hu/g)	WL – 711	24	0.11	0.19	0.30	0.54	0.69
		48	0.11	0.55	0.69	0.73	0.85
	WL – 1562	24	1.01	1.57	1.97	2.05	2.40
		48	1.01	1.99	2.43	2.50	2.63
Free amino acids (mg glycine/g)	WL – 711	24	0.47	0.52	0.55	0.65	0.74
		48	0.47	0.72	0.85	0.88	1.05
	WL – 1562	24	0.32	0.36	0.40	0.45	0.58
		48	0.32	0.39	0.43	0.51	0.61

LSD (0.05)	Varieties	Sprouting	Sprouting period	Blending level
Diastatic activity	N.S.	150	61	138
Damaged starch	N.S.	N.S.	0.75	N.S.
Proteolytic activity	0.18	0.31	0.12	0.28
Free-amino acids	0.12	0.22	0.09	0.20

It was interesting to note that during the 24 hr sprouting, the damaged starch decreased whereas 48 hr sprouted samples gave higher values than the control samples in both the cultivars. The blended samples also followed the same pattern when 10-30 per cent grains were added to the sound stock before milling. Similar pattern of damaged starch was also reported by Lukow and Bushuk . The changes in the damaged starch occurred due to the changes in the hardness of grains upon sprouting.

Sprouting and its duration had pronounced effect on the proteolytic activity in both the cultivars. The blending of sprouted samples (10-30 per cent) made significant changes in the proteolytic activity of resultant flours. Free amino acids which are indirect indications of proteolytic activity also underwent similar changes and progressive increase was found with increased level of blending in both the cultivars.

Baking properties: Table 5 depicts the changes in the baking properties of flours with different blending levels of sprouted wheats. Bake-absorptions were considerably reduced with 24 and 48 hr sprouting; however, 24 hr sprouted wheat of 'WL-1562' gave slightly higher values. Sprouting of grains and blending level of 10-30 per cent produced substantial increase in the loaf volume in both the cultivars. There was an interaction between variety and blending level on loaf volume. The colour of the crust darkened with increased level of blending the sprouted wheat with sound stock. The crumbs of the breads were white to creamish which turned towards yellowish at higher levels.

Cookie spread factor non-significantly increased with increased amount of sprouted grains in the grist before milling. The blending level upto 30 per cent proved better in making large volume cakes; however, organoleptically the cake from fully sprouted wheat was better in texture and internal score.

The whole meal flour from sprouted grains had significantly lower water absorption than sound wheats but the duration of sprouting had no impact on the water absorption capacity of these flours. Blending sprouted wheat at levels of 10-30 per cent progressively decreased the water absorption of samples with increased amounts of sprouted grains in the samples. Upto 20 per cent of 2-day sprouted grains in the

TABLE 5. EFFECT OF SPROUTING AND BLENDING THE SPROUTED GRAINS WITH UNSPROUTED SOUND WHEAT ON BAKING CHARACTERISTICS OF FLOUR

	Variety	Sprouting period (hr)	Values at indicated % blending levels				
			0	10	20	30	100
Bake absorption (ml)	WL - 711	24	62.5	61.0	60.0	59.5	59.0
		48	62.5	60.0	59.0	58.0	57.0
	WL - 1562	24	60.0	63.0	62.5	62.5	61.0
		48	60.0	62.0	61.5	61.0	57.0
Bread loaf vol (ml)	WL - 711	24	450	470	480	500	490
		48	450	480	510	30	505
	WL - 1562	24	510	590	600	605	540
		48	510	515	510	515	560
Cookie spread factor (W/T)	WL - 711	24	5.96	6.37	6.99	7.48	7.99
		48	5.96	5.80	6.37	6.74	7.54
	WL - 1562	24	6.49	6.50	7.29	6.34	6.04
		48	6 49	5.59	5.90	7.48	7.68
Cake vol (ml)	WL - 711	24	740	740	745	760	770
		48	740	785	790	785	680
	WL - 1562	24	790	845	810	800	760
		48	790	790	780	830	630
Wholemeal water absorption (%)	WL - 711	24	58.5	57.6	57.4	57.0	56.5
		48	58.5	57.5	57.5	57.1	56.0
	WL - 1562	24	63.4	62.8	61.9	61.5	59.8
		48	63.4	61.3	61.0	60.7	57.1

LSD (0.05)

	Varieties	Sprouting	Sprouting period	Blending level
Bake absorption	1.14	1.97	0.81	N.S.
Bread loaf volume	11	19	7	17
Cookie spread factor	N.S.	N.S.	N.S.	N.S.
Cake volume	N.S.	N.S.	N.S.	N.S.
Whole meal water absorption	0.90	1.56	N.S.	1.43

sample produced non-sticky dough and puffed full during baking. The increased level of sprouted grains in the grist produced sticky doughs which were difficult to handle and did not puff fully during baking of *chapatis* on traditional iron pan.

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Comparative Efficacies of Gibberellic Acid (GA) and Ethrel Mixture and Other Established Chemicals for the Induction of Sprouting in Seed Potato Tubers

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Comparative efficacies of several growth promotary chemicals viz. gibberellic acid, ethrel, thiourea, rindite and carbon disulphide were assessed for breaking dormancy and/or sprouting of seed potato tubers. Although all the chemicals tested were effective, they were considered as inferior to GA + E treatment for commercial applications because of their toxic nature or need for higher concentration and/or longer duration of treatment. A dip in a mixture of GA + E (60 + 40 ppm) for 10 min for whole tubers was good. Further field trials showed an increase in tuber yield with GA + E and rindite treatments compared to untreated tubers.

Freshly harvested potato tubers remain dormant for a few months and do not sprout even when the temperature and humidity conditions are favourable for sprouting. This period of dormancy however, varies with the variety and the temperature and humidity conditions at which they are held[1]. The number of crops raised per year varies in different parts of the country. In India, particularly in Karnataka, the potato crop is raised twice a year[2]. Hence, dormancy is a real problem, since physiologically young and dormant tubers produced from the previous crop have to be used as seed (as in the case of cultivation of potatoes in Nilgiri hills). Planting of such dormant tubers and waiting for the break of dormancy in soil is an undesirable practice not only because of the time lost but also of erratic germination and uneven crops[2].

Hence, breaking of dormancy in seed tubers before planting is very essential. Dormancy of the tuber can be broken by physical or chemical means. A little wounding[3] or keeping the dormant tubers at high temperature and relative humidity conditions can break the dormancy[4]. There are several reports regarding use of chemicals for the breaking of dormancy. The most important chemical agents in commercial practice are rindite, carbon disulphide, gibberellic acid and thiourea[5-8]. Although all the three chemicals break dormancy, only ethylene chlorhydrin does not have multisprouting effect[6]. Gibberellic acid releases lateral buds from apical dominance and therefore increases the number of sprouts, besides producing elongated sprouts[8]. Ethrel (2-chloroethyl phosphonic acid) has also been reported to break dormancy[9]. In our earlier paper, we have reported that gibberellic acid (GA_3) and ethrel act synergistically in breaking dormancy and produce sprouts which are sturdy[10]. The objective of the present study was to assess the efficacy of the various chemicals in comparison with that of GA + E mixture so that a suitable recommendation can be made for the cultivation of potatoes in the tropics.

Materials and Methods

Potatoes: Potato tubers (*Solanum tuberosum*. L. cultivar 'Kufri Jyoti') were obtained from Devanahalli, Bangalore district where the crop had been raised from certified seed, employing the 'seed plot technique' as described in our earlier paper[11]. The tubers were cured in the field for 5 days and then treated with various dormancy breaking chemicals.

Chemicals: The following chemicals with the indicated source of procurement were used.
Carbon-di-sulphide (LR, Ranbaxy, India). Carbon tetrachloride (AR, Glaxo Laboratories, India); Ethrel (2-chloroethyl phosphonic acid)–4 lb-gal, (Amchem. products, Inc., USA.) Ethylene dichloride (Sisco Research Lab., Bombay) Ethylene chlorhydrin (Koch Light Ltd., Bombay), Gibberellic acid – GA_3 (Lobo Chemie Indo Austranat Co., Bombay), Thiourea – CP (Purex), Rindite – a mixture of ethylene chlorhydrin, ethylene dichloride and carbon tetrachloride in the ratio of 7:3:1.

Treatments: In all the experiments, the seed tubers (whole/cut) were given dip or vapour treatment.

The tubers were dipped in solutions of the above chemicals, individually or in various combinations for different periods of time. Tubers dipped for a similar length of time in water served as control. The relative concentrations of gibberellic acid and ethrel used in these experiments were 60 and 40 ppm as reported earlier[10]. After treatment the tubers were spread, surface dried and packed in 0.2 per cent ventilated 100 gauge polyethylene bags. Five replicates with 25 medium size tubers per replicate were maintained for each treatment. Tubers were stored at ambient temperature and humidity conditions (22-30°C and RH 50-80 per cent).

Carbon disulphide at the rate of 30 g for 400 kg of potatoes was given as vapour treatment to dormant tubers, in a closed box of volume (0.125 m^3). Tubers were kept in the carbon disulphide atmosphere for 10 days after which they were removed and stored at ambient conditions in 0.2 per cent ventilated 100 gauge polyethylene bags.

Visual observations: During the storage period, the treated and untreated tubers were observed at intervals for sprouting behaviour. Parameters like per cent number of sprouted tubers per piece, mean number of sprouts per tuber and sprout yield were recorded.

Field experiments: The tubers which were cured in the field for 5 days after harvest and cold stored for 3 months at 2 ± 1°C were treated with growth promoters like GA + ethrel (GA + E), Rindite (R) and Thiourea (T). The tubers dipped in water for similar length of time served as control. After treatment, the tubers were kept for one week at ambient conditions before planting as single eye pieces. The concentration of GA + ethrel was 60 + 40 ppm and of rindite and thiourea was 10,000 ppm. The tubers receiving GA + E treatment were dipped for 10 min while in the other two cases, the treatment was for 1h. The treated tubers were cut into single eye seed tuber pieces and planted in a field at Devanahalli, Bangalore district. Plot of 9 m^2 were used for planting these tubers with 5 replicates (plots) for each treatment under completely randomised design.

Results

Comparative efficacy of different chemicals for breaking the dormancy: Among the various treatments to whole tubers, GA + ethrel treatment was more effective in inducing sprouting. The per cent of tubers sprouted at the end of 15 days was more and there was also a significant increase in the number of sprouts per tuber and sprout yield.

Ethrel stands next to GA + ethrel treatment regarding per cent of sprouted tubers; it is equal to GA treatment as far as number of sprouts per tuber is concerned. Eventhough, number of sprouts per tuber were high or almost same as that of GA treatment, it was found inferior to GA with respect to sprout weight. In the GA + ethrel combinations, the sprout number increased and the formed sprouts were short and sturdy which is a desirable characteristic (Table 1).

In the case of treatment to single eye cut tuber pieces (Table 2), all the treatments increased sprouting percentage when compared to control. Here, thiourea (10,000 ppm) was as effective as GA + ethrel (60 + 40 ppm) treatment. However, since GA + ethrel treatment is effective at low concentration with short duration, it would be preferred for commercial cultivation.

TABLE 1. COMPARATIVE EFFICACY OF DIFFERENT GROWTH PROMOTERS FOR THE INDUCTION OF SPROUTING IN DORMANT WHOLE TUBERS

Chemical		Treatment		Sprout details at indicated days of storage		
Name	Concn. (ppm)	Nature	Duration (min)	15*	45	45
				Sprouted tubers (%)	Mean sprouts/ tuber	Sprout wt (g fresh/100 tubers)
Gibberellic acid (GA)	100	Dip	10	59.00(50.21)^{a}	5.81^{a}	9.58^{a}
Ethrel (E)	10	Dip	10	69.00(56.83)^{ab}	5.35^{a}	5.89^{ab}
Thiourea (T)	10,000	Dip	60	58.20(50.01)^{a}	5.80^{a}	7.11^{ab}
Carbon disulphide (CS_2)		Vapour	10 days	39.20(38.76)^{c}	4.83^{ab}	6.96^{ab}
GA + E	60+40	Dip	10	77.00(61.72)^{b}	7.75^{c}	16.69^{c}
Control	-	-	-	12.00(18.48)^{d}	3.55^{b}	3.71^{b}
SEm (24 df)				±3.38	±0.44	±1.25

Figures in parantheses indicate the transformed values (arc-sine), means in the same column followed by different superscripts differ significantly according to Duncan's New Multiple Range Test ($P< 0.05$)

*Tubers of all treatments reached 100 per cent sprouting at the end of 45 days of storage.

TABLE 2. COMPARATIVE EFFICACY OF DIFFERENT GROWTH PROMOTERS FOR THE INDUCTION OF SPROUTING IN DORMANT CUT TUBER PIECES

Chemicals		Treatment		Sprouted pieces* (%)	Mean sprouts/ tuber**	Sprout wt** (g fresh/ 100 pieces)
Name	Concn. (ppm)	Nature	Duration (min)			
Gibberellic acid (GA)	100	Dip	10	71.12(58.09)^{ab}	4.67^{ab}	7.78^{a}
Ethrel (E)	10	Dip	10	77.00(61.84)^{ab}	6.52^{b}	6.46^{a}
Thiourea (T)	10,000	Dip	60	94.00(79.91)^{d}	8.34^{c}	15.09^{b}
Rindite (R)	10,000	Dip	10	85.46(64.78)^{ac}	6.47^{b}	18.78^{c}
Carbon disulphide (CS_2)		Vapour	10 days	61.63(51.77)^{b}	5.76^{b}	6.83^{a}
GA + E	60+40	Dip	10	88.00(71.71)^{cd}	7.39^{c}	12.36^{d}
Control	–	–	–	38.75(38.21)^{e}	3.85^{a}	2.53^{e}
				±8.51	±1.26	±1.57
S.Em				(25 df)	(25 df)	(24 df)

Figures in parantheses are the transformed values (arc-sine transformation).
Means in the same column followed by different superscripts differ significantly ($P<0.05$) according to Duncan's New Multiple Range Test.
*After 15 days of storage. Tuber pieces reached 100 per cent sprouting at the end of 30 days of storage
**After 30 days of storage.

TABLE 3. TREATMENTS OF TUBERS (WHOLE TUBER) WITH DIFFERENT CONCENTRATIONS FOR DIFFERENT DURATIONS ON THE INDUCTION OF SPROUTING

Chemicals		Sprouted tubers* (%) at indicated time (min) of treat.			Sprouts/tuber** (No.) at indicated time (min) of treat.			Sprout wt.** (g/100 tubers) at indicated time (min) of treat.		
Name	Concn. (ppm)	10	30	60	10	30	60	10	30	60
Control	0	47.1 (5.4)			9.2 (0.6)			21.9 (2.6)		
Rindite	10,000	85.2 (3.6)	98.4 (1.5)	86.3 (2.2)	8.7 (0.4)	9.8 (0.4)	9.6 (0.8)	27.6 (2.3)	25.6 (3.8)	31.1 (3.8)
	25,000	72.4 (1.5)	94.0 (3.4)	83.2 (7.9)	11.3 (1.4)	10.6 (0.8)	9.6 (0.8)	22.1 (1.5)	29.7 (5.7)	44.3 (4.6)
	50,000	78.5 (4.1)	nd	nd	8.5 (1.3)	nd	nd	26.3 (1.0)	nd	nd
Ga + E	6 + 4	96.1 (2.2)	73.2 (9.1)	96.4 (3.5)	9.0 (1.1)	9.7 (0.7)	11.28 (0.4)	26.0 (3.2)	28.0 (3.9)	35.0 (1.0)
	15 + 10	88.5 (5.4)	92.4 (4.3)	93.3 (3.8)	10.1 (1.5)	9.9 (0.4)	10.4 (0.5)	35.0 (4.5)	37.0 (2.5)	39.0 (3.0)
	30+20	86.0 (4.8)	91.6 (5.0)	85.5 (2.6)	7.9 (0.5)	9.2 (0.4)	10.4 (0.9)	35.2 (4.3)	37.2 (3.8)	36.7 (3.3)
	60+40	90.7 (4.4)	95.0 (5.0)	100.0 (0.0)	11.4 (0.6)	11.2 (0.9)	9.9 (0.9)	37.0 (1.2)	41.6 (4.5)	55.7 (1.4)
R+GA+E	10,000+ 30+20	92.8 (4.1)	92.1 (2.6)	91.1 (4.8)	11.6 (1.0)	12.4 (1.6)	14.3 (2.1)	45.6 (2.3)	62.0 (4.9)	80.0 (5.2)
T+GA+E	10,000+ 30+20	89.2 (3.5)	94.8 (5.1)	87.4 (4.1)	9.9 (0.9)	11.2 (1.1)	11.5 (0.2)	35.0 (3.0)	50.0 (7.5)	56.0 (4.0)
R+GA	10,000+ 50	73.9 (4.5)	85.8 (3.1)	89.7 (4.3)	10.2 (1.8)	10.7 (1.4)	10.9 (1.7)	29.9 (5.7)	34.8 (5.6)	47.6 (8.5)
T+GA	10,000+ 50	84.8 (4.1)	91.4 (4.1)	96.4 (2.0)	11.2 (0.7)	11.8 (1.0)	10.1 (0.9)	28.0 (0.9)	45.7 (3.0)	45.0 (2.3)
R+E	10,000	nd	81.9 (2.4)	nd	nd	12.1 (1.2)	nd	nd	35.1 (2.3)	nd

Figures in parentheses indicate S.Em.
nd = not done
Each treatment was tried at three durations of dipping, namely 10, 30, and 60 min, except R (50,000 ppm) and R+E (10,000+40 ppm) treatments which were tried only for 30 and 10 min, respectively.
*After 15 days. **After 45 days

TABLE 4. DURATION AND CONCENTRATION OF GROWTH PROMOTERS ON THE INDUCTION OF SPROUTING IN DORMANT POTATO TUBERS (WHOLE)

Treatment	Duration of treat. (min)	Sprouted tubers* (%)	Mean sprouts/ tuber**	Sprout wt** (g fresh/ 100 tubers)
GA + E	10	90.75(74.58)[a]	11.46[a]	37.05[a]
(60 + 40)	30	95.00(82.00)[a]	11.27[a]	41.65[a]
ppm	60	100.00(88.19)[a]	9.99[a]	55.76[b]
R+GA+E	10	92.86(77.94)[a]	11.65[a]	45.70[a]
(10,000+30+20)	30	92.16(75.43)[a]	12.44[a]	62.01[b]
ppm	60	91.11(75.21)[a]	14.33[a]	80.33[c]
Control	-	47.16(43.34)[b]	9.25[a]	21.96[d]
S.Em.	-	±9.67(20 df)	±2.24(20 df)	±6.63(18 df)

Figures in parentheses are the transformed values (arc-sine)
Means of the same column followed by different superscripts differ significantly according to Duncan's New Multiple Range Test ($P < 0.05$).
*After 15 days of storage. **After 45 days of storage.

Effect of duration and concentration of growth promoters on the induction of sprouting: An experiment employing rindite, thiourea, gibberellic acid and ethrel in various combinations and varying the concentration and duration of treatment (containing 33 treatments as detailed in Table 3) was conducted. Among them, the treatments GA + ethrel (60 + 40 ppm) and R + GA + E (10,000 + 30 + 20 ppm) were most effective (Table 4). With the increase in duration, there was not much difference regarding per cent of sprouted tubers as well as mean number of sprouts per tuber. But, sprout yield increased with increased duration of treatment.

TABLE 5. EFFECT OF TREATMENT OF SEED TUBERS WITH RINDITE, THIOUREA AND (GA + E) MIXTURE ON THE TUBER YIELD IN FIELD TRIALS.

Treatment*	Duration of treatment@ (min)	Tuber Yield (kg/plot)
Gibberellic acid + Ethrel (60 + 40 ppm)	10	30.4[a]
Rindite (10,000 ppm)	60	33.4[a]
Thiourea (10,00 ppm)	60	30.1[a]
Control	-	27.4[a]
	SEm(16 df)	±1.4

@ The whole tubers were treated with the growth promoters 1 week before planting.
* Tubers cured for 5 days in field after harvest were cold stored (2 ± 1°C) for 3 months before treatment.

Field performance of seed tubers treated with growth promoters: Field trials conducted with seeds treated with sprout inducement chemicals like GA, ethrel, rindite and thiourea showed a tendency towards increase in yield (Table 5).

Discussion

Thiourea and carbon disulphide are commonly used for induction of sprouting in seed potato tubers in India, especially in Nilgiri hills where three crops are raised – summer, autumn and winter crop[2]. Rindite alone and in combination with gibberellic acid is used generally in Western countries[5,6]. However, higher concentrations and longer duration of treatment is needed with these chemicals in order to be effective. The results presented earlier show that sprout induction with GA + ethrel treatment is more effective compared to some of these conventional chemicals – carbon disulphide and thiourea, besides requiring high concentration as well as duration of treatment is longer. In this study also, rindite alone or in combination with GA + E was as effective as GA + E alone. However, rindite treatment should be done carefully as the vapours are toxic to operators. Further, the tubers are to be subjected to treatment for an hour. In view of this, GA + E treatment is preferable to treatment with rindite. Further, our study showed that tubers treated with GA + E have produced better yield than control tubers.

From these studies, it is concluded that treating seed tubers with GA + E for sprout induction is safest, less

expensive and most efective and can replace the other chemical treatments in future.

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Effect of Postmortem Conditioning Treatments to Sheep Carcasses on Some Biophysical Characteristics of Muscles

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Female sheep (age: 4-5 yr) of Bannur breed were sacrificed and carcasses subjected to postmortem conditioning treatments, viz., Achilles tendon suspension (conventional) at 2-3°C for 24 hr (C_1) or pelvic suspension at RT (26 ± 2°C) for 7 hr followed by chilling at 2-3°C for 17 hr (C_2). The data collected on SM and ST muscles from conditioned (C_1 or C_2) carcasses were compared with those obtained on fresh (F) muscles dissected out within 1 hr postmortem. The results indicated that conventional Achilles tendon suspension (under chilling conditions) exerts different restraining influence on thigh muscles permitting contraction in SM and stretching in ST muscles whereas pelvic suspension (under delayed chilling condition) stretched both muscles, effect being more in ST than in SM muscle. Pre-rigor muscles when cooked shortened most and cold contracted muscles least and stretched muscles shortened more on cooking than (cold) contracted ones; the interaction conditioning × cooking was highly significant ($P < 0.01$). Pressure cooking induced greater thermal shrinkage than cooking without pressure. Contraction or stretching of muscles on account of carcass suspension posture had no significant ($P>0.05$) effect on cooking loss and WHC of muscles.

Postmortem chilling produces contracture in the striated muscles of both ovine and bovine species and this is associated with a very pronounced toughening of cooked meat[1-4]. A detailed study of the shortening-temperature relationship revealed that muscle contraction is minimum at 15°C and increases on either side of this temperature[5]. Further, conditioning at 15-16°C coupled with altered carcass posture has been found to be effective in overcoming toughening due to cold contraction effect in beef[6] and in mutton[7]. Cooking also induces contraction of muscles and the extent of thermal shortening is found to influence the tenderness quality of muscles[8]. Also water holding capacity (WHC) of muscles is found to be associated with the contraction/stretching of muscles during postmortem conditioning[9] and with the shrinkage of muscles during cooking[10]. Most of the work on these lines has been carried out using various time-temperature schedules of postmortem holding of carcasses/muscles (especially of beef animals) and cooking of muscles. The purpose of this investigation was to find out the influence of holding carcasses at ambient temperature (26 ± 2°C) prevailing in tropical regions and cooking practices ("well done" or "very well done") followed in India on various biophysical and physico-chemical characteristics affecting eating quality of meat. The results of biophysical characteristics are reported here.

Materials and Methods

Experimental animals: Female sheep of local variety of Bannur breed possessing 8 incisors in the lower jaw and judged to be 4-5 yr of age[11] were procured from the local market, slaughtered and dressed in the Training Abattoir attached to the laboratory according to the procedure outlined by Dani *et al*[7]. The dressed and washed carcasses were subjected to the following conditioning treatment:

C_1: Achilles tendon suspension (conventional) at 2-3°C for 24 hr – Direct chilling.

or C_2: Pelvic suspension (Hip-free suspension from a hook inserted through obturator foramen with the limbs hanging free at RT (26 ± 2°C) for 7 hr followed by chilling at 2-3°C for 17 hr (Tender stretch suspension) – Delayed chilling.

The thigh muscles, Semi Membranosus (SM) and Semi-tendinosus (ST), were dissected out from C_1 and C_2 – carcasses and data obtained on these muscles were compared with those obtained on fresh muscles (F) dissected out within 1 hr postmortem from unconditioned carcasses. Longissimus dorsi muscle was used to monitor the pH fall during conditioning.

Experimental design: Completely randomised design was used to obtain data on pH, length (contraction/stretching), moisture content and WHC of uncooked muscles from animals slaughtered during the course of the investigation. These data are pooled, analysed and reported here.

The data on thermal shortening in length, cooking loss in weight, moisture content and WHC of cooked

muscles were obtained from the experiment planned on a replicated 3 × 2 factorial basis using 18 animals (live wt. 21.2 ± 2.1 kg) with the conditioning as the first factor and cooking as the second.

Two procedures of cooking adopted were:

Cooking without pressure – "Well done" (H_1): Each muscle from left thigh was placed in a HDPE pouch, immersed in water bath, heated to boil and allowed to remain at this temperature for 30 min. The pouches were taken out from water bath and muscles allowed to cool.

Cooking with pressure – "Very well done" (H_2): Each muscle from right thigh was placed in a 250 ml beaker and covered with a petri dish. The muscles were pressure cooked by raising the steam pressure to 1 kg/cm^2 and maintaining this pressure for 20 min Allowed to cool.

Measurement of pH: The glass combination electrode was pierced directly into the incision made in the muscle and pH measured at the muscle temperature with a Radiometer pH meter[12].

Water holding capacity (WHC) of muscles: WHC of uncooked as well as cooked muscles was measured using minced samples by filter paper method outlined by Wierbicki and Deathrage[13] with the modification that the sample was pressed for 2 min instead of 1 min. The per cent bound water obtained by deducting per cent free water from 100 is reported as WHC of muscle.

Statistical analyses: The data were analysed using analysis of variance technique appropriate to the above mentioned designs[14]. Duncan's New Multiple Range Test[15] was adopted to separate the treatment means.

Results and Discussion

Muscle contraction/stretching: Direct chilling with conventional Achilles tendon suspension (C_1) caused significant contraction (about 21.4 per cent) in SM muscle and stretching (about 13 per cent) in ST muscle and altered posture, i.e. suspension through obturator foramen (pelvic) coupled with delayed chilling (C_2) resulted in substantial stretching of both muscles (about 24 per cent in SM and 33 per cent in ST). Both muscles were found to be stretched even at 7 hr RT (26 ± 2°C) under pelvic suspension (Table 1). This observation that the SM muscle contracts and ST stretches under the same suspension through Achilles tendon suggests that the skeleton exerts a different restraining influence on muscles as also observed by Herring *et al*[16]., in bovine muscles. Among the muscles, ST was found to be more susceptible to stretching than SM muscle under pelvic suspension.

The findings on thermal shortening indicate that conditioning treatment is found to affect very significantly ($P<0.001$) the ability of thermal contraction of muscles. The pre-rigor (F) muscles shortened highest followed by C_2 and C_1 – treated

TABLE 1. LENGTH, pH, MOISTURE AND WHC OF UNCOOKED MUSCLES

Conditioning treatment	Muscle	Length (cm)	pH	Moisture (%)	WHC
F	LD	–	6.79(42)a	–	–
	SM	14.12(19)a	6.68(9)a	76.68(6)ab	96.80(6)a
	ST	11.70(19)b	6.42(8)b	78.53(5)c	89.37(5)b
7 hr RT	LD	–	6.00(9)c	–	–
	SM	15.42(6)c	5.97(6)cd	76.48(6)a	62.44(6)c
	ST	14.33(6)a	5.87(6)cde	78.28(6)c	65.08(6)cd
C_1	LD	–	5.81(11)de	–	–
	SM	11.10(19)d	5.76(9)ef	75.16(7)a	67.91(7)cd
	ST	13.22(19)e	5.76(9)ef	77.60(7)c	77.68(7)e
C_2	LD	–	5.62(17)fg	–	–
	SM	17.49(19)f	5.57(12)g	76.55(10)a	66.89(10)cd
	ST	15.56(19)c	5.60(12)fg	77.56(11)bc	69.30(11)d
	SD	±0.86 (118df)	±0.17 (138df)	±0.96 (50df)	±4.80 (50df)

Figures in the parenthesis indicate the number of animals.

Means of the same column followed by different superscripts differ significantly ($P<0.05$) according to Duncan's New Multiple Range test. F, 7 hr. RT, C_1 and C_2 are as described in text.

TABLE 2. SHORTENING (%) IN MUSCLE LENGTH ON COOKING WITH PRESSURE (H_2) AND WITHOUT PRESSURE (H_1)

Treatment	Semi-membranosus			Semi-tendinosus		
	H_1	H_2	Means	H_1	H_2	Means
F	50.80	51.22	51.01a	48.06	49.12	48.59a
C_1	28.07	31.54	29.80b	36.28***	42.15***	39.22b
C_2	40.11	46.82	43.46c	41.68	49.49	45.48c
Means	39.66 ×	43.19y		42.01 ×	46.85y	
S.Em.	(25df)	±0.83			±0.93	

Statistical analyses carried out on 3 × 2 factorial experiment.
Each observation is a mean of 6 animals.
***Very highly significantly (P<0.001) different from corresponding value for SM muscle
F, C_1 and C_2 as described in text.

TABLE 3. LOSS IN WEIGHT (%) WHEN COOKED WITHOUT PRESSURE (H_1) AND WITH PRESSURE (H_2)

Treatment	Semi-membranosus			Semi-tendinosus		
	H_1	H_2	Means	H_1	H_2	Means
F	41.67	43.60	42.64a	44.14	48.22*	46.18a
C_1	45.43	48.10	46.76b	42.09*	47.23	44.66a
C_2	43.89	47.40	45.64b	42.24	47.24	44.74a
Means	43.66 ×	46.37y		42.82 ×	47.56y	
S.Em.	(25df)	±0.83			±0.99	

Statistical analyses carried out on 3 × 2 factorial experiment.
Each observation is a mean of 6 animals.
*Significantly (P<0.05) different from corresponding value for SM muscle.
F, C_1 and C_2 as described in text.

muscles (Table 2). Method of cooking (H_1 or H_2) also affected thermal shortening of muscles highly significantly (P<0.001). Of the two cooking procedures used, cooking with steam pressure (H_2) induced greater shrinkage than cooking without pressure (H_1) and among the muscles, ST suffered thermal shrinkage to a greater extent than SM under a given treatment condition (Table 2).

The interaction conditioning × cooking was found to be highly significant (P<0.01) and greater thermal shrinkage was observed in stretched (about 40 per cent) under H_1 and 47 per cent under H_2 for SM) than in contracted (about 28 per cent under H_1 and 32 per cent under H_2 for SM) muscles. This observation is in line with earlier reports[17-19]. Further, it may also be seen that more the stretching of muscle during carcass conditioning more it shrinks on cooking as seen by the results of ST muscle; C_1 muscles (13 per cent stretched) shrunk on cooking about 36 per cent under H_1 and 42 per cent under H_2 as against 42 per cent under H_1 and 49 per cent under H_2 for C_2 – muscles (33 per cent stretched).

pH and water holding capacity (WHC): The mean initial pH value of 6.79 of LD muscle decreased to 6.0 at 7 hr postmortem when carcasses held under pelvic suspension at RT (26 ± 2°C) were transferred to chill room (2-3°C) (Table 1). The pH at 24 hr is significantly (P<0.05) lower in C_2-muscles (5.57 to 5.62) compared to C_1-muscles (5.76 to 5.81) on account of delayed chilling of carcasses resulting in faster pH fall. No significant differences were observed among conditioned muscles (LD, SM and ST).

Like pH, WHC of uncooked muscles was also affected markedly (P<0.001). The initial pre-rigor value was found to be highest (Table 1) which got lowered in conditioned muscles. It was lowest at 7 hr RT (pH ~ 6.0) due to onset of rigor in muscles which then increased at 24 hr (both in C_1 and C_2 muscles), the increase being significant (P<0.05) in the case of ST muscle. This is in accordance with earlier findings of Hamm[20] and also reported subsequently by many researchers[21-23].

As regards effect of contraction and stretching of muscles during conditioning on their WHC, the results of SM muscle which underwent contraction under C_1 and stretching under C_2 – treatment indicated that the contracted muscle had a slightly higher WHC than its stretched counterpart but the differences were marginal (P>0.05). Bouton *et al.*,[22] on the other hand, reported that contracted beef muscles had significantly reduced WHC than their stretched counterparts.

No significant (P>0.05) differences in cooking loss were observed among the conditioned (C_1 and C_2) muscles (Table 3) but cooking loss was significantly higher (P<0.001) for prerigor (F) SM muscle agreeing with its higher WHC (compared to C_1 – and C_2 – muscles). However, in the case of ST muscle, cooking loss had remained unaffected by conditioning treatments possibly on account of large variations among replicates. Cooking with pressure (H_2) resulted in

TABLE 4. WATER HOLDING CAPACITY OF MUSCLES COOKED WITHOUT PRESSURE (H_1) AND WITH PRESSURE (H_2)

Treatment	Semi-membranosus			Semi-tendinosus		
	H_1	H_2	Means	H_1	H_2	Means
F	69.08	77.26	73.17a	66.33	82.26	74.30a
C_1	65.74	73.80	69.77b	66.24	79.29	72.92a
C_2	61.83	73.49	67.66b	64.40	78.19*	71.30a
Means	65.55 ×	74.85y		65.55×	80.01y	
S.Em.	(25df)	±1.11			±1.49	

Statistical analyses carried out on 3 × 2 factorial experiment.
Each observation is a mean of 6 animals.
*Significantly (P<0.05) different from corresponding value for SM muscle.
F, C_1 and C_2 as described in text.

TABLE 5. MOISTURE (%) IN MUSCLES COOKED WITHOUT PRESSURE (H_1) AND WITH PRESSURE (H_2)

Treatment	Semi-membranosus			Semi-tendinosus		
	H_1	H_2	Means	H_1	H_2	Means
F	63.96	63.04	63.50a	64.95	62.64	63.80a
C_1	61.06	58.94	60.00b	64.72**	62.52**	63.62a
C_2	63.74	61.45	62.50a	66.16	64.40*	65.28b
Means	62.92 x	61.14y		65.28 x	63.19y	
S.Em.	(25df)	±0.64			±0.59	

Statistical analyses carried out on 3 × 2 factorial experiment.
Each observation is a mean of 6 animals.
*,**Significantly (P<0.05, P<0.01) different from corresponding value for SM muscle.
F, C_1 and C_2 as described in text.

significantly (P<0.001) greater cooking loss in either muscles compared to cooking without pressure (H_1) as was also observed in beef muscle by Bouton *et al*[24]. However, the interaction conditioning × cooking was found to be non-significant (P>0.05).

The WHC of muscles (Table 4) cooked pre-rigor (F) was again highest as observed in uncooked ones and conditioning treatments (C_1 and C_2) lowered WHC markedly (P<0.05) in SM muscle and marginally (P>0.05) in ST muscle. Among the two treatments, C_2 provided muscles with lower WHC than C_1 but the difference was found to be non-significant (P>0.05) in muscles cooked either way. The muscles cooked with pressure (H_2) had significantly higher (P<0.001) WHC than those cooked without pressure (H_1) despite significantly greater (P<0.001) cooking loss (Table 3) and lower (P<0.01 for SM and P<0.001 for ST) water contents (Table 5) of muscles cooked with pressure (H_2). This apparently ambiguous result was found to be due to the larger portion of labile water released already on cooking with steam pressure resulting in cooked meat containing relatively small quantity of water and that of the smaller quantity of water remaining in cooked meat only a very small fraction had been expressed out from meat on application of mechanical force (pressing meat on filter paper). This is evident from the very slightly greater total area observed than the meat film area traced on the filter paper. However, the interaction conditioning × cooking was not significant (P>0.05) both for moisture content and WHC of either muscles.

In conclusion, the conventional Achilles tendon suspension exerted different restraining influence on thigh muscles resulting in contraction of SM and stretching of ST muscles whereas pelvic suspension stretched both muscles. The contraction or stretching of muscles marginally (P>0.05) affected cooking loss and WHC but significantly (P<0.001) affected the thermal shortening of muscles. Also, cooking with pressure induced greater thermal shrinkage than cooking without pressure. The interaction conditioning × cooking regarding thermal shortening was highly significant (P<0.01).

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Studies on SO_2 Treatment of Minced Goat Meat. II. Effect on Spoilage Bacteria and Some Food Pathogens

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The effect of SO_2 on different functional groups of organisms and some food pathogens was studied. *Lactobacilli* were affected to the maximum extent followed by coliforms, faecal coli, streptococci and salt tolerants (7.5% NaCl). Amongst the pathogens studied, *S. enteritidis* and *Y. enterocolitica* were killed. *S. aureus*, though found initially to undergo bactericidal action, appeared to overcome the detrimental action of SO_2.

Earlier studies[1] had indicated bacteriostatic action of SO_2 on spoilage microflora in general, resulting in delayed spoilage changes in minced goat meat. Further studies were, therefore, undertaken to study the action of SO_2 individually on different groups of bacteria: coliforms, faecal coli, faecal streptococci, lactobacilli, salt tolerants (7.5% NaCl),. aerobic mesophiles and psychrotrophs, Gram negative mesophiles and psychrotrophs, and pathogens like *salmonella, Yersinia* and *Staphylococcus*. Results are presented in this paper.

Materials and Methods

Effect of SO_2 on different functional groups of spoilage bacteria present in minced goat meat: Collection of the minced goat meat samples, their transportation and treatment with 450 ppm. of SO_2 were done as described earlier[1]. One set consisting of treated and untreated minced meat samples, collected from the same source at the same time was stored at 7°C and the other at 15°C. The samples from the former set were drawn on 0, 3, 5, 7, 9 and 11 days while from the latter on 0, 2, 4 and 6 days of storage and were processed for estimation of different groups of bacteria as summarised in Table 1.

Effect of SO_2 on survival and growth of certain food pathogens: Effect of 450 ppm of SO_2 on pathogens like enterotoxigenic *S. aureus* (enterotoxin A) in minced goat meat, *S. enteritidis* and *Y. enterocolitica* in nutrient broth (NB) at storage temperature of 16, 37 and 7°C respectively was studied. The cells of the respective organisms were harvested in sterile normal saline solution (NSS) from 18 hr growth culture on nutrient agar and were used to contaminate artificially minced meat or nutrient broth as the case may be. The SO_2 treated and untreated minced meat (collected at the same time from the same source) and the nutrient broth samples were suitably divided and inoculated separately with the cells of *S. aureus, S. enteritidis* and *Y. enterocolitica* to get concentrations of about 10^2, 10^3, 10^4 and 10^5 CFU/ml or g of the sample and were stored at appropriate temperatures as mentioned above. The samples from the inoculated substrates irrespective of the treatment were drawn on 2, 4, 6, 8 and 10 days of storage to estimate the number of pathogens as per the methods indicated in Table 1. The number of pathogens were also estimated on 0-day on an average within 2 hr of adding the inoculum to the substrates.

All the experiments were repeated thrice and the average values are reported. Wherever necessary, the data were subjected to appropriate statistical analysis[6].

Results and Discussion

The treatment of minced goat meat with 450 ppm of SO_2 and storage at 7°C adversely affected the growth of all groups of spoilage bacteria though to a varying extent; maximum effect was on lactobacilli. their

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TABLE 1. MEDIA, METHODS OF PLATING, QUANTITY OF INOCULUM USED AND INCUBATION TEMPERATURE AND TIME FOLLOWED TO STUDY THE GROWTH OF DIFFERENT GROUPS OF BACTERIA

Bacteria	Medium	Plating	Quantity of inoculum (ml)	Incubation		References
				temp. (°C)	time (hr)	
Mesophiles	SPC agar	Pour plate	1	30	48-72	2
Psychrotrophs	SPC agar	Pour plate	1	7	6-7 days	2
Gram negative (mesophiles)	CVTC	Pour plate	1	32	48	3
Gram negative (psychrotrophs)	CVTC	Pour plate	1	7	6-7 days	3
Lactobacilli	MRS agar	Surface spread	0.1	37	48	4
Salt tolerants	Carters'	Surface spread	0.1	37	48	5
S. aureus (fried egg appearance)	Carters'	Surface spread	0.1	37	48	5
Coliforms	Violet red bile agar	Pour plate	1	37	48	5
Faecal streptococci	Slanetz and Bartley's	Surface spread	0.1	37	48	5
S. enteritidis	Brilliant green agar	Surface spread	0.1	37	24-48	–
Y. enterocolitica	Modified McConkeys's lactose agar	Surface spread	0.1	37	32-96	–

CVTC: Crystal violet tetrazolium chloride agar.

count decreased significantly by the 9th day of storage (spoilage day) indicating their destruction probably due to SO_2 (Fig.1). The results are significant especially for vacuum packed meats since lactobacilli predominate in the spoilage of such foods. Coliforms, salt tolerants, faecal coli and streptococci more or less maintained their number without any significant change during storage (Fig.1). Coliforms, aerobic mesophiles and psychrotrophs, and Gram negative mesophiles and psychrotrophs revealed a significantly prolonged lag phase of growth as compared to that observed in untreated samples. It might be observed that lactobacilli, faecal coli and streptococci and salt tolerant bacteria form a part of the flora of aerobic mesophiles and psychrotrophs. Similarly, faecal coli constitute a portion of Gram negative mesophiles and to some extent of Gram negative psychrotrophs also. The results, therefore, indicate that unlike in untreated meat samples in which all the groups of bacteria were actively involved in causing spoilage (their count had revealed significant increase continuously throughout the spoilage period), in treated meat only a portion of the microflora was involved in causing the spoilage. It would, therefore, be interesting to pinpoint the species which would bring out the spoilage of the SO_2 treated meat stored at 7°C under different conditions of packing.

The antimicrobial action of SO_2 at 15°C was, in general, similar to that observed at 7°C except in case of lactobacilli, salt tolerant and faecal streptococci which revealed significant increase in their count though after a comparatively prolonged lag phase of growth.

On the pathogens, the SO_2 treatment appeared to have bactericidal effect in general and on *S. enteritidis* and *Y. enterocolitica* in particular (Fig.2). Bactericidal action was found to commence immediately especially in case of *S. aureus* and *Yersinia* as they revealed a significant reduction in their count within 3 hr (zero day) of inoculation. The reduction in count continued during storage till a stage of 'no-detection level' ($\lesssim$1 CFU/ml of NB or $\lesssim$100 CFU/g) of minced meat as per the method followed) was reached indicating bactericidal action of SO_2. This stage, in general, was observed to be reached earlier in samples with low initial number of *Salmonella* and *Yersinia* and continued till the end of the storage period. But in case of *S. aureus* no definite pattern was observed and the organisms appeared to overcome the effect of SO_2 as their count increased after the stage of no detection level (Fig.2).

No comparisons, however, could be drawn between the action of SO_2 on the three pathogens, since the menstrum (the substrate) in which the organisms were present and the temperature of incubation differed. Further detailed studies on the effect of SO_2 on pathogens under the conditions in which meat is packed and stored are necessary to evaluate bactericidal action of SO_2.

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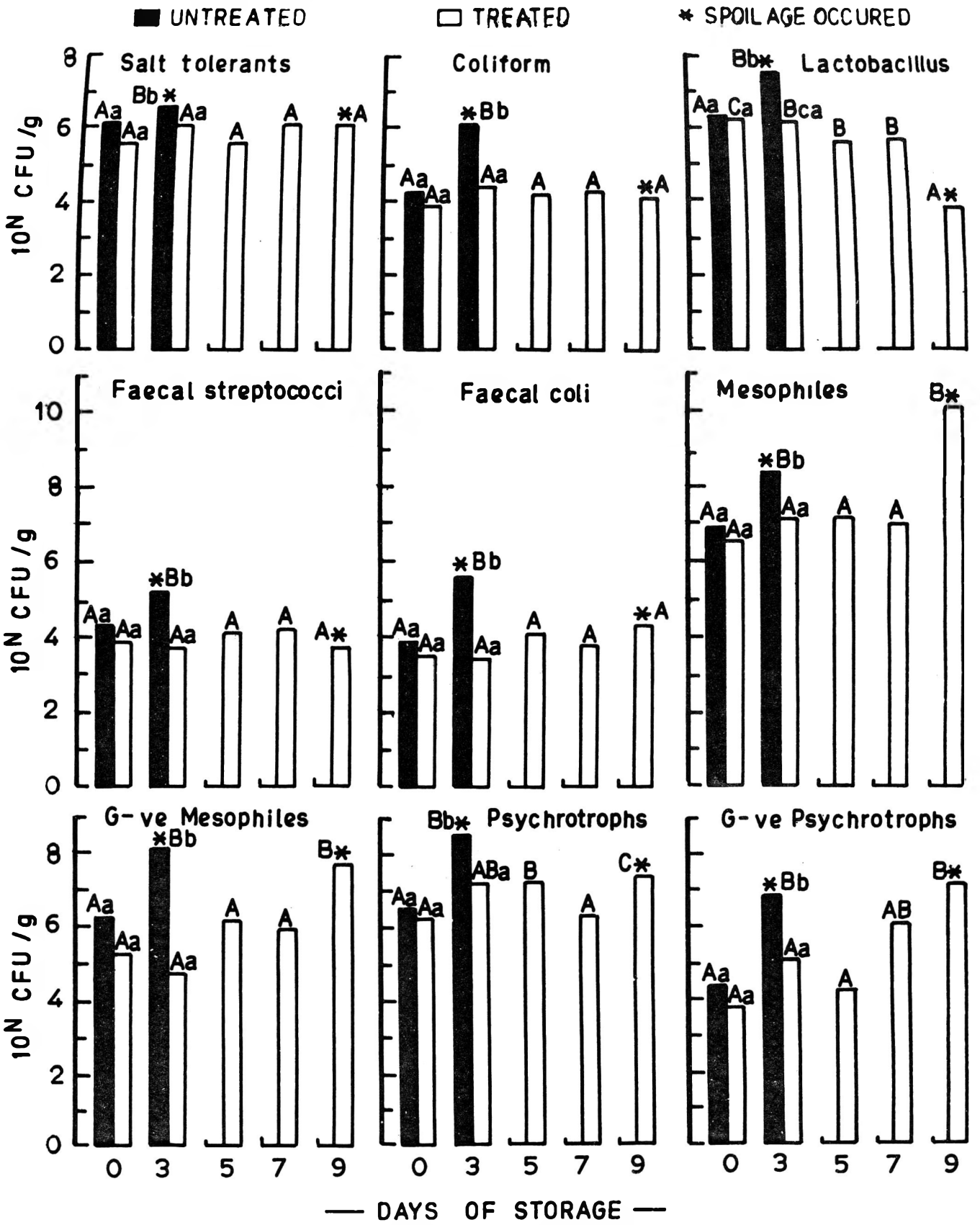

Fig. 1. Effect of SO_2 on different functional groups of bacteria in minced goat meat stored at 7°C.
Capital letters indicate comparison between days and small letters indicate between treatments. Figures with same superscript do not differ.

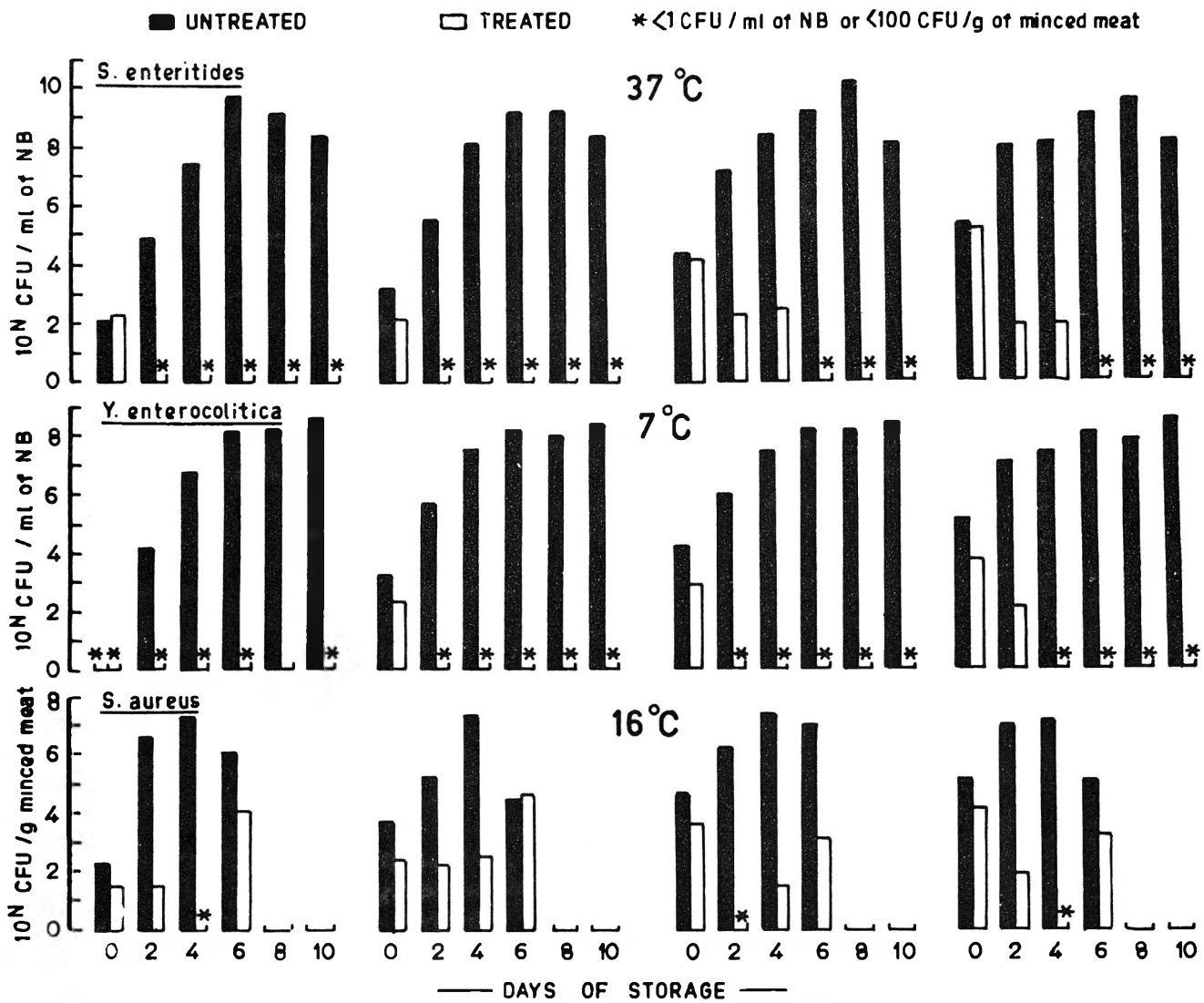

Fig. 2. Effect of 450 ppm SO_2 on survival and growth of food pathogens at different initial concentration levels.

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Composition and Digestibility of Fermented Fish Foods of Manipur

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The chemical composition, total bacterial counts and digestibility of Hentak (H) and Ngari (N), two fermented fish foods consumed in Manipur were determined. The compositions of Hentak and Ngari were: cholesterol, 2.67 and 8.37 mg/g; calcium, 12.60 and 6.88 mg/g; iron, 1.29 and 0.51 mg/g; and total viable bacterial counts, 4.8×10^8 and 5.0×10^7 cells/g respectively. Pepsin action for 2 hr followed by trypsin action for 20 hr *in vitro* digested 71.89 per cent and 82.28 per cent of total protein, liberating 5 and 4 essential amino acids from Hentak and Ngari respectively. Hentak appears to be a better food in view of its higher calcium, iron, essential amino acids, lipids and lower cholesterol content.

Many small sized freshwater fish are not readily acceptable to consumers because of low meat yield, bony nature and poor taste. Though, these trash fish are consumed by some people in the fresh condition, they are generally sun-dried. As the nutritive value of trash fish is as good as the popular table varieties, attempts have been made to prepare protein-rich products acceptable for consumption from such fish[1].

The technique of solid substrate fermentation of oriental foods for enhancement of flavour has been known for many centuries[2]. In India, though there are reports on ensiling of trash fish[3], literature on fermented fish for human consumption is lacking. 'Hentak' and 'Ngari' are fermented fish products widely consumed in Manipur. Hentak is a paste prepared by fermentation of a mixture of sun-dried fish powder and petioles of aroid plants. It is given in small amounts to mothers in confinement and patients in convalescence. Ngari is another fish product liked by the Manipuris because of its special flavour. It is a compulsory item in curry preparations. In the present paper, the proximate composition, total viable bacterial counts and *in vitro* digestibility of these fermented products are reported.

Materials and Methods

Sun-dried *Puntius sophore* (6.5 – 7.5 cm standard length) and *Esomus danricus* (5.0 – 6.0 cm standard length) were purchased from the Imphal market.

Preparation of 'Hentak': Sun-dried *E. danricus* were crushed to powder. Petioles of *Alocasia macrorhiza* were cut into pieces, washed with water and then exposed to sunlight for an hour. An equal weight of the cut pieces was then crushed with fish powder and then incubated at room temperature in earthen pots for 7 days. The paste, then ready for consumption was taken for analysis.

Preparation of 'Ngari': Sun-dried *P. sophore* were washed briefly with water and then spread on a tray and exposed to sunlight for about 1 hr. Fishes were blotted dry and then filled and pressed hard in an earthen pot. To the inner wall of the pot, a layer of mustard oil was applied before filling the fishes. The pot was sealed airtight and then stored at room temperature for 4 months. After the incubation, the lid was opened. The Ngari, which is then ready for consumption was taken for analysis.

Analyses: The methods of analyses for different parameters are given below. One gram sample was homogenized with 10 ml of distilled water and the pH determined by pH meter[4]; total ash, estimated by igniting the sample at 550°C in a muffle furnace[5]; water content by air oven method[6]; total N, by micro-Kjeldahl method[7]; non-protein N, by subtracting the value of protein N from total N; total lipid, by extraction with chloroform-methanol[8]; cholesterol, by colorimetric determination at 570 nm after treatment of the lipid with Libermann-Burchard reagent; total soluble sugar, by anthrone method[9] and total amino acids, by ninhydrin method[10]. Calcium was estimated by precipitating it in the form of oxalate and then titrating against potassium permanganate[11]; and iron content, by the modified method of Wong[12].

Total bacterial count: Total viable bacteria/gram wet weight of the samples were counted by plating in nutrient agar with tryptone[10].

Digestibility in vitro: The samples and 0.2 per cent pepsin solution were prepared according to AOAC[13] Six conical flasks were taken in each of which 1 g of powdered defatted sample, and 150 ml of pepsin

solution were added. The flasks were shaken well and then incubated at 45°C. At the end of 2 hr, all the flasks were removed. The contents of 3 flasks were filtered. To each of the remaining 3 flasks, 0.3 g of trypsin was added after adjusting the pH of the suspension to 8.2 by adding conc. NaOH. Incubation was continued at 45°C for another 20 hr and then filtered. During incubation, the contents were shaken every half an hour. Protein N was estimated from the residues separately after pepsin and after combined pepsin and trypsin actions. Values of digestible N were calculated by subtracting the values of N in the residue from those of the sample. Protein values were obtained by multiplying the corresponding values of N by 6.25. Digestible protein was expressed as percentage of total protein of the sample.

Amino acids liberated after pepsin and combined pepsin and trypsin actions were identified from the filtrates by two dimensional TLC[14]. Percentage of total amino acids liberated after 2, 4, 8, 12 and 22 hr of digestion were estimated by the ninhydrin method[10].

Results and Discussion

As seen from Table 1, Ngari and Hentak were nearly neutral. Ash, iron and calcium contents of Hentak were much higher. Incorporation of *Alocasia* in the preparation of Hentak may be responsible for these higher values. Inclusion of both scales and bones in the processing also accounts for the higher calcium contents in both the preparations. Lipid contents were similar; however, cholesterol content in Ngari was much higher. Total amino acid and soluble sugar contents of the 2 preparations were comparable. Moisture values were low which make them suitable substrates for solid state fermentation. Viable bacterial load was more in Hentak because of the aerobic condition which was not the case with Ngari.

TABLE 1. CHEMICAL AND MICROBIOLOGICAL ANALYSES OF HENTAK AND NGARI (ON WET WEIGHT BASIS)*

	Hentak	Ngari
pH	6.7 ± 0.50	7.50 ± 0.34
Ash (%)	11.43 ± 0.30	5.49 ± 0.86
Water content (%)	36.30 ± 1.00	36.03 ± 0.23
Total N (%)	5.34 ± 0.18	6.14 ± 0.03
Non protein N (%)	1.32 ± 0.01	3.60 ± 0.12
Total lipids (%)	13.60 ± 1.00	13.34 ± 1.49
Cholesterol (mg/g)	2.67 ± 0.07	8.37 ± 0.27
Total soluble sugars (mg/g)	1.53 ± 0.07	1.12 ± 0.12
Total amino acids (mg/g) (expressed as glycine)	6.00 ± 1.60	8.05 ± 0.20
Calcium (mg/g)	12.60 ± 0.97	6.88 ± 0.25
Iron (mg/g)	1.29 ± 0.07	0.51 ± 0.07
Total viable bacterial count/g	4.8×10^8	5.0×10^7

*Mean ± S.D. of six determinations

TABLE 2. PERCENTAGE OF DIGESTIBLE PROTEIN

	Total protein (N × 6.25) (%)	Digestibility of protein (%)	
		Pepsin action	Pepsin + trypsin action
Hentak	25.13	46.82	71.89
Ngari	15.88	56.36	82.28

Average of three determinations (3 samples)

Very little portion of protein was hydrolysed by pepsin action for 2 hr in both the preparations. Trypsin could digest larger amount of protein (Table 2). There was a gradual rise in the amino acid liberation during trypsin action (Table 3). Four essential amino acids, viz., lysine, phenylalanine, leucine and threonine were released from Ngari and five, viz., lysine, phenylalanine, leucine, valine and methionine were released from Hentak during enzyme action on the protein.

In the Orient, there has been a clear understanding that processes closely allied to putrefaction and biodegradation can be converted into palatable and nutritious food by selecting appropriate conditions and exercising proper control[15]. Hentak and Ngari are the foods of such type which are used as condiments along with monotonous rice dishes as it is practised widely in South East Asia.[16] Though, various microorganisms in foods are assumed harmful, Moo-Young *et al.*,[2] described the use of yeasts and filamentous fungi in the preparation of oriental foods.

The traditional preference of Hentak to Ngari in supplementing the diet of patients is justly supported by the finding that the former has higher iron, calcium, essential amino acids and lipids with lower cholestrol level and also a desirable flavour. However, nutritive status of the foods with the added aroid plant with high calcium oxalate content needs further investigation. Also, identification of microorganisms including saprophytic fungi is essential as some of

TABLE 3. TOTAL AMINO ACIDS LIBERATED ON PEPSIN FOLLOWED BY TRYPSIN DIGESTION

	Amino acids liberated at indicated time (hr) of digestion				
	2	4	8	12	22
Hentak	0.6 ± 0.16	0.7 ± 0.00	1.4 ± 0.02	1.4 ± 0.0	2.11 ± 0.00
Ngari	0.8 ± 0.02	1.8 ± 0.02	2.1 ± 0.11	2.5 ± 0.0	3.53 ± 0.14

% of net wt.

them have been found to produce toxins harmful to man.

Acknowledgement

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Studies on Microencapsulation of Cardamom Oil by Spray Drying Technique

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The characteristics of the spray dried microcapsules of cardamom oil using gum acacia, dextrin white and maltodextrin as wall materials have been determined by chemical analysis and scanning electron microscopy. It was seen that while gum acacia gave spherical capsules with dents but better oil retention, less surface oil and practically no cracks, maltodextrin gave slightly more roundish capsules and had cracks on the surface. Various combinations of the two wall materials have also been tried of which the combination of maltodextrin and gum acacia in the ratio 30:70 gave the best quality capsules.

Encapsulation can be defined as the technique of packing minute particles of a core material within a continuous polymer film, which is designed to release its contents in a predictable manner under a predetermined set of conditions[1]. When the particle size of the capsule is less than 5000 μm, they are generally classified as microcapsules and the process microencapsulation[2].

In flavour industry, encapsulation is the most popular modern technique of converting a volatile aroma concentrate to a stable powder form. A number of methods are reported for microencapsulation of flavours but the most popular technique employed in industry is spray drying.

Though, literature available on microencapsulation of flavour in general is adequate, there is dearth of data[3] on microencapsulation with special reference to spice oils. Some studies carried out on microencapsulation of cardamom oil by spray drying technique with gum acacia and starch derivatives as wall materials are reported in this paper.

Materials and Methods

The cardamom oil was obtained in the laboratory by steam distillation of cardamom grown in Idukki District of Kerala State. In all cases, pure samples of gums or carbohydrate derivatives were used. Gum acacia was supplied by SISCO, Bombay, dextrin white by Romali, Austria and matltodextrin by Spectrochem. Bombay. Tween-80 (poly oxyethylene sorbitan mono oleate) was obtained from Loba Chemie, Bombay.

Emulsion preparation: Wall materials or their suitable blends were hydrated for 24 hr prior to spray drying. The gum solutions were prepared in warm (50-60°C) water to aid dispersion and if necessary, blended for 1 min in a laboratory blender to get a uniform dispersion. Cardamom oil was added and again blended for 1 min. After the preliminary standardisation experiments, the wall material to oil ratio was kept at 4:1. The globule size of the emulsion was found to be less than 2 μm.

Spray drying: The blended emulsion was spray dried without delay in a Buchi 190 mini spray drier, equipped with a nozzle of 0.5 mm diameter and with inside chamber dimensions of 44 cm height and 10.5 cm diameter. Pumpable atomisable viscosity in this spray drier was found to be 90 ± 5cps (30°C) by trial experiments. Drying conditions were standardised at an inlet temperature of 155 ± 5°C and an exit air of 100 ± 5°C with an atomisation pressure of 6 atmospheres. Infeed material was introduced with stirring to the drier at room temperature. Under these conditions, the spray drier was found to evaporate about 0.25 kg of water per hr.

Viscosity measurement: Viscosity of aqueous solutions of the different wall materials was determined using Redwood viscometer.

Dextrose equivalent: Reducing sugar content was determined by Shaeffer – Somogyi method[4] and expressed as dextrose equivalent (DE).

Scanning electron microscopy: Particle size and nature of the capsule surface were examined in a Jeol-JSM 35 C model scanning electron microscope after the products were sputtered with gold. Dents were clearly visible at 1800 magnification while cracks could be detected only when the magnification was more than 3000.

Optical microscopy: The size of the dispersed

phase droplets in the emulsion was determined in a Carl-Zeiss Jena-Ergaval biological microscope.*

Oil analysis: The volatile oil content in the emulsion and in the dried product was estimated by Clevenger distillation method[4]. The chemical composition of the oil samples was examined with a Hewlett Packard 5840A model gas liquid chromatograph; the conditions were column OV-17; 6 ft detector FID, temperature programme, 80-5-200°C.

For surface oil determination, a known weight of the product was stirred for 10 min without destruction of capsules with petroleum ether (60-80°C), solvent decanted and residue was dried. The difference in volatile oil content before and after washing with petroleum ether was taken as content of surface oil.

Moisture estimation: Moisture was determined by the toluene distillation method[4].

Results and Discussion

One of the main constraints of spray drying involving a gum or starch material is the limitation imposed by high viscosity and consequent difficulty to pump it through the nozzle. Several gums like guar gum, gum tragacanth, gelatin, sodium alginate and modified carbohydrates like carboxy methyl cellulose and hydroxy propyl methyl cellulose showed viscosity of about 300 cps with solutions of 1 to 3 per cent concentration and hence were not acceptable as wall material. Only gum acacia, dextrin white and maltodextrin showed promise as high solids carriers. Table 1 represents the viscosity and ease of pumping of these wall materials with the 0.5 mm nozzle used. It can be seen that in the case of gum acacia, a concentration up to 26 per cent could be used. In the case of dextrin white and maltodextrin, the highest concentration that could be used was 21 and 36 per cent respectively. The dextrose equivalent (DE) of dextrin white was 2.3 and that of maltodextrin was 19.8.

According to theories on flavour retention during spray drying, volatiles are lost only until the drying droplet forms a semipermeable film[5]. The higher the solids content in the infeed emulsion, shorter the time required for film formation. The highest solids levels used are generally those that can still be pumped and atomised[6]. However, it has also been reported that each encapsulant has a characteristic optimum infeed concentration for maximum retention[7].

With a view to reducing viscosity, addition of Tween-80 upto a level of 0.8 per cent was tried; reduction of neither the globule size nor viscosity was noticed. However, in the case of 100 per cent maltodextrin as wall material, addition of Tween-80 was found to be beneficial to get a stable emulsion (Fig. 1a). The results of oil retention in the case of both gum acacia and maltodextrin showed practically no difference with different wall material to oil ratios and hence a ratio of 4:1 was followed in subsequent experiments. The generally recommended[6,8] ratio of wall material to oil for encapsulation of other flavours also is 4:1.

The scanning electron micrographs of the microcapsules obtained with different wall materials and cardamom oil are shown in Fig. 1 to 4. Maltodextrin while giving a more spherical shape with

TABLE 1. VISCOSITY BEHAVIOUR OF THE SELECTED WALL MATERIALS

Wall material	% solids in water	Viscosity (cps)
Gum acacia	23	48*
	26	87+
	30	305Y
Dextrin white	17	42*
	21	85+
	26	145Y
Maltodextrin	26	20*
	31	52*
	36	93+
	40	NCS

Values are means of 3 replications
*easily pumpable; + pumpable Y not pumpable
NCS: Not completely soluble

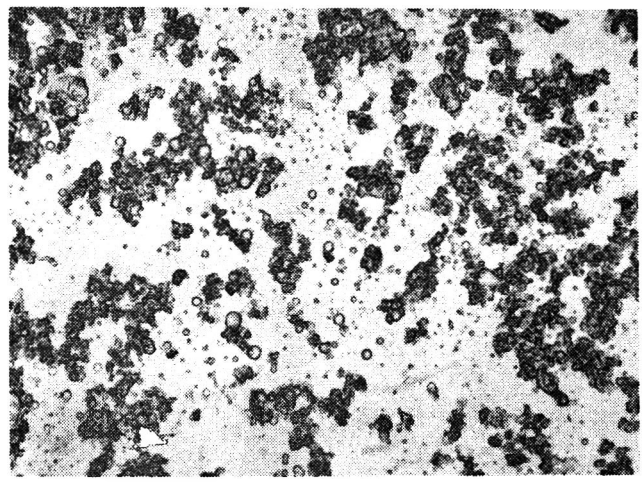

Fig. 1a: Photomicrograph of cardamom oil droplets dispersed in maltodextrin solution.

*Photomicrograph of the dispersed oil droplets was taken in a Nikon 'Optiphot' model biological microscope.

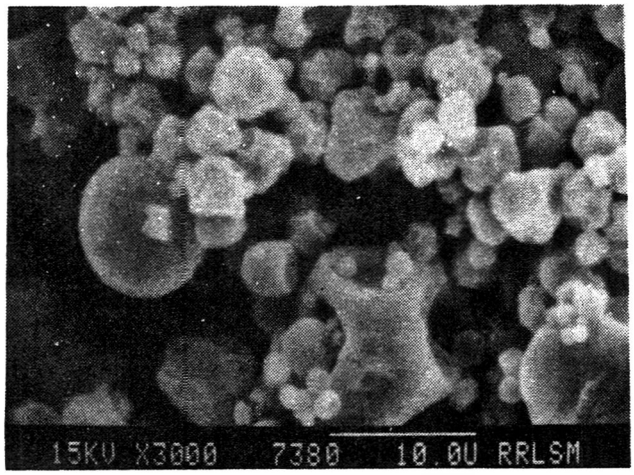

Fig. 1: SEM of microcapsules with gum acacia as wall material. The surfaces are free of cracks but characterised by dents.

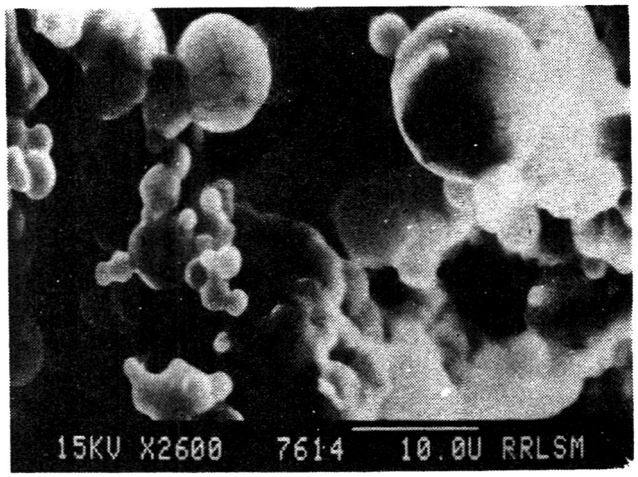

Fig. 3: SEM of microcapsules with maltodextrin as wall material. The surfaces are free of dents but have cracks and capsules are spherical.

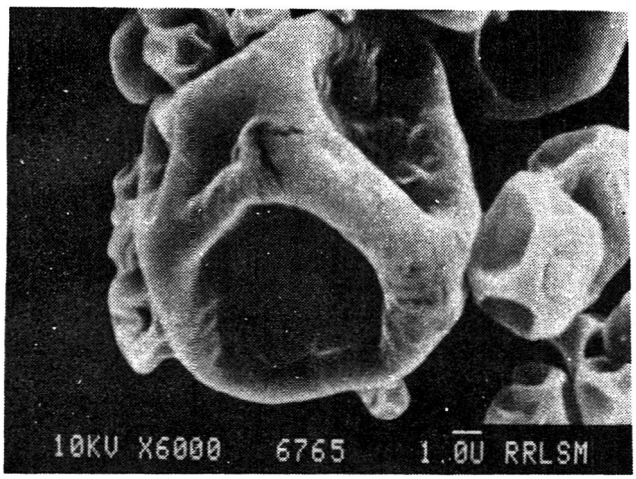

Fig. 2: SEM of microcapsules with dextrin white as wall material. Deep dents and cracks are visible on the surface.

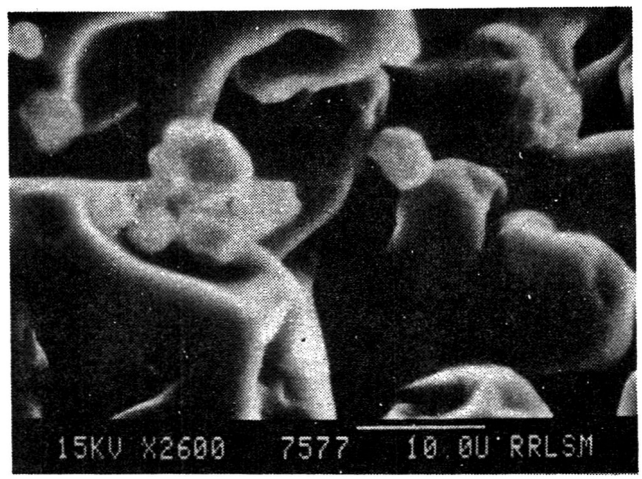

Fig. 4: SEM of microcapsules with gum acacia-maltodextrin mixture (1:1) as wall material. the surfaces have no cracks but have dents.

minimum dents, showed a tendency for cracks to develop under the experimental conditions used. When gum acacia and maltodextrin were used in equal proportions, cracks were not seen. The development of cracks could also be due to the drying conditions employed especially the rate of drying. However,

TABLE 2. OIL RETENTION CHARACTERISTICS AND PARTICLE SIZE RANGE IN MICROCAPSULES

Wall material		Wall material	% oil retention		GC data of recovered oils				Particle size range of micro capsules
					Emulsion		Product		
Gum acacia (%)	Malto-dextrin (%)	(g per 100g of solution) (g)	Emulsion	Product	Cineole (%)	Terpenyl acetate (%)	Cineole (%)	Terpenyl acetate (%)	
100	0	26	87.5	65.4	30.2	42.0	25.5	43.5	1 – 18
70	30	33	84.2	68.3	29.7	43.4	24.3	44.9	1.2 – 16
60	40	33	84.7	68.6	30.6	42.5	25.9	46.1	1.5 – 25
50	50	33	90.2	60.2	30.6	42.0	21.0	47.3	0.9 – 20
40	60	33	90.2	61.1	31.0	40.7	27.0	42.0	1.1 – 22
30	70	33	91.1	57.9	27.3	43.1	26.5	45.9	0.8 – 19
0	100	36	87.5	39.2	27.4	40.7	18.7	48.9	1 – 28

Composition of cardamom oil: cineole 31.2%; terpenyl acetate 42.2%
The data are the average of three replications.

TABLE 3. MOISTURE AND SURFACE OIL IN MICROCAPSULES

Wall material					
Gum acacia (%)	Malto-dextrin (%)	Moisture (%)	Oil in capsules (% v/w)	Surface oil (% v/w)	Encapsu-lated oil (% v/w)
100	0	5.5	16.6	1.8	14.8
70	30	5.2	16.6	3.5	13.1
60	40	4.8	16.8	4.1	12.7
50	50	5.0	14.5	4.7	9.8
40	60	4.4	14.5	5.3	9.2
30	70	4.0	13.5	6.0	7.5
0	100	4.2	9.0	6.6	2.4

Values are the means of three replications

maltodextrin showed an increasing tendency for the development of cracks with increase in concentration even in combination with gum acacia upto 60 per cent level in the mixture. More roundish shape and less of dents tend to give maltodextrin capsules more free-flowing property[9].

The particle size showed a range of 2 to 25 μm and the size appeared to be higher in the case of maltodextrin (Table 2). All the powders generally showed a moisture content of 4 to 5.5 per cent (Table 3). Despite the relatively low moisture content, the product was not very free-flowing. Use of anticaking agent[10] and granulation technique[11] are known to improve free-flowing properties.

The retention of oil in the emulsion in both gum acacia and maltodextrin was fairly high in the range of 85 to 90 per cent (Table 2). However, in the final product, gum acacia showed a clear superiority in oil retention. Dextrin white gave results almost similar to maltodextrin both in retention of oil and characteristics of microcapsules. Because of the fact that maltodextrin allowed a solution strength of 36 per cent with satisfactory pumpable viscosity as against only 21 per cent in the case of dextrin white, in experiments using combination of starch derivative and gum acacia, only maltodextrin was used. Five different combinations were tried with a view to use higher solids content and thereby obtain quality microcapsules without cracks and maximum retention of flavour. Use of gum acacia with maltodextrin upto 30 per cent level showed an improvement of 18 per cent in the retention of oil. The maximum retention of oil was observed with two different combinations of maltodextrin and gum acacia i.e., 30 to 70 and 40 to 60 as shown in Table 2.

Gas chromatographic examination of the oil recovered from the emulsion and the spray dried microcapsules revealed patterns essentially similar to the original oil. (Fig. 5 to 7). However, a marginally

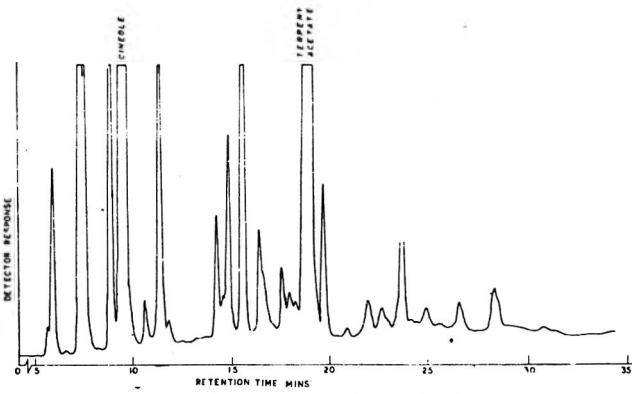

Fig. 5: GC pattern of cardamom oil.

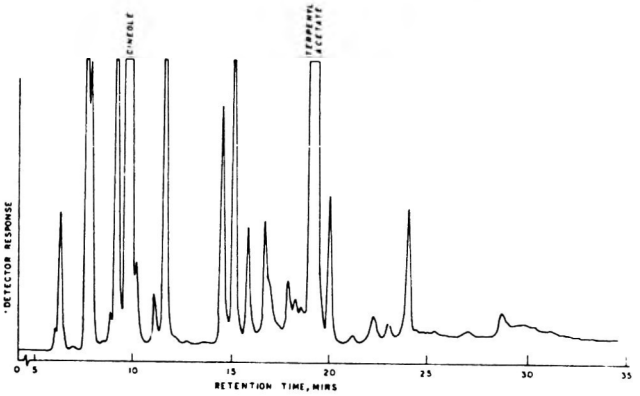

Fig. 6: GC pattern of cardamom oil recovered from emulsion. Wall material: Gum acacia – maltodextrin (1:1).

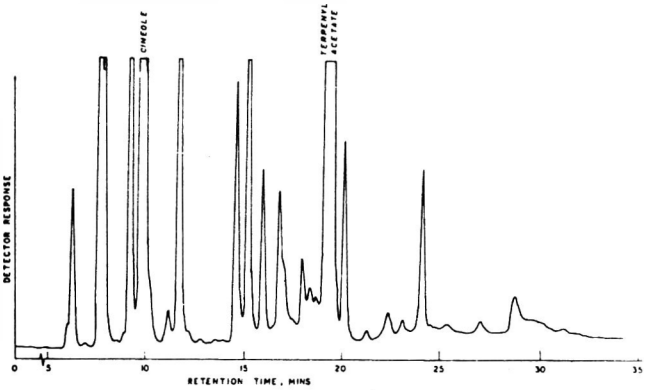

Fig. 7: GC pattern of cardamom oil recovered from microcapsules. Wall material: Gum acacia – maltodextrin (1:1).

higher retention of terpenylacetate and lower retention of cineole have been indicated (Table 2). It may be remembered that of the two constituents, cineole is more volatile. The above observation can be explained only on the basis of a higher evaporation loss in the early phase of spray drying before the membrane effect of dried wall material could come into effect. It may also be noted that cineole has a lower molecular weight than terpenylacetate and thereby relatively decreasing its chance for retention by the membrane effect of the wall material after drying as well.

In ideal encapsulation, the entire oil will be within the wall. Any oil on the surface of the capsule is liable to be evaporated and subjected to the action of atmospheric oxygen. Washing of the surface with a non-polar solvent like petroleum ether is recommended as a procedure for removing the surface oil[10,12]. More surface oil is noticed in the case of maltodextrin compared to gum acacia (Table 3). Part of the surface oil so accounted may be due to extraction of the oil from inside the capsule through cracks. Inefficiency of maltodextrin as an encapsulating matrix has been reported in the case of other flavours also[13,14].

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The Use of Gamma Irradiation for Improving Microbiological Qualities of Spices

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The ability of gamma irradiation in reducing microbial contamination was investigated in turmeric, black pepper and chilli. The initial total plate count varied from 10^4 – 10^6, coliform count from 10^2 – 10^5, spores from 10^2 – 10^5 and yeast and moulds from 10^2 – 10^3 per gram of the sample. Treatment with γ–rays (^{60}Co) at dose levels of 1-10 kGy were studied. From the data, it was concluded that dose levels of 4-5 kGy were sufficient to reduce the bioburden to acceptable levels.

Spices constitute an important group of agricultural commodities which are virtually indispensable in the culinary art all over the world. The meat industry and soup manufacturers use the largest quantities of spices[1]. There has been increasing demand around the world for spices during the last few years because of the increased production of convenience foods. Production of most of the spices is restricted to a few developing countries. They play an important role in their national economy. Presently, India contributes to 20-30 per cent of the world trade.

However, a common problem faced by both importing and exporting countries is that spices are often heavily contaminated with microorganisms which form a major source of contamination in the food products to which they are added. Excessive numbers of aerobic mesophilic spores, mesophilic anaerobes and flat sour thermophiles, introduced through spices into foods that are subsequently canned, have been responsible for their spoilage[2]. Spices have been incriminated for softening of dill pickles and spiced fruit in acidified syrup[3]. Palumbo *et al*[4]. observed that quality of processed meats get adversely affected by the bacteria and moulds introduced through spices. Even when probability of contaminated spice causing spoilage is low, it can certainly introduce members of micro-organisms which are important from public health point of view[5]. The presence of varying numbers of *Penicillium* species, *Aspergillus flavus* and *A. glaucus* in spices has caused concern over the possibility of the occurrence of aflatoxin[6].

A number of methods have been tried to reduce the microbial load in spices to acceptable levels, such as heating[7], fumigation with ethylene oxide or propylene oxide[8] and ethanol vapour[9]. However, these suffer from disadvantages like loss of flavour or of toxic effect of the chemical. Therefore, the present trend in the international scene is to switch over to gamma irradiation. As the process does not increase the temperature of the product, the losses in the volatile oil of spices[10] are very low. The results presented here show the effect of γ–irradiation on different types of microbes in turmeric powder, black pepper and chilli powder and the practical dose levels needed for spices to be used in convenience foods.

Materials and Methods

Spice samples: Black pepper (whole), (*Piper nigrum*), turmeric (*Curcuma longa*) and chilli (*Capsicum*) (in powdered form) were procured from the local market. They were distributed in 200 g samples in high density polyethylene pouches (400 gauge).

Irradiation: The sealed bags of spices were irradiated at Bhabha Atomic Research Centre, Bombay, India at room temperature (30°C) under ambient conditions. Irradiation doses selected for evaluation were 1, 2, 3, 4, 5, 7.5 and 10 kGy delivered from a ^{60}Co source. Unirradiated spices served as control.

Microbiological analysis: Spice samples were ground aseptically using sterile mortar and pestle and physiological saline containing 0.1 per cent peptone as the diluent. Samples were analysed in duplicate for total plate count, mesophilic and thermophilic spores, coliforms, and yeasts and moulds as per standard methods[11]. Dextrose Tryptone Agar (DTA) was used

*Bhabha Atomic Research Centre, Bombay.

for enumeration of total plate counts and spores, and Violet Red Bile Agar (VRBA) for coliforms[12]. Yeast and mould counts were observed on Potato Dextrose Agar (PDA) supplemented with 100 ppm chloramphenicol to inhibit bacteria[13]. Media materials used were of Bacteriological/Analar grade.

Results and Discussion

Microbial contamination of spices: The microbial profile of turmeric, black pepper and chilli were as follows. Total plate counts varied from 5.91 to 6.11 logs for turmeric, 4.95 – 6.87 logs for black pepper and 5.66 – 6.25 logs per g for chilli. Coliform counts were lowest (2.36 logs/g) in chilli, whereas pepper and turmeric showed the counts in range of 4.78 – 5.18 logs per g. Yeasts and moulds contamination varied from 3.25 – 3.49 logs per g in all the three spices. Mesophilic and thermophilic spores were 2.48 – 5.18 logs and 1.7 – 4.7 logs per g respectively in pepper. Turmeric powder harboured 4.48 – 4.75 logs spores whereas in chilli powder it ranged from 2.11 – 4.71 logs per g. The moisture content in samples ranged from 6.5 – 7.0; 8.2 – 8.3 and 9.92 per cent in black pepper, chilli powder and turmeric powder, respectively.

The variation in contamination level of spices may be due to various reasons. It can be attributed to the variation in micro-organisms indigenous to the spice bearing plants and soil where they are grown, added with post harvest contamination from dust, non-potable water and also with the methods of harvesting and processing[14,15]. Since coliforms are generally short-lived in the atmosphere, the high counts in black pepper and turmeric powder indicate contamination of recent origin. The fungal contamination is often reported as incidental or as a result of growth during drying and storage[16].

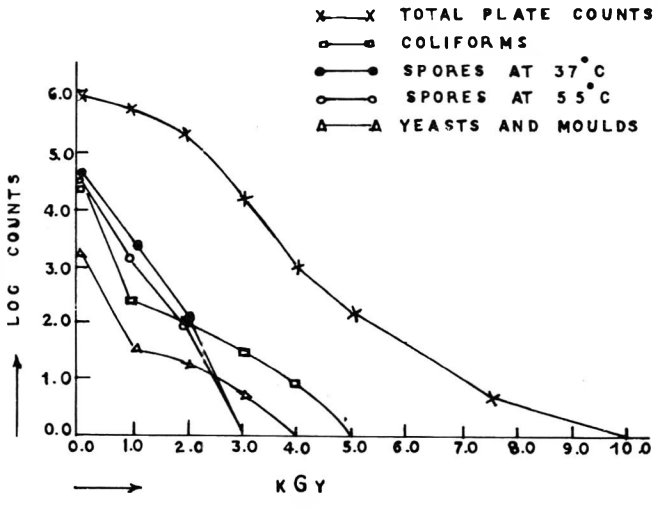

Fig. 1. Effect of irradiation on microflora of turmeric powder.

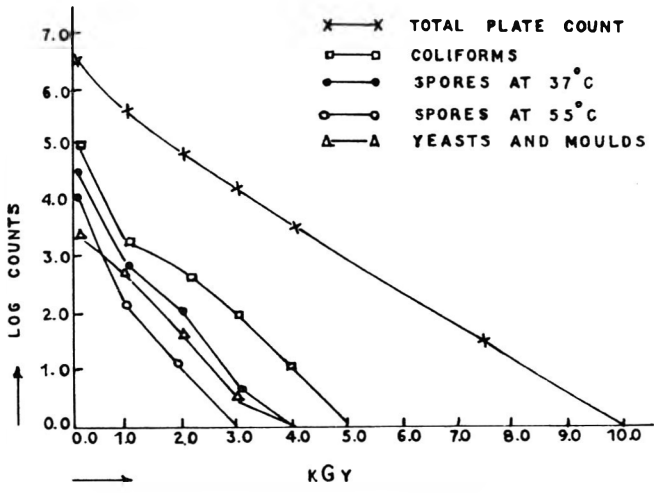

Fig. 2. Effect of irradiation on microflora of black pepper.

Radiation survival of microflora: The effect of irradiation on microflora of spices are shown in Fig. 1, 2 and 3. The survival curves are distinct and characteristic for each type of microflora. Both mesophilic and thermophilic spore formers yielded straight line inactivation curves in turmeric (Fig. 1). Total plate counts and coliform counts showed biphasic, and yeast and moulds triphasic survival curves during irradiation. Complete removal of spores, yeasts and moulds, coliforms and total bacterial count was observed at irradiation doses of 3.0, 4.0, 5.0 and 10 kGy, respectively.

In black pepper, microflora showed almost straight line survival curve excepting for coliforms which tended to show a biphasic curve (Fig. 2). Irradiation doses of 3, 4 and 5 kGy have been able to take care of thermophilic spore formers, mesophilic spore formers and yeasts and moulds and coliforms respectively. Total plate counts required 10 kGy for their complete elimination. Total bacterial counts of chilli powder showed nil counts at 7.5 kGy and other micro-organisms showed inactivation at 3.0 kGy (Fig 3). Survival curve

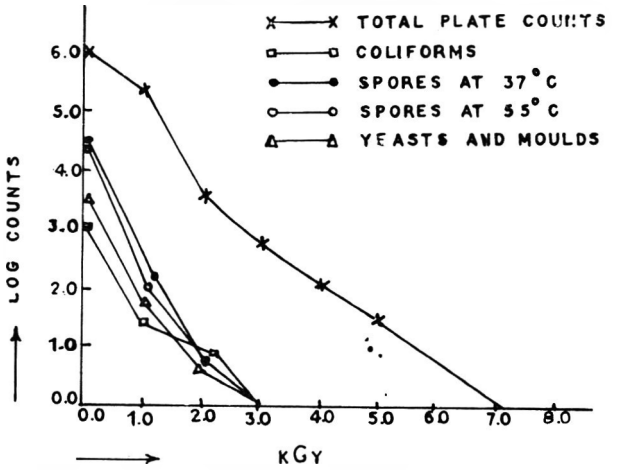

Fig. 3. Effect of irradiation on microflora of chilli powder.

TABLE 1. D_{10} VALUES* OF MICROFLORA IN SPICES

Part survival curve	Total plate count	Coliforms	Spores		Yeast & Moulds
			37°C	55°C	
		Turmeric			
A	3.22	0.41	0.65	0.65	0.55
B	1.15	1.21			5.0
					3.2
Overall	1.44	1.04	0.65	0.65	1.23
		Black pepper			
A	1.53	0.52	0.85	0.70	1.14
B		1.22			
Overall	1.53	0.96	0.85	0.70	1.14
		Chilli			
A	1.47	0.61	0.68	0.70	1.11
B	0.55	2.00			1.44
C	1.56	1.00			
Overall	1.25	1.00	0.68	0.70	1.21

*Values calculated from Fig. 1-3

for total bacterial counts and coliforms have been triphasic and for yeasts and moulds biphasic. However, as in other spices, both types of spore formers showed straight line survival curves as a result of irradiation treatment.

D_{10} values for microflora of spices showed that they ranged from 1.25 – 1.53 kGy for total bacterial counts, 1.14 – 1.23 kGy for yeasts and moulds and 0.96 to 1.04 kGy for coliforms (Table 1). Spores which are known to have the highest resistance for other treatments such as high temperature, showed highest sensitivity for irradiation in spices with their D_{10} values varying from 0.65 – 0.85 kGy. This finding is rather surprising and the least expected. It does not fall in line with the observations made by other workers[17] who report that D_{10} value equal to or more than 1 kGy is indicative of spore formers and only exceptional vegetative forms or moulds have values higher than 1 kGy[18]. Grecz *et al.*[19] showed that for bread, the decimal reduction for spores was in the range of 1.1 – 1.5 kGy while for total plate count the value was 0.7 – 0.8 kGy. The contrasting observation in the present experiments may be perhaps due to the difference in the types of contaminating microbes and their previous history. However, at the moment it is rather difficult to conclude any specific reason for it. There are some isolated reports in literature[18] which mention about highly radiation sensitive spores and highly resistant vegetative cells which strengthen our observations.

The biphasic and triphasic survival curves observed for total plate counts, coliforms, and yeasts and moulds indicate the heterogencity of population with respect to irradiation sensitivity. For instance, in case of coliforms, the population in portion A of the curve shows D_{10} values ranging from 0.41 – 0.61 kGy which show high sensitivity of this population to irradiation as compared to the rest of the population showing D_{10} of 1.00 – 2.00 kGy. The spore forming bacteria uniformly represented straight line curves indicating homogeneous population as far as irradiation sensitivity is concerned.

From the data presented above, it can be concluded that irradiation treatment at 4-5 kGy is capable of killing most of the micro-organisms in spices especially the spore formers which are mainly responsible for spoilage of thermal processed foods. Hence, this dose is enough in practice to decontaminate spices.

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EFFECT OF VARIOUS GRAIN PARAMETERS ON POPPING QUALITY OF SORGHUM

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Nineteen cultivars of grain sorghum (*Sorghum bicolor*) of kharif (July-October) harvest were assessed for their physical characteristics and their relationship with popping quality. The results indicated that there were significant genotypic differences in kernel weight, kernel volume, bulk density, true density, seed hardness, floaters percentage, seed moisture, volume of pop, expansion ratio and popping percentage. Expansion ratio had significant positive effect due to seed hardness and bulk density, whereas, seed hardness had strong positive correlation with pop yield. Pop volume did not show any relationsip with any of the physical parameters tested.

Sorghum (*Sorghum bicolor* L.) grains are consumed in different forms in India, namely unleavened bread, dumpling and boiled rice like products[1]. Popped sorghum is a very popular, traditional snack food in central India[2]. Puffed cereals mixed with oil and spiced or sweetened are popular snack foods[3]. Popping improves the flavour and modifies texture[4]. Good popping sorghum grains will be small in size, have medium thick pericarp, hard endosperm and a very low germ/endosperm ratio[5]. Popped sorghum grains can be used in weaning food formulations[6]. There is a large variation among sorghum cultivars for popping characteristics[7,8]. In our study, grains of different cultivars of sorghum were evaluated for their popping quality as affected by their physical characters.

Sorghum grains of Nineteen cultivars as listed were selected for the study. The seeds of kharif (July-October) harvest were obtained from the Sorghum Research Station, Marathwada Agricultural University, Parbhani. After cleaning, the grains of each cultivar were evaluated for various quality parameters such as thousand kernel weight, bulk density, true density, per cent floaters[5] and moisture content[9]. Seed hardness was measured by Hardson's seed hardness tester at their original moisture content and colour by munsel colour charts[10]. Popping was performed at 18 per cent moisture and 240 ± 5°C temperature[7].

The popped samples were evaluated for per cent popping and expansion ratio[11]. One thousand pop volume was measured by randomly selecting 1000 pops from the bulk of the popped kernels for each cultivar. Statistical analysis of data was performed by using completely randomized design with 3 replications[12] Relationship of physical parameters with per cent popping and expansion ratio was assessed by multiple regression equations using least square technique[13] The partial regression coefficients (Bi) were determined by using formula.

$$Bi = bi \frac{Sxi}{Syi}$$

Where,
bi = Regression coefficients
Sxi = Standard deviations of independent variables xi
Syi = Standard deviations of dependent variables yi

There were significant genotypic differences in all the characters studied (Table 1). This indicates the presence of variability amongst the genotypes in these characters[1,5] 'CSH-1' kernels had highest bulk volume followed by 'HES-11', 'HES-9' and 'HES-7' However, true volume was highest in 'HES-12' followed by 'HES-9' and 'HES-7'. The ranges of bulk density and true density were 0.61 to 0.79 and 1.14 to 1.44, respectively. Seed hardness was highest in 'CSH-9' and lowest in 'HES-11'. Seed hardness was inversely proportional to per cent floaters. Moisture content in the seed of all the genotypes was in the range 9.5 to 12.0 per cent. Most of the genotypes were yellowish except 'HES-11' which was red and 'HES-12' which was white.

Maximum volume of one thousand pops was obtained from 'HES-6' (865 ml) followed by 'CSH-9' (750 ml) and 'HES-9' (710 ml) and lowest volume was observed for 'HES-5' (400 ml). Highest expansion ratio was observed in 'HES-6' cultivar and popping percentage was highest in 'HES-5' followed by 'CSH-6' and 'CHS-9'. Most of the genotypes which exhibited higher breaking strength, gave good expansion ratio and pop yield[1].

The results of simple correlation (Table 2) revealed that 1000 kernel weight, bulk and true kernel volume and per cent floaters had strong negative significant

TABLE 1. PHYSICAL PARAMETERS AND POPPING QUALITY OF SORGHUM GRAINS

Cultivar	1000 kernel Weight (g)	1000 kernel Bulk vol. (ml)	1000 kernel True vol. (ml)	Bulk density (g/ml)	True density (g/ml)	Seed hardness (kg)	Floaters (%)	Expansion ratio	Popping (%)
HES-1	22.97	29.0	18.0	0.79	1.28	6.25	20	19.8	88
HES-2	21.39	34.0	18.0	0.63	1.19	6.75	34	18.2	87
HES-3	27.47	38.0	21.5	0.72	1.28	5.75	31	17.0	77
HES-4	25.34	41.5	22.2	0.61	1.14	6.00	46	14.6	90
HES-5	14.11	18.0	10.9	0.78	1.30	5.75	8	22.2	98
HES-6	27.16	36.0	21.5	0.75	1.26	5.50	31	24.0	62
HES-7	31.20	43.1	25.7	0.73	1.21	5.50	34	10.7	53
HES-8	26.43	36.5	21.7	0.72	1.22	5.50	23	15.1	53
HES-9	30.84	43.5	26.0	0.71	1.23	4.82	55	16.3	55
HES-10	25.96	39.0	20.5	0.67	1.27	6.00	51	15.4	77
HES-11	29.54	45.0	24.5	0.66	1.21	4.00	100	9.3	46
HES-12	31.95	38.0	26.5	0.65	1.17	4.25	96	14.6	91
NSS-1	25.20	36.5	20.0	0.69	1.26	6.00	14	13.7	63
NSS-2	25.02	35.5	19.7	0.70	1.32	6.00	37	13.2	67
NSS-5	28.80	35.5	20.7	0.73	1.24	7.25	44	14.0	80
CSH-1	26.75	46.0	23.0	0.62	1.25	6.75	46	16.4	84
CSH-5	23.06	31.0	18.5	0.74	1.25	5.75	54	20.0	87
CSH-6	26.59	37.5	18.5	0.71	1.44	7.25	16	18.5	97
CSH-9	27.12	39.5	22.0	0.69	1.23	7.75	40	20.0	93
SE	±0.516	±0.554	±0.427	±0.02	±0.04	±0.548	±2.298	±0.997	±2.353
CD at 5%	1.535	1.646	1.269	0.04	1.11	1.629	6.806	2.963	2.993
CV %	2.805	2.088	2.873	3.321	4.06	13.029	7.891	8.640	4.404

correlation with expansion ratio of popped grains. Seed hardness and bulk density however, had significant positive effect on expansion ratio of popped grains. Kernel weight, bulk and true kernel volume had significant negative correlation and seed hardness had strong positive correlation with popping percentage. Murty *et al.*[5]. found significant negative relationship of per cent popping with grain weight, grain volume and per cent floaters. Pop volume (y_1) did not show any relationship with any of the physical parameters tested. However, Murty *et al*[5]. found negative relationship of pop volume with grain weight, grain volume and per cent floaters.

TABLE 2. CORRELATION MATRIX OF POPPING CHARACTERS AS AFFECTED BY PHYSICAL PARAMETERS OF SORGHUM GRAINS

Parameter	Expansion ratio (y_2)	Per cent popping (y_3)
1000 kernel wt (x_1)	−0.592**	−0.486*
Bulk kernel vol (x_2)	−0.577**	−0.463*
True kernel vol (x_3)	−0.613**	−0.519*
Bulk density (x_4)	0.478*	−0.042
True density (x_5)	0.367	0.216
Seed hardness (x_6)	0.487*	0.549*
Floaters % (x_7)	−0.548*	−0.203

* Significant at 5% level.
** Significant at 1% level.

The results of multiple correlation regression indicated that none of the physical parameters had significant effect on pop volume and expansion ratio when all the physical parameters were considered simultaneously. None of the physical parameters had significant effect on popping yield except seed hardness and bulk kernel volume. The partial regression correlation coefficients showed that seed hardness (Bi= 0.9464 ± 0.2542) had significant positive effect on per cent popping whereas, bulk kernel volume (Bi = −1.673 ± 0.4990) had significant negative effect on pop yield.

It is concluded that the sorghum cultivars which have more seed hardness and lower kernel weight, lower bulk and lower true kernel volume were better for good expansion ratio and pop yield.

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INVESTIGATION OF AFLATOXIN CONTAMINATION IN COMMERCIAL GROUNDNUTS

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Groundnuts stored and sold in Ahmednagar city and nearby villages were analysed for the presence of aflatoxin B_1(AFB1) aflatoxin B_2(AFB2), aflatoxin G_1(AFG1) and aflatoxin G_2(AFG2). Out of 96 samples analysed, 75 samples showed the presence of AFB1. Among them, 37 samples showed AFB1 above acceptable limit (120 μ/Kg). Three samples showed presence of AFB2, while AFG1 and AFG2 were not detected. Storage in open containers with other cereals and high moisture content (70-80°% relative humidity) in storing rooms was observed in most of the shops.

Numerous agricultural commodities are affected by aflatoxin contamination in developing countries of tropical and subtropical regions. Davis and Dioner[1] and Loper and Christensen[2] have suggested that in these regions, mould growth is favoured by conditions of humidity, temperature, cultivation, harvesting, transport and storage. Insect pest and sometimes drought and excess of rain may increase the extent and severity of fungal attack of crops, also if groundnuts are not dried to a safe moisture level of 9 per cent or less[3,4]. Aflatoxin B_1 (AFB1) is the most toxic and carcinogenic mycotoxin produced by *Aspergillus flavus* and frequently occurs in major foods such as maize, groundnut and cottonseed. Aflatoxin B_2 (AFB2), aflatoxin G_1 (AFG1) and aflatoxin G_2 (AFG2) are also produced. Several studies conducted on aflatoxin contamination in groundnuts in India, had shown fairly high level of aflatoxin[5]. The present survey was undertaken to see if aflatoxin B_1 was present in stored groundnuts obtained in markets from Ahmednagar city and nearby villages. The presence of AFB2, AFG1, and AFG2 was also checked.

Ninety six samples of groundnuts weighing 500 g were collected from different shops located in different areas in Ahmednagar city and nearby villages. City area included shops from slum area, retail and wholesale dealers. Standard AFG1(10μg/ml) and AFB2, AFG1 and AFG2 were obtained from National Institute of Public Health, the Netherlands. The extraction and estimation of aflatoxin from groundnuts were carried out as per the standard AOAC methods.[6,7] Microbiological assay was carried out by suspending 10 g of sample in 100 ml sterile water and plating on Potato Dextrose Agar (Potato extract 200 ml, dextrose 10g., distilled water to make final volume to 1 liter. pH.6.0) after serial dilutions, plates were incubated at room temperature. The number of colonies of *Aspergillus* species and *Aspergillus flavus* were counted on the 5th day.

Seventy five samples showed presence of AFB1. Among these, 38 had AFB1 less than 120μg/kg, that is the concentration permitted in groundnut flour in India.[5] Three samples showed presence of AFB2 while AFG1 and AFG2 were not detected in any of the samples tested. (Table 1). All samples collected from slum area and majority of samples from villages showed presence of AFB1. High humidity (70-80 per cent RH) and unhygienic condition and pest infestation was observed in these shops. The occurrence of aflatoxin was found to be low in the samples collected from shops of middle-class localities and wholesale dealers. Prevalence of low humidity (40-55 per cent RH) and use of closed metal or wooden containers for storage were observed in these shops. Microbiological assay indicated that *Aspergillus flavus* was the dominant organism involved in toxin production as compared to other *Aspergillus* species (Table 1).

TABLE 1. DISTRIBUTION OF AFLATOXIN IN GROUNDNUT SAMPLES COLLECTED FROM DIFFERENT LOCATIONS

Sampling location	Samples analysed (No.)	*A. flavus* (% of Aspergillus sp)	*A. flavus* Colonies ($\times 10^3$/g)	No. of samples with AFB1	No. of samples with AFB1 <120 μg/kg	No. of samples with AFB2
Slum area	28	80	12 – 30	28	11	3
Villages	34	68	6 – 25	30	12	–
Retail shops	18	52	3 – 15	7	5	–
Wholesale shops	16	70	5 – 12	10	–	–

AFG1 and AFG2 were not detected in any sample.

Ingestion of aflatoxin by humans at low level (ppb) is unavoidable; even at these levels aflatoxin presents considerable hazard[8-9]. About eighteen countries have regulations which prescribe maximum acceptable limits for aflatoxin in food in the range of 0-50 μg/kg of food[10]. During 1974, an outbreak of acute hepatitis in Rajasthan and Gujarat occurred and more than 100 persons died. High concentration of aflatoxin was found in their food ranging from 6.5 to 15.5 μg/-g.[11-13]. Though such high concentration may not be found all the time, it is better to minimize the fungal infection and reduce the aflatoxin content. This could be achieved by proper storing conditions. Groundnuts for retail sale could be sealed in plastic bags. This would avoid pest infestation as well as provide protection from moisture.

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INCIDENCE OF SPORE FORMING BACTERIA IN MILKS HEATED TO ABOVE 100°C

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An investigation was undertaken to isolate and identify aerobic mesophilic and thermophilic spore forming bacteria from cow's and buffalo milk samples heat treated at 109°C for 15 min, 115°C for 10 min and 121°C for 5 min. Among mesophilic isolates, *B. subtilis* was predominant, followed by *B. licheniformis* and *B. cerues*. Among thermophiles *B. stearothermophilus* was predominant followed by *B. coagulans* and *B. licheniformis*.

Considerable attention has been given to spore forming bacteria in milk because they bring about spoilage in pasteurized[1], boiled[2] and ultra high temperature[3] heat treated milks. These organisms are highly heat resistant and their numbers in the product depend upon the initial load in raw milk and the processing temperature employed. The present investigation was undertaken to isolate and identify aerobic mesophilic and thermophilic spore forming bacteria from heat treated cow's and buffalo milk samples.

Milk: Both cow's and buffalo milk samples required for the study were obtained from Dairy Experimental Station, A.P. Agricultural University, Rajendranagar. The samples were preheated separately at 80°C for 10 min to destroy the vegetative cells, transferred into previously sterilised 250 ml sample bottles and crown-corked. The bottles were then heated in an autoclave at 109°C for 15 min, 115°C for 10 min and 121°C for 5 min. The heating up time and cooling down time were maintained uniform. The bottles were removed from the autoclave, cooled under tap water and stored at room temperature for 30 days.

As and when the samples were spoiled, they were pour-plated on milk starch agar using suitable dilutions. The plates were then incubated at 37°C and 55°C for 48 hr for mesophilic and thermophilic spore forming bacteria, respectively.

Characterisation of isolates: The isolates (0-5) obtained from the plates were purified and characterised according to standard methods[4,5]. The average count of mesophilic spores in raw cow's and buffalo milk samples was 1602/ml and 219/ml respectively. Similarly, the thermophilic spore counts were 156/ml and 170/ml for cow's and buffalo milk respectively.

Out of the 25 isolates of mesophilic spore forming bacteria obtained from cow's milk samples heated to 109°C for 15 min, *B. subtilis* (8), *B. licheniformis* (5), *B. cerues* and *B. circulans* (4 each), *B. coagulans* and *B. megatherium* (2 each) were identified (Table 1).

TABLE 1. DISTRIBUTION OF AEROBIC MESOPHILIC SPORE FORMING BACTERIA IN HEAT TREATED MILK SAMPLES

Species	Cow's milk			Buffalo milk		
	109°C/ 15 min	115°C/ 10 min	121°C/ 5 min	109°C/ 15 min	115°C/ 10 min	121°C/ 5 min
B. subtilis	8 (32)	6 (37)	5 (38)	10 (41)	7 (46)	6 (40)
B. licheniformis	5 (20)	5 (19)	2 (15)	5 (21)	2 (13)	3 (20)
B. cereus	4 (16)	2 (13)	1 (7)	3 (13)	1 (7)	2 (13)
B. coagulans	2 (8)	2 (13)	1 (5)	3 (13)	2 —	—
B. megatherium	2 (8)	1 (6)	1 (8)	— —	1 (7)	— —
B. polymyxa	—	—	1 (8)	1 (4)	—	—
B. pumilus	—	1 (6)	—	—	—	1 (7)
B. firmus	—	—	—	—	1 (7)	1 (7)

*The cultures were incubated at 37°C for 48 hr.
Figures in parentheses indicate percentages.

TABLE 2. DISTRIBUTION OF AEROBIC THERMOPHILIC SPORE FORMING BACTERIA IN HEAT TREATED MILK SAMPLES

Species*	Cow's milk			Buffalo milk		
	109°C/ 15 min	115°C/ 10 min	121°C/ 5 min	109°C/ 15 min	115°C/ 10 min	121°C/ 5 min
*B. stearothermophilus***	17 (71)	10 (63)	10 (67)	15 (75)	8 (67)	9 (69)
B. coagulans	4 (17)	2 (12)	3 (20)	3 (15)	3 (25)	3 (23)
B. licheniformis	3 (12)	4 (25)	2 (13)	2 (10)	1 (8)	2 (8)

*Cu tures were incubated at 55°C for 48 hr.
**Figures in parentheses indicate percentages.

When the temperature was raised to 115°C for 10 min, 16 isolates were obtained and these were identified as *B. subtilis* (6). *B. licheniformis* (3), *B. coagulans* and *B. cereus* (2 each) *B. circulans, B. megatherium* and *B. pumilus* (1 each). Out of the 13 isolates obtained from cow's milk heat treated at 121°C for 5 min, *B. subtilis* (38 per cent), *B. licheniformis* and *B. circulans* (15 per cent) and *B. cereus, B. coagulans, B. megatherium* and *B. polymexa* (8 per cent each) were identified. Similarly, among buffalo milk samples *B. subtitis* was the predominant species representing 42 per cent followed by *B. licheniformis* and *B. cereus* (11 per cent) and others.

From Tables 1 and 2, it may be observed that *B. subtilis* was the predominant species among mesophilic spore forming bacteria isolated from heat treated cow's and buffalo milk samples followed by *B. licheniformis*. The predominance of *B. subtilis* has also been reported earlier[6,7]. However, Martin[8] and Khalafalla *et al.*[9] reported *B. licheniformis* and *B. megatherium* as the predominant species respectively.

B. subtilis causes sweet curdling of milk by the liberation of a rennet like enzyme[10,11]. Hence, the presence of this organism in the final product is undesirable and should be eliminated. *B. stearothermophilus* was the predominant thermophilic spore forming bacteria and this organism is known to cause acid coagulation of proteins of sterilised milk and milk products. All the thermophilic isolates obtained from cow's as well as from buffalo milk samples belonged to *B. subtilis, B. coagulans* and *B. licheniformis*. The percentage occurrence of these organisms in the milk samples is shown in Table 2. *B. stearothermophilus* was the predominant thermophilic spore forming bacteria in all the heat treated milk samples examined in the study. The observations were similar to those reported by Grinslid and Clegg[12] and Choi[13].

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EFFECT OF MOISTURE ON SOLUBILITY, FREE FATTY ACID CONTENT AND β-CAROTENE OF SPRAY-DRIED, FREEZE-DRIED AND FOAM-MAT-DRIED EGG POWDER DURING STORAGE

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Spray-dried, foam-mat-dried and freeze-dried egg powders with different moisture levels (2-12%) were stored for 60 days at 19-25°C and 37°C. There was a loss of solubility, an increase in free fatty acids content and loss of carotene depending upon storage temperature. On the basis of these changes, it is suggested that about 2% moisture would be optimum for storage of egg powder for 60 days.

Browning is a major problem during the storage of egg powder and results in loss of solubility and increase in toughening. Hydrolytic reactions and enzymatic hydrolysis of lipids are also possible even at extremely low moisture levels and at higher temperatures during storage[1,2]. Other deteriorative reactions are a function of temperature and moisture content.

Brooks and Hawthorne[3] concluded that change in pH and the increase in free fatty acids (FFA) content was a function of water content and that below 4.5 per cent water, there was little change either in pH or FFA. Hay and Pearce[4] have indicated that the moisture content of egg powder should be below 2.8 per cent and preferably 1.4 per cent if quality comparable to that of fresh egg powder is to be maintained during storage. Dutton and Edwards[5] have concluded that the destruction of carotenoid pigments and formation of browning increases as the moisture content and storage temperature increase. In the present study, the effect of different moisture levels (2-12%) on the loss of solubility, free fatty acid and β-carotene contents of egg powders made by three different techniques namely spray-drying, freeze-drying and foam-mat-drying during a maximum storage periods of 60 days was investigated.

All the three types of egg powders described above were prepared and packed as per methods described by Rao *et al*[6]. They were first exposed to different relative humidities until the required moisture content (2 to 12 per cent) was attained. The samples were then stored in glass bottles with air tight lids at room temperature (19-25°C) and at 37°C for different periods. Solubility was determined as described in the Indian standards methods[7]. FFA and β-carotene contents were determined according to AOAC[8].

Tables 1, 2 and 3 present the data on the effect of moisture, temperature and storage period on solubility, free fatty acid contents, and β-carotene contents of spray-dried, foam-mat-dried and freeze-dried egg powders respectively.

Solubility: It is seen from the tables that there was no significant change in solubility of spray-dried egg powder upto a moisture level of 6 per cent when stored for a period of 60 days at 19-25°C and 37°C. Above 8 per cent moisture level, the solubility decreased as early as 10 days storage at both the temperatures and were unacceptable. In the case of foam-mat-dried egg powder, there was a loss of solubility below optimum level of 85% at moisture levels of above 8 per cent at room temperature (19-25°C). Thereafterwards with the increase in moisture level, solubility further decreased. The solubility decreased more at

TABLE 1. CHANGES IN SOLUBILITY, FREE FATTY ACIDS AND β-CAROTENE OF SPRAY-DRIED EGG POWDER AT DIFFERENT MOISTURE LEVELS

Moisture (%)	Solubility (%) at indicated temp. and days						FFA at 60 days (%oleic acid)		β-carotene at 60 days (μg %)	
	RT (19-25°C)			37°C						
	10	20	60	10	20	60	19-25°C	37°C	19-25°C	37°C
2.24	91.7	93.5	90.0	90.0	90.0	90.0	0.60	0.80	52	52
3.03	85.7	85.7	85.7	85.7	85.7	87.7	0.84	0.87	42	46
4.04	87.7	85.7	85.7	85.7	85.7	85.7	0.84	1.03	32	48
5.54	87.7	85.7	87.7	87.7	87.7	85.7	0.91	0.95	40	40
6.04	85.7	85.7	85.7	97.8	87.8	87.8	0.95	0.84	40	40
7.08	85.7	97.7	85.6	87.7	87.7	83.3	0.84	1.06	40	40
8.27	81.0	81.0	81.0	81.0	81.0	75.9	1.03	1.26	28	28
9.98	78.6	78.6	75.9	75.9	75.9	70.1	1.12	1.14	28	28
11.50	78.6	78.6	70.1	70.1	70.1	66.9	1.18	1.29	26	24

Initial values : solubility, 95.3%; FFA, 0.59%, β-carotene, 68 μg %.

TABLE 2. CHANGES IN SOLUBILITY, FREE FATTY ACIDS AND β-CAROTENE CONTENTS OF FOAM-MAT-DRIED EGG POWDER AT DIFFERENT MOISTURE LEVELS

Moisture (%)	Solubility (%) at indicated temp. and days						FFA at 60 days (% oleic acid)		β-carotene at 60 days (μg %)	
	RT (19-25°C)			37°C						
	10	20	60	10	20	60	19-25°C	37°C	19-25°C	37°C
2.12	93.5	93.5	91.7	93.5	90.0	90.0	0.72	0.74	60	60
4.00	90.0	81.0	81.0	85.6	81.0	85.6	1.23	1.40	56	50
5.54	87.7	78.6	78.6	85.6	78.6	78.6	1.29	1.41	56	48
6.17	85.6	78.6	78.6	78.6	75.9	66.9	1.29	1.43	56	46
7.00	85.6	81.0	78.5	75.9	75.9	66.9	1.41	1.42	52	44
8.00	83.3	78.6	78.6	75.9	73.1	66.9	1.41	1.42	44	48
10.21	78.6	75.1	75.1	75.1	70.1	66.9	1.41	1.46	50	40
11.67	73.1	73.1	70.1	73.1	70.1	70.1	1.41	—	46	32
12.12	73.1	66.9	70.1	70.4	70.1	70.1	1.52	1.52	46	32

Initial values : solubility, 95.3%; FFA, 0.71%, β-carotene, 68 μg %.

TABLE 3. CHANGES IN SOLUBILITY, FREE FATTY ACIDS AND β-CAROTENE CONTENT OF FREEZE-DRIED EGG POWDER AT DIFFERENT MOISTURE LEVELS

Moisture (%)	Solubility (%) at indicated temp. and days						FFA at 60 days (% oleic acid)		β-carotene at 60 days (μg %)	
	RT (19-25°C)			37°C						
	10	20	60	10	20	60	19-25°C	37°C	19-25°C	37°C
1.87	93.5	93.5	93.5	93.5	90.0	90.0	0.68	0.70	64	58
3.04	93.5	90.0	90.0	90.0	85.6	85.6	0.69	0.72	64	58
4.15	87.7	87.7	85.6	85.6	83.3	81.0	0.69	0.74	64	58
6.20	85.3	85.3	85.3	81.0	73.1	70.1	0.70	0.78	64	58
7.19	85.6	81.0	78.6	73.1	70.8	70.1	0.70	0.78	62	58
10.55	75.9	66.9	59.6	73.1	66.9	69.9	0.75	0.83	60	24
11.06	70.1	59.6	55.5	61.4	59.6	55.5	0.78	—	60	28
12.29	61.4	59.6	50.8	66.9	50.8	50.8	0.78	—	32	20

Initial values : solubility, 95.3%; FFA, 0.68%, β-carotene, 76 μg %.

37°C even at 5.5 per cent moisture level. Freeze-dried egg powder reached a solubility of 85.3 per cent at 6 per cent moisture level within a period of 60 days of storage at room temperature (19-25°C) and at 3 per cent moisture level at 37°C. The sample was unacceptable after 6 per cent moisture level within a storage period of 60 days. This observation made with freeze-dried egg powder is intriguing.

Free fatty acids: The free fatty acid contents of spray dried egg powder increased as the solubility decreased at all moisture levels studied and at both room temperature and at 37°C. The free fatty acid contents in foam-mat-dried egg powder were considerably higher than those of spray-dried egg powder for corresponding moisture levels. The changes in FFA were higher at higher moisture levels at both room temperature and at 37°C. Freeze-dried egg powder recorded a very low FFA and it was almost independent of increases in moisture as well as temperatures of storage. However, there was a slight increase in FFA at the end of the storage period of 60 days. Similar observation was also made by Potthast[9].

β-carotene content: Increasing losses of β-carotene were observed with increase in moisture as also at the higher temperature throughout the storage period in spray-dried egg powder. With foam-mat-dried egg powder, the losses of β-carotene were gradual with increasing moisture level; again the losses were more at 37°C than at room temperature. There was a reduction of 16 μg of β-carotene content at 11 per cent moisture level at ambient temperature and 34 μg at 12 per cent moisture at the end of 60 days. On the other hand, in freeze-dried egg powder at 37°C the β-carotene loss was 48μg at 11 per cent and 56 μg at 12 per cent moisture, respectively at the end of 60 days storage.

Browning: During the 60-day storage period, significant browning was not observed in all the three types of egg powders.

The data presented here indicate the practical importance of preventing uptake of moisture by dried

egg powder during transport and storage. It is also seen that during storage at any given temperature, the parameters studied namely, solubility, β-carotene retention and FFA values are greatly influenced by moisture content of the product.

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BACILLUS CEREUS IN MEATS : INCIDENCE, PREVALENCE AND ENTEROTOXIGENICITY

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An investigation was undertaken to study the incidence and enterotoxigenicity of *B. cereus* in meats. It was observed that 35% fresh buffalo meats, 100% *kabab* and 30% of the curry samples harboured 9.65×10^4, 1.07×10^3 and 6.4×10^2 *B. cereus*/g respectively. Though the levels of *B. cereus* population decreased in cooked meats, the prevalence (% positive samples) increased. *Bacillus cereus* counts varied between 1.10×10^2–10^4/g in cooked meats viz. *kabab* and curry. Guinea pig skin response of *B. cereus* cell free filtrates revealed that 90% of the strains were enterotoxigenic. On the whole, 30% of the fresh buffalo meat and 60% of the cooked meats (kabab and curry) harboured enterotoxigenic *B. cereus*.

Meat and meat products have been reported to be one of the important vehicles of *B. cereus* food poisoning[1]. Enumeration of *B. cereus* is an essential criterion to assess the safety of foods because large number of viable cells are required to produce food poisoning syndrome. Sherikar[2] reported objectionable levels of *B. cereus* contamination from a variety of ready-to-eat meat products. Almost all the *B. cereus* strains tested by Turnbull[3] were capable of producing enterotoxin. The present paper reports the incidence, prevalence and enterotoxigenicity of *B. cereus* in fresh buffalo meats and cooked meat products.

Fresh buffalo meat samples were collected from the slaughter house and the *kabab* and curry (cooked meat) samples were obtained from road side restaurants of the local market. Meat homogenate was prepared according to ICMSF[4]. Thereafter, 10-fold serial dilutions were prepared upto 10^{-6} for *B. cereus* quantitation. Indian standard method[5] was followed to identify and enumerate *B. cereus* in buffalo meats. Dilutions in 0.1 ml quantities were spread evenly over freshly prepared and dried surface of phenol red egg yolk polymyxin agar and incubated at 30°C for 48 hr. After obtaining presumptive counts of *B. cereus*, at least 5 representative colonies per plate were picked up and confirmed by further testing. Confirmed counts were computed from the presumptive counts based on per cent of confirmed colonies.

Glatz and Goepfort's procedure[6] was followed to test the enterotoxigenicity of *B. cereus* using Cell Free Filtrate (CFF). The development of characteristic weak and strong dermonecrotic reaction was recorded after 6 and 24 hr. No lesion was observed at the site of saline inoculation.

The levels of *B. cereus* incidence/g of meat were divided arbitrarily into 5 categories as follows : very low, 1-100; low, 10^2 + 1-10^3; medium 10^3 + 1-10^5; high 10^5 + 1-10^6 and very high exceeding 10^6/g. Range and mean counts of *B. cereus* are presented in Table 1. On the whole, mean counts varied from 6.4×10^2/g

TABLE 1. INCIDENCE AND ENTEROTOXIGENICITY OF *BACILLUS CEREUS* ISOLATED FROM MEAT AND MEAT PRODUCTS

Product	Samples tested (No.)	Samples +ve (No.)	% +ve	Counts/g	Samples harbouring enterotoxigenic strains		
					No.	% of +ve samples	% of total samples
Fresh meat	20	7	35	9.65×10^4 ($4.44 \times 10^3 - 4.0 \times 10^5$)	6	86	30
Kabab	10	10	100	1.07×10^3 ($2.0 \times 10^2 - 4.5 \times 10^3$)	9	90	90
Curry	10	3	30	6.4×10^2 ($2.1 \times 10^2 - 1.2 \times 10^3$)	3	100	30
Total	40	20	50	3.44×10^4 ($2.0 \times 10^2 - 4.0 \times 10^5$)	18	90	45

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in cooked products to 9.65×10^4/g in fresh meats. The spore forming aerobic bacilli occurred at "medium" (10^3 + 1-10^4/g) to "high" (10^5 + 1-10^6/g) levels in 35 per cent of the fresh buffalo meats. If the carcasses are not chilled, the population may multiply to dangerous levels exceeding 1.0×10^6/g in about 2 hr. Products prepared from such highly contaminated meats will contain large number of *B. cereus* cell/g. Though the level of *B. cereus* population decreased in cooked meats, the prevalence rates (per cent positive samples) increased. *Bacillus cereus* counts varied between $1.01 \times 10^2 - 10^4$/g in all samples of *kabab* as compared to 30 per cent of meat curry. In all, 65 per cent of the cooked and 50 per cent of the total samples contained *B. cereus* at different levels. On the whole, 30 per cent of fresh buffalo meat, 90 per cent of *kabab* and 30 per cent of meat curry harboured enterotoxigenic *B. cereus*. Out of 20 strains, 90 per cent proved to be enterotoxigenic giving weak and strong dermonecrotic reactions.

Meat and meat products constitute an important vehicle of *B. cereus* food poisoning. Levels of *B. cereus* contamination were found to be lowest in meat curry as compared to *kabab* and fresh meat. It remained at lower levels in meat curry obviously due to thorough cooking and minimum exposure to external environment, whereas *kabab* was neither thoroughly cooked nor prevented from post-cooking contamination. Smykal and Rokoszewska[7] reported prevalence of *B. cereus* in 18.6 per cent samples – mainly meats. Some of the buffalo meat samples harboured *B. cereus* at low levels even under present conditions of production and processing. This indicated that there was possibility of minimising the incidence and prevalence of *B. cereus* in meat and meat products.

High heat resistance of *B. cereus* spores, inadequate heating of the product and improper storgae might result in heavy build up of *B. cereus* population in cooked meat products. Quite a large proportion of *B. cereus* have been reported to be enterotoxigenic by Goepfert[8] and Glatz and Goepfert[6] also. They reported about 86 to 88 per cent of the strains to be enteropathogenic. Similarly, 100 per cent strains of *B. cereus* isolated from meats and poultry were reported to be enterotoxigenic[3]. Hence, most of the strains must be considered potentially dangerous.

Prevalence of enterotoxigenic *B. cereus* in meat products were very high. This is very important in view of the conditions of sanitation and the manner in which these meat products are prepared and stored in the open market. Presence of *B. cereus* in large numbers, particularly in ready-to-eat meat products, must be considered a potential hazard. On the basis of the present investigation, it is clear that low *B. cereus* counts in meat and meat products are attainable. On the whole, 65 per cent of the fresh meats and 70 per cent of the cooked meat product (curry) were free from *B. cereus*. Seventy per cent of the *kabab* samples harboured *B. cereus* at levels less than 10^3/g and that *B. cereus* has not been reported to cause food poisoning when present at levels less than 10^4/g in any food[4]. It is suggested that these points may be considered while fixing permissible limits for *B. cereus* in meat foods.

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SELECTIVE MEDIUM FOR THE PRODUCTION OF LIPIDS BY *RHODOTORULA GRACILIS* CFR-1

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Out of the four *Rhodotorula* spp. tested for their oleagenicity, *R. gracilis* CFR-1 was found to be a potential lipid producer. Using Enebo's medium as a reference, a selective medium (glucose 40, NH_4NO_3 0.285, yeast extract 1.5, $MgSO_4$ $7H_2O$, 0.4, $CaCl_2$ $2H_2O$, 0.4, KH_2PO_4 0.75 g/l. pH 5.0) was developed and it increased the oleagenicity of *R. gracilis* CFR-1 to more than 65%.

Rate of lipid synthesis in an oleagenous microorganism depends mostly on high carbon/nitrogen (C/N) ratio, balanced supply of elements such as hydrogen, oxygen, phosphorous, sulphur and potassium in the medium[1-3], under suitable environmental conditions. In the present study, four different *Rhodotorula* spp.: *R. rubra* 3174, *R. glutinis* 3353, (National collection of Industrial microorganisms, National Chemical Laboratory, Pune), *R. gracilis* – CFR-1 and an unidentified species of *Rhodotorula* (departmental isolate) were tested for their oleagenicity in Enebo's medium[4]. Of these, *R. gracilis* CFR-1 was found to be a potential fat producer. The present paper describes the development of a culture medium for increased oleagenicity of *R. gracilis* CFR-1, based on biomass and fat production while optimising some of the environmental and nutritional factors.

The cultures were maintained in potato dextrose agar slants. Each slant was washed with 12 ml of sterile distilled water to get a suspension of cells and 2 ml aliquot was added as inoculum to each of the 50 ml of Enebo's medium (glucose 40, $(NH_4)_2SO_4$ 1.0, $MgSO_4$ $7H_2O$ 1.0, $CaCl_2$ $2H_2O$ 0.5, NaCl 0.5, KH_2PO_4 1.0, $FeCl_3 6H_2O$ 0.005, yeast extract 0.5 g/l, pH 5.5) in 250 ml capacity conical flasks. The flasks were incubated at 28±1°C on a rotary shaker (rpm 250) for 96 hr. The harvested cells were dried at 110°C overnight, weighed, hydrolyzed with 1 N HCl for one hr, washed free of acid and dried prior to fat extraction using petroleum ether in a soxhlet apparatus. The residual sugar in the medium was estimated by the DNS method[5].

Upon screening, *R. gracilis* CFR-1 gave higher yields of biomass (10.4 g/l) and fat content (50.3 per cent) with a fat coefficient value of 13.0 (based on fat produced per 100 g of sugar supplied) (Table 1).

TABLE 1. MICROBIAL LIPID PRODUCTION IN FOUR DIFFERENT SPECIES OF *RHODOTORULA*

Test organism	Biomass (g/l)	Lipid content (%)	% sugar assimilation	Fat co-efficient*
R. rubra 3174	10.1	9.2	73.0	2.25
R. gracilis CFR-1	10.4	50.3	64.0	13.00
R. glutinis 3353	9.4	12.5	76.0	3.00
Rhodotorula sp.	11.6	13.8	97.0	4.00

*Fat coefficient = g lipid produced per 100 g glucose supplied.

In order to exploit the full potential of the fermentation process, it was essential to optimise different environmental factors and composition of the growth medium. Preliminary trials indicated that 96 hr and 4 per cent inoculum were optimal. The optimum pH was at 5. It is interesting to note that the final pH values of the media were within the range of 2.3 to 2.8 which may be due to excretion of certain acidogenic metabolites to the medium during fermentation.

It is known that a balance of metabolic activities can be manipulated to favour the production of lipids through fermentation. Table 2 shows effect of optimum concentration of different compounds on biomass and fat synthesis in the test organism. A high C/N ratio is considered to be one of the most important factors for oleagenicity in microorganisms[6]. The optimum level of glucose was at 4 per cent. The nature and concentration of nitrogen sources influenced biomass and fat production. Suitability of three sources of nitrogen – NH_2CO-NH_2, NH_4NO_3 and $(NH_4)_2$ SO_4, were tested at 0.1 to 1.0 g/l nitrogen levels. As the levels of nitrogen increased, the cell biomass decreased in value (13.9 to 9.6 g/l) for urea, whereas for NH_4NO_3 it

TABLE 2. OPTIMUM CONCENTRATIONS OF DIFFERENT COMPOUNDS FOR BIOMASS PRODUCTION AND FAT COEFFICIENT VALUES IN *RHODOTORULA GRACLIS* CFR-1

Compounds	Optimum concn. (g/l)	Biomass produced (g/l)	Fat coefficient
Glucose	4.00	11.0	13.8
KH_2PO_4	0.75	12.7	20.0
$MgSO_4$ $7H_2O$	0.40	14.1	21.7
$CaCl_2$ $2H_2O$	0.40	10.2	16.1
NH_4NO_3	0.285	10.0	17.5
Yeast extract	1.50	12.3	20.2

increased with increasing concentrations up to 0.5 g/l followed by a gradual decrease. On the contrary, with NH_4NO_3, as the nitrogen level increased, the lipid content decreased from 70 to 26 per cent. Similar observations were also made for $(NH_4)_2\ SO_4$ wherein, after 0.2 g/l nitrogen, lipid content declined from 46 to 20.1 per cent. In the case of $(NH_4)_2\ SO_4$, there was a declining trend of biomass production and sugar assimilation from 0.2 g/l nitrogen level.

Studies on the effect of yeast extract as an organic source of nitrogen, showed that at 1.5 g/l level, a higher per cent of fat (65) with a fat coefficient of 20.2 was obtained. Absence of yeast extract caused poor responses of biomass production (6.6 g/l), fat coefficient value (5.8) and sugar consumption rate (20 per cent). The C/N ratio of the finally formulated medium is 65:1 which gives reasonably high yields of both biomass and fat production.

Supply of inorganic nitrogen alone may not be sufficient for increased oleagenicity of an organism. In the present investigation, a complex organic source (yeast extract) along with an inorganic salt (NH_4NO_3) increased oleagenicity of *R. gracilis* CFR-1. Absence of these two components in the medium led to poor fat production and sugar consumption. It is reported that certain yeasts accumulate more lipid when an organic form of nitrogen source is given[3]. According to Evans and Ratledge[8], in certain yeasts various organic nitrogenous compounds stimulate lipid synthesis. Almost all the reported media in the literature have a combination of a complex organic source and nitrogen salt[5,9-11]. The exact biochemical reason for such a phenomenon is yet to be established.

Apart from high C/N ratio, optimised concentration of elements are also required for oleagenicity of microorganisms[2]. Relatively little is known about the importance of the concentration and composition of various minerals for lipid production. Woodbine[9] and Weete[2] reviewed the importance of inorganic elements on biomass and fat synthesis of fungi. In the present case, the optimum concentrations of $MgSO_4\ 7H_2O$ and $CaCl_2\ 2H_2O$ were found to be 0.4 g/l whereas for KH_2PO_4 it was 0.75 g/l. Influence of KH_2PO_4 was also tested separately and in combination with K_2HPO_4 (1:1 ratio) and they gave almost identical results. Absence of these salts led to low sugar consumption (about 50 per cent of the total supplied). The salts, K_2HPO_4 and KH_2PO_4 were found to be equally good in the medium for fat synthesis. KH_2PO_4 was preferred to K_2HPO_4 as the latter caused precipitation during autoclaving of the medium. KH_2PO_4 is used as a buffering agent, as sources of K, H, P and O in the medium and it helps maximum sugar assimilation by the organism. Pan *et al*[11] reported that proper addition of KH_2PO_4 or $(NH_4)_2\ SO_4$ could ensure complete sugar assimilation by *R. gracilis*. NaCl and $FeCl_36H_2O$ (components of Enebo's medium) did not have any effect on the oleagenicity. Based on these results, a selective medium was formulated with the optimum concentrations of different compounds with a pH of 5.0 and repeated experiments showed increased oleagenicity of *R. gracilis* CFR-1 to more than 65 per cent.

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BIOCHEMICAL COMPOSITION OF STORED PIGEON PEA (*CAJANUS CAJAN* L) IN RELATION TO BRUCHID DAMAGE*

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The composition of pigeon pea seeds stored for one year in wooden, steel and mud bins was studied in relation to field borne infestation by *Callosobruchus chinensis* L. The highest increase in insect population and consequent damage to seeds occurred in the mud bin followed by steel and wooden bin. There was a significant increase in crude protein, crude fibre, total ash as well as reducing sugars, but decrease in total carbohydrates including non-reducing sugars, crude fat and physiological energy of the seed at one year storage.

Seeds undergo biochemical changes during storage which result in losses in the constituents. Infestation of seeds by insects further aggravates these losses. Insect development in stored seeds and consequent damage to seeds depends on the storage ecosystem of which granary structure is one of the main components. An attempt has been made in the present investigation to find out the changes in the composition of pigeon pea seeds stored in different types of storage structures in relation to bruchid infestation.

Three types of bins (wooden, steel and mud bin) were used to store the seeds. The bins were fumigated with EDB to eliminate the hidden infestation, if any. The bins were opened and exposed to the sun for 3 days. After 15 days, the bins were filled with pigeon pea seed var. 'UPAS-120' weighing 50 kg. The bins were covered with their respective lids to make them considerably air tight. The bins were kept for one year at laboratory conditions having temperatures varying from 18 to 36.5°C and RH from 50 to 93 per cent. Samples were drawn at 0, 3, 6, 9 and 12 months of

TABLE 1. COMPOSITION OF PIGEON PEA SEEDS DURING STORAGE IN RELATION TO INFESTATION BY *C. CHINENSIS*

Storage period (months)	Insect count (No/100 g seed)	Seed damage (%)	Moisture (%)	Total ash (%)	Crude fibre (%)	Crude protein (%)	Crude fat (%)	Total carbohydrates (%)	Physiological energy (KCal)	Reducing sugars (%)	Non-reducing sugars (%)
					Wooden bin						
0	3.0	3.02	12.40	4.75	7.06	22.57	2.66	62.96	366	0.77	6.71
3	4.23	3.23	12.50	4.79	7.07	22.65	2.53	62.96	365	0.79	6.36
6	8.65	3.57	12.65	4.82	7.09	22.90	2.41	62.78	364	0.88	6.04
9	10.23	4.20	12.84	4.82	7.15	23.21	2.30	62.62	364	1.09	5.75
12	25.00	7.14	13.65	5.00	8.05	23.74	2.18	61.03	359	1.29	4.90
					Steel bin						
3	34.34	27.50	12.95	5.26	8.84	24.31	2.01	59.58	354	1.38	4.31
6	73.00	68.00	14.00	5.41	9.82	25.61	1.59	57.57	347	1.57	3.44
9	110.00	77.41	14.36	5.50	10.91	26.92	1.49	55.08	341	1.43	3.56
12	140.00	80.31	15.05	5.70	11.28	27.57	1.18	54.27	338	1.52	3.05
					Mud bin						
3	40.00	33.49	13.08	5.41	9.28	24.53	1.83	58.95	350	1.59	4.00
6	89.34	70.61	14.21	5.59	10.32	25.81	1.40	56.88	343	1.78	3.13
9	138.67	80.00	15.00	5.74	11.88	27.08	1.31	53.99	336	1.66	3.21
12	179.00	83.62	15.32	5.89	12.48	28.17	0.98	52.48	331	1.70	2.83
CD_1 at p = 0.05*	7.52	2.26	0.15	0.09	0.10	0.21	0.09	0.44	1.02	0.16	0.22
CD_2 at p = 0.05**	7.91	2.06	0.16	0.14	0.11	0.15	0.16	0.61	1.32	0.17	0.22

*Between 2 sub-plots at the same level of main plot
**Between 2 sub-plots at the different level of main plot.
All the values reported are average of six replicates each.

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storage and analysed. Insect species present in the seeds were identified. Insect count (number of live or dead insects per 100 g seeds) and seed damage (per cent seeds damaged by bruchids) were measured. Proximate composition and reducing and non-reducing sugars were estimated on dry weight basis according to AACC procedure[1]. The data collected were analysed statistically by CRD-split plot[2].

The observations on insect infestation and composition of seeds, presented in Table 1, show that initial infestation in seeds by *Callosobruchus chinensis* L. (3.02 per cent) developed to a considerable extent in the mud bin followed by steel and wooden bins. A similar trend was observed with respect to seed moisture. The lowest insect count in wooden bins might have been partly due to retention of residual EDB by wood for longer period unlike steel and mud structures and partly because of comparatively lower seed moisture. The significant increase in seed moisture in mud bin might have occurred due to its porous nature which allowed atmospheric moisture to interact. Steel, being a good conductor of heat, was affected by fluctuations in laboratory temperature and led to condensation of water vapours produced during seed and insect metabolic activity, inside the bins. It has been reported that infestation increased with the increase in seed moisture. Consequently, large insect populations produced more water as a result of their metabolic activity which further increased seed moisture[3]. In general, seed protein, ash, fibre and reducing sugars increased whereas carbohydrates, fat physiological energy and non-reducing sugars decreased significantly in all the bins. With respect to composition, all the bins differed significantly from one another at 3, 6, 9 and 12 months storage. The changes observed could be attributed partly to metabolic changes occurring in the seed and mainly to consumption of seed contents by the bruchids during the course of storage[4-6]. It has been demonstrated that pulse beetle feeds deep inside the cotyledons leaving the seed coat portion as such[3]. Since the seed coat is rich in minerals and fibre, these constituents increased when calculated on per cent basis. The loss in carbohydrate occurred due to its consumption during respiration by seed and bruchid. Degradation of seed starch to sugars by amylases and phosphorylases of seeds and inter-conversion of reducing and non-reducing sugars resulted in changes in sugar content[7]. The losses in carbohydrates were responsible for significant increase in protein content on per cent basis. Significant drop in physiological energy occurred due to significant decline in seed fat and carbohydrates.

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SPECTROPHOTOMETRIC DETERMINATION OF THIRAM, ZIRAM AND ZINEB IN FORMULATIONS, WATER, GRAINS AND VEGETABLES USING OXIDISED HAEMATOXYLIN

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A highly sensitive spectrophotometric method has been developed for the determination of thiram, ziram and zineb, by heating them with oxidized haematoxylin in water bath at 70°C for 5 min under neutral conditions. The absorption maximum of the coloured species so formed is at 555 nm. The method is simple, reproducible and accurate within ± 1% and applicable to the assay of thiram, ziram and zineb in fungicidal formulations, water samples, grains and vegetables.

Thiram (tetramethyl thiuram disulphide), Ziram (Zinc bis dimethyl dithiocarbamate) and Zineb (Zinc ethylene bis dithiocarbamate) are widely used anti-fungal agents. Most of the visible spectrophotometric methods[1-6] for their determination are based on the estimation of acid decomposition products, carbon disulphide and hydrogen sulphide and are tedious and time consuming. A few visible spectrophotometric methods[7-11] have been described for the direct determination of these fungicides. Herein, a new visible spectrophotometric method has been described using hematin (6a, 7-dihydro-3,4.6a-10-tetrahydroxy benz (b) indeno (1,2-d) Pyran-9 (6H)–one); oxidised form of haematoxylin) as a chromogenic agent for the first time.

Spectral absorbance measurements were made with a Systronic model MK 1 spectrophotometer. The pH measurements were made with an Elico LI 120 digital pH meter.

Methanolic solution of 0.2 per cent haematoxylin (Aldrich, AR) and aqueous solution of 0.4 per cent chloramine-T (BDH, AR) were prepared freshly. The buffer solution (pH 7.0) was prepared by mixing 390 ml of 0.067M of potassium hydrogen phosphate (BDH, AR) and 610 ml of 0.067M of disodium hydrogen phosphate (BDH. AR).

The stock solutions of fungicide (2 mg/ml) were prepared separately by dissolving 100 mg in 50 ml of methanol (thiram) or pyridine (ziram and zineb). The working standard solutions were prepared by diluting the stock solution with methanol to get the desired concentration.

To a series of 25 ml graduated test tubes, 1 ml each of haematoxylin and chloramine-T and 15 ml of pH 7.0 buffer solution were added successively. The mixture was kept aside for 20 min. Then added aliquots of fungicidal solutions containing 6 – 84 μg of thiram, 5 – 40 μg of ziram or 15 – 120 μg of zineb and kept in a water bath at 70°C for 5 min. The test tubes were removed from the bath, cooled and diluted to 25 ml with distilled water and the absorbance read against a corresponding reagent blank at 555 nm within the stability period (20 min). The amounts of thiram, ziram and zineb were deduced from their respective standard calibration curves.

Twenty five mg of each well mixed formulation was dissolved in a manner similar to standard, filtered and the filtrate was treated as above.

Various amounts of thiram, ziram and zineb were mixed with grain (20 g) as a water suspension, the grain dried in the sun. A 20 g of blended vegetable free from fungicide was uniformly mixed with various amounts of thiram, ziram and zineb separately. Field water samples were spiked with various amounts of ziram and zineb. The samples were extracted with chloroform (for thiram and ziram) or pyridine (for zineb). The extracts were evaporated *in vacuo* to dryness. The procedure for standard curve was then followed.

The optimum conditions incorporated in the procedure for the determination of thiram, ziram and zineb were established through control experiments based on the maximum colour development and its stability.

The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity for each fungicide are given in Table 1. The precision and accuracy were found by the analysis of eight replicate samples containing known amounts of fungicides and the results are summarised in Table 1.

TABLE 1. OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY

	Thiram	Ziram	Zineb
Beer's law limits (μg/ml)	0.2 – 3.4	0.2 – 1.6	0.6 – 4.8
Molar absorptivity (l/mol/cm)	5.51×10^4	1.07×10^5	3.9×10^4
Sandell's sensitivity (μg/cm^2 /0.001 absorbance unit)	0.004	0.0028	0.007
% relative standard deviation	0.70	0.77	1.11
% range of error (95% confidence limit)	0.57	0.64	0.93

TABLE 2. DETERMINATION OF DITHIOCARBAMATES IN FUNGICIDAL FORMULATIONS

Technical grade sample	Quantity indicated on the label (%)	Dithiocarbamate found* (%)	
		Proposed method	Reported method
Thiram (thiride)	80	79.0	78.5
Ziram			
(Ziride)	80	78.2	78.0
(Cuman)	27	26.3	25.9
Zineb			
(Dithane-Z78)	75	73.9	73.5
(Hexathane)	27	25.9	25.5

*Each value is the average of three determinations.

Comparison of the values of formulation analysis of the fungicides with the method proposed by Rangaswamy *et al*[8-10] reveals good accuracy of the data (Table 2).

After addition of any of the three fungicides (5 mg) to wheat, rice, potato, tomato, cabbage and water (except thiram) recovery was in the range of 95-98 per cent.

The other insecticides such as DDT, BHC, and dieldrin did not interfere. The components such as molybdate, Cu^{2+} usually present in formulation and degradation products (carbon disulphide, hydrogen sulphide, amine) of dithiocarbamates did not interfere even when they are present in 50 – 100 fold excess.

The method is applicable to residues of grain, vegetables and water samples, due to its sensitivity and avoidance of interfering substances and degradation products. The coloured species appear to be a charge-transfer complex between hematin (o-quinone derivative, formed *in situ* from haematoxylin and chloramine-T; acceptor) and the fungicide (donor, due to presence of sulphur atoms) as in the case of quinone and organosulphur compound (i.e. chloranil and pencillin G[12]).

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BOOK REVIEWS

Changing Food Technology: Manfred Kroger and Ralph Shapiro (Ed); Technomic Publishing Co. Inc., Lancaster, Penn., USA, 1987; pp:170; Price: not indicated.

This is a compilation of selected papers of Fourth Eastern Food Science and Technology Conference where the emphasis was Innovation and Communication. Partners for progress in the Food Industry.

The papers are classed into three sections. The first one, viz "Process automation for quality and cost control" deals with the need to automate has been related to the need to reduce the number of processing centres to achieve a fixed target and to reduce manual operation. Hitherto, automation was mostly considered as "hard" automation based on mechanization of a process. Total plant automation would involve "soft" automation using microprocessors at all levels of instrumentation and control systems. The pros and cons of computer control and various in-line and on-line sensors to satisfy various needs are also discussed.

The second section is grouped broadly under "Laboratory productivity and new food products". With the competition becoming more and more complex, there will be pressure to automate laboratories at the same time exploiting human resources more effectively. Increased competition will also raise corporate expectation of creation of own technologies and need to include R&D in Company's strategic planning processes. Regarding incorporating nutritional research results into new product formulation, there is need to base claims on scientifically sound benefits which is communicated correctly. Product development opportunities for the 80's include health foods, elite foods, convenience foods, foods for older generation and even gourmet foods. Future Government regulations are important in deciding the product formulation and for this, technologists should understand the rule making process and collaborate with scientific cell of Government.

The third section "Future processing technologies and packaging trends" has an excellent coverage on membrane technology. This paper is covered in detail with impressive figures to support various points. In fact, this is the best paper of the publication and occupies nearly 25% of the book. The paper covers operational functions of RO, UF and MF crossflow technologies, characteristics of different membranes and emerging applications in the food industry.

Food irradiation is considered as an innovation in food preservation. The technology required for irradiation is straight forward and does not cause environmental impact. This is despite conflicting reports appearing in media. Instantized food ingredients are shown to be superior to other kinds of powders due to better wettability, dispersability and flowability. There is a paper on packaging where it is seen also as a medium for communicating information of products.

There is also an appendix consisting of 12 graduate student reports which were presented in the poster session.

The early sections are narrated more in a speaking style. The discussion is not in detail. No doubt, this is a compilation of papers presented orally, but as far as long range usefulness goes, it would have been better if coverage was precise and adequate. A notable exception to this is the paper on cross flow membrane technology which is very well covered.

Printing and quality of paper are excellent. The publication is a useful material for reading but may not qualify as an authoritative reference material.

A.G. MATHEW
KISSAN PRODUCTS LTD.
BANGALORE.

Storage Lives of Chilled and Frozen Fish and Fish Products: Proceedings of Meetings at Aberdeen, U.K., Oct. 1-3, 1985, International Institution of Refrigeration, Paris, France, 1985; Price: not indicated.

To the student, teacher and the research worker alike, the study of the keeping quality of chilled and frozen fish and fish products is both a common place and a perennial subject. The expanse and depth of the subject matter, therefore, tend ordinarily to be lost sight of. The publication under review is an eye-opener in this regard, being a collection of all papers presented at Torry Research Station, Aberdeen, during the meetings convened in Oct. 1985 by the International Institution of Refrigeration. At the meetings, "more attentive examination was made of the means to provide longer storage and better overall

preservation qualities of fish and marine products". The publication is, in a way, a compilation which updates the current state of art in fish preservation by chilling and freezing, and points as well to the present trends and needs of research on the topic.

The papers are presented in eight sections, viz.

1. What is shelf life?, 4 papers.
2. Chemical changes in frozen storage, 5 papers.
3. Storage of frozen fish and shell fish, 5 papers.
4. Other aspects of storage, 5 papers.
5. Possible methods of extending chilled storage life, 6 papers.
6. Chilled storage studies on fish and shell fish, 5 papers.
7. Improving and monitoring traditional handling of chilled fish, 5 papers.
8. Poster presentations, 16 papers.

In addition, there are two summary reports on discussions on chilled storage and frozen storage, and frozen storage, followed by conclusions which identify "the scope for improvement and development of research in this area". There is little doubt that "this book is a valuable documentary source" for all those interested in preservation of marine foods by chilling and freezing.

N.V. SRIPATHY
C.F.T.R.I. REGIONAL CENTRE
MANGALORE

Fermented Foods of the World – A Dictionary and Guide: by G. Campbell-Platt, Butterworths (Services) Ltd. Borough Green, Sevenoaks, Kent TN15 8PQ, England; 1987; pp:291; Price: £ 35.00 in U.K.

The book is a pioneering work on fermented foods of the world. The author's intention was to provide a reference source book on fermented foods, for all students, researchers, product development workers, and scientists, working in the area of food science and technology.

The introduction on fermented foods is succint, and satisfactory, with additional references for a detailed study of the available literature. The author's definition of fermented foods – a model of brevity and exactitude – is worth quoting: "Fermented foods may be defined as those foods which have been subjected to the action of microorganisms or enzymes, so that desirable biochemical changes cause significant modification to the food".

Fermented foods of the world have been brought together in one cohesive dictionary. About 3500 individual foods are listed, classified into some 250 groups. Each product is described and its consumption and production given. The nutritive value is given on dry matter basis, which makes comparison easy between foods of different moisture content. This is followed by the microbiology and biochemistry of the product and references pertinent to the region where the fermented product is normally produced and eaten.

The sections "Food by region" and "Foods by class' help in identification of a fermented product and firmly docket it in the mind.

There is a need of more dictionaries or anthologies of this type, in other areas of traditional food technology, particularly sun-dried and dried paste goods of the world.

SAROJANI. K. DASTUR
C.F.T.R.I., MYSORE.

Technology Advances in Refrigerated Storage and Transport: Proceedings of the Conf. of the International Institute of Refrigeration, 177, Boulevard Malesherbes, F75 017, Paris, held on November 17-21, 1985 at Orlando, Florida (USA), pp:336, Price: not indicated.

The development of Refrigeration Industry has radically changed the food industry. Prior to advent of refrigeration, only as much perishable food as could be consumed, was produced. Refrigerated storage plus refrigerated transportation have enabled the food producers and processors to develop new markets, introduce new products, and help feed areas of the world that had previously depended upon foods indigenous to those particular areas only.

Although the technical advances that brought about those early developments were monumental and gave us an entirely new system of food usage and transportation, today's technical community may be on the brink of further new developments, but what is more important is the transfer of appropriate technology since technology exchange is crucial to the efficient and expeditious development of new technology, be that in the area of energy conservation, indoor air quality or new refrigeration equipments/systems. Much remains to be done, however, to improve the efficiency of the present systems as well as processes. There is pressing need for development of inexpensive refrigeration methods and equipment to improve the quality of life in the poorer countries. So far, little has been done to develop simple bulk-handling and storage techniques.

With these objects in view, the Orlando (Florida)

meetings of IIR Commissions D_1, D_2, and D_3 were sponsored and organised by the U.S. National Committee for the IIR from November 17-21, 1985. About 210 delegates from 23 countries attended this meeting. The texts of 37 interesting communications (of which 22 were from the host country USA alone) of authors from 11 countries presented and discussed at this conference, alongwith a substantial summary of discussions on each paper, constitute the present publication under review which is the *61st Volume* of the IIR series entitled: "Refrigeration Science & Technology". However, this publication does not cover the recommendations of the conference of D_1, D_2 and D_3. The 37 papers have been categorised under the following five sections:

Section 1 – Refrigeration and the Food Supply (3 papers)

Section 2 – Refrigerated Storage (11 papers)

Section 3 – Refrigerated Transport (14 papers)

Section 4 – Chilling and Freezing of Products (5 papers)

Section 5 – Time-Temperature Monitoring (4 papers)

Section "Refrigeration – a key technology for the future" by Dr. Lorentzen (Norway) in Section 1 covers a number of ways to improve living conditions in the world, notably the Third World. It is essential to develop new energy systems for rational conservation of perishables. Greater realism is needed in all discussions of the possible role of refrigeration in the developing world. Dr. Gac, Director, IIR discusses at length the role of IIR in development of nations, after covering briefly the history of refrigeration, usages of refrigeration and need for development of cold chain. The 3rd paper covers an over-view of refrigeration in developing countries and highlights new and practical ideas for economic growth by using –

(1) Solar – Driven Absorption Refrigeration System,

(2) Wind-Mill driven Ice-making machine for fish.

Two commercial units of the latter machine have been ordered by India. The cost per unit is US $11,000 and cost of production of ice (for fish) is about 39 P/kg which can be further lowered by enlarging the unit size.

In Section 2, the very first paper has a novel title and utility. It is "Air-supported structures as an alternative to conventional construction for refrigerated ware houses" for chill storage of fruits and vegetables. Its main objects are (a) to reduce the cost of construction, (b) to increase the speed of construction, and (c) to provide flexibility for moving the structure to another site, if necessary. It is 120′ wide, 200′ long and 38′ high; total surface area = 24,000 Sq.ft and 620 160 CU.ft. as volume and 2°C (36°F) storage temperature is a working reality. The structure consists of three layers – an outer skin, a central fabric layer and an inner linen. This new development deserves commercial trials, notably in developing countries. The next fascinating paper is on the use of winter-cold for food refrigeration, including ice-production for the pre-cooling of vegetables. This relatively inexpensive passive system which by-passes the compressors in mechanical refrigeration system, holds considerable promise to save electrical energy. The other contributions of topical interest are: "A refrigeration system called 'Stored Energy Carbon Dioxide ($SECO_2$) System ; 'Comparison of the conventional Vs Automated Refrigerated Storage Systems; The Design and Operation of a high-rise refrigerated automated storage faciltiy; Re-building a 35 year old cold store; Refrigerated port facilities into cold stores; Cooling Towers – the neglected energy source; Micro-computer monitoring of commercial cold stores and last "Freezing Economy in both the Mechanical *Vs* Cryogenic Freezing Systems".

Section 3 covers the largest number (14 papers) on "Refrigerated Transport" of which, the following are of more topical interest; Future trends in cryogenic refrigeration of transport vehicles; Carbon dioxide as an expendable refrigerant for frozen foods; New test facility for refrigerated transport vehicles; and 'Adaptation of Space Technology to transport refrigeration.' The requirement for a small, compact, efficient cryogenic refrigerator in space applications has led to the development of equipment based on new thermodynamic cycles and concepts. Significant savings can be made in energy requirements and in improved product deliveries by the adaptation of space technology to transport refrigeration. A modified sterling Transport Refrigeration system has been developed and its working nicely illustrated. The other interesting papers are: "Advanced technological developments in combined refrigerated transport", "Refrigeration loads and insulation requirements for holds of wooden Fishing Vessels", "Shipping perishables by air", 'Transport refrigeration units' working under humid conditions; Temperature distribution in frozen and chilled commodities in insulated and refrigerated ISO containers and rates of temperature change in refrigerated and in ventilated commodities in ISO containers.

'Chilling and Freezing of Products' is dealt with in 5 papers under Section 4. They cover 'Forced-air cooling systems for *fruits* and *vegetables*; Economic feasibility of IQF vaccum packed *tuna steaks;* Practical applications of cooling performance data for perishable commodities; Transient heat and mass transfer for *oranges* in closed containers and 'Factors affecting the

cooling rate of Avocados packed in corrugated cartons'.

The four interesting papers on 'Time-Temperature Monitoring' under the last Section 5 are: 'Using the autology TTM (Time-Temperature Monitor) to monitor foods in transit', Experience with Monitor-Mark product temperature exposure indicators; Computerized freshness monitoring system' and 'Application of Time-Temperature indicators in food storage and distribution.

In a nut-shell, the publication is of immense practical interest to Refrigeration Industry and notably its ware-housing and transport sectors. Many papers should be of great value to R&D establishments, teachers and the taught in Food Science and Technology and specially in refrigeration systems and refrigeration/freezing of perishables. The printing, the quality of paper and the get-up, simple and elegant deserve special mention. This compendium should be a valuable asset to all libraries in Food Science & Technology.

J.S. PRUTHI
CRISHMA FOOD CONSULTING SERVICES
NEW DELHI - 21.

Principles for the Safety Assessment of Food Additives and Contaminants in Food: Title No. 70 under Environmental Health Criteria Series, World Health Organisation, Geneva; 1987; pp: 174; Price: Sw.fr.14.

The use of food additives has proliferated enormously during this century and presently food additives have become an indispensable part of our life in that they are highly essential for modern food processing, preservation and making it appealing. Today, about 2,800 substances are intentionally added to foods to produce a desired effect, while as many as 10,000 compounds find their way into various foods during processing, packaging and storage. The modern processes of refining and preserving food have increasingly been shown to pose unforeseen toxicological problems boh acute and chronic and may even lead to cancer. The monograph – 'Principles for the Safety Assessment of Food Additives and Contaminants in Food' reviews the basis for decision making by the Joint Expert Committee on Food Additives (JECFA) of FAO and WHO. Safety evaluation of food additives is a 2-stage process. The first stage involves the collection of relevant data of studies on experimental animals (and observations in man, if available). The second stage involves the assessment of data to determine the acceptability of the substance as a food additive.

Both the chemical characteristics of food additives and their toxicological evaluation – the two primary aspects in food safety evaluation are adequately dealt with in this monograph. The recommendations of procedures for testing of chemicals in food and their safety assessment are discussed in broad terms taking into account the latest scientific advances in the relevant fields. After a brief introduction of the genesis and tasks of JECFA (Chapter-1), the periodic reviews of the past decisions on food safety by JECFA are recalled (Chapter-2). Chapter-3 deals with 'Criteria for testing and evaluation of food additives'. Proper safety evaluation and use of food additives require that they be chemically characterised. Chapter-4 on 'Chemical composition and the development of specifications' reviews required data relating to identity and purity of additives, and also possible reaction products of additives and contaminants that may arise during processing and storage. JECFA specifications are then elaborated taking these factors into account. Chapter-5 on 'Toxicity test procedures and evaluation' discusses protocols for testing various toxic manifestations encountered – functional, non-neoplastic, neoplastic and reproduction/developmental. The use of metabolic and pharmacokinetic studies in safety assessment is also presented in detail. Influence of age, nutritional status, etc in the experimental design is discussed. This chapter also deals with evaluation of the toxicology data to set up Acceptable Daily Intake (ADI). Chapter-6 is on 'Principles related to evaluation of specific amounts, such as flavouring agents, food contaminants, etc and those consumed in large amounts, such as sorbitol, xylitol, modified starch, etc are dealt individually.

There are 87 references and 6 annexures. Annex-2 gives very useful and detailed guidelines on statistical aspects of toxicity studies. This comprehensive monograph published by WHO in the 'Environmental Health Criteria' series is very useful to research workers engaged in the field of toxicology of food additives and to the regulatory authorities and policy makers on food safety.

K. SRINIVASAN
C.F.T.R.I., MYSORE.

Dictionary of Biotechnology in English Japanese and German: By R. Schmid and S. Fukui, Springer – Verlag, Berlin, 1986; pp:1328; Price: 2080 Y

Biotechnology has, in recent years, assumed tremendous importance in industry and academia. An

exclusive dictionary on Biotechnology is therefore, well timed. The volume under review is a three language dictionary: English; German; Japanese, and it contains around 6000 technical and scientific terms presented in three parts, each having the terms of one of the languages arranged alphabetically with translations to the other two languages. Thus, the volume is, in fact, three dictionaries under one cover, which enables quick and easy location of expressions in any of the three languages required for translation to the other two. The dictionary is unique in that phrases like 'maximum specific growth rate', 'reagent for clinical laboratory' etc have been included.

The dictionary should prove valuable to translators of scientific literature. In particular, it should be very useful to those who need translations of Japanese and German scientific literature to English like those of us in India who are more conversant with English than the other two languages.

RICHARD JOSEPH
C.F.T.R.I., MYSORE.

AFST(I) News

Ludhiana Chapter

The Annual General Body Meeting was held in August 1988. The following office bearers were elected for 1988-89.

President	: Dr. J.S. Sidhu
Vice-President	: Dr. A.S. Bawa
Hon. Secretary	: Dr. F.C. Shukla
Hon. Jt. Secretary	: Shri. K.S. Minhas
Hon. Treasurer	: Dr. (Mrs) Usha Bajwa

Award

Dr. M.B. Bera, member, AFST(I) Jabalpur chapter has been honoured with Young Scientist Award by M.P. State Council of Science and Technology (MPCOST) and Department of Science and Technology, Government of India at Jiwaji University, Gwalior for his research work on Rice bran.

Hisar Chapter

The Annual General Body Meeting was held on 21st March 1988. The following office bearers were elected for 1988-89.

President	: Dr. D.S. Dahiya
Vice-President	: Dr. H.K.L. Chawla
Hon. Secretary	: Mrs. N. Khetarpaul
Hon. Treasurer	: Dr. (Mrs) Asha

The Editor expresses his gratitude to the following referees for the valuable service in the evaluation of papers submitted to the JOURNAL during 1987-88

Achaya, K.T.
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Zakia Banu
Zia Uddin.

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Publications of the Association of Food Scientists and Technologists (India) CFTRI Campus, Mysore – 570 013, India

Title	India		Foreign	
	Price	Postage	Price	Air Mail
	Rs.	Rs.	US$	US$
1st Indian Convention of Food Scientists and Technologists, 1978 (Proceedings)	25	9	12	6
Symposium on the Status and Prospects of the Confectionery Industry in India, 1979 (Proceedings)	30	13	12	8
Symposium on By-products from Food Industries: Utilization and Disposal, 1980 (Proceedings)	30	9	12	6
2nd Indian Convention of Food Scientists and Technologists, 1981 (Proceedings)	40	13	15	7
3rd Indian Convention of Food Scientists and Technologists, 1983 (Proceedings)	25	9	15	5
4th Indian Convention of Food Scientists and Technologists, 1984 (Proceedings)	35	9	25	6
Symposium on Recent Developments in Food Packaging, 1986 (Proceedings)	65	13	35	8
Prof. V. Subrahmanyan Commemorative Issue, 1980	30	13	12	8
Production and Processing of Meat and Poultry Products, 1986 (Proceedings)	100	9	45*	
2nd International Food Convention & Exhibition (IFCON'88) – Food Technology Overview	100	13	45*	
2nd International Food Convention & Exhibition, (IFCON' 88) – Abstract of Papers	100	13	45*	

*Includes postage

INSTRUCTIONS TO AUTHORS

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

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

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

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

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

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

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

   Citation should be as follows (note the underlines also):

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

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

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

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

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

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

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

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