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RESEARCH PAPERS

Isolation and Utilization of Proteins from Whey Systems of Buffalo Milk on Pilot Scale. I. Application of Sodium Hexametaphosphate Complex for Isolation

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Manuscript received: 21 *March* 1978; *revised:* 24 *January* 1979

Efficacy of two processes employing sodium hexametaphosphate (SHMP) and "Ferripolyphosphate Complex" (FPP) for the recovery of proteins from different systems of buffalo milk was investigated on pilot scale. Recovery of proteins by processes employing SHMP and FPP were found to range between 61 and 63%, and 88 and 89% respectively. The electrophoretic behaviour of whey protein components remained unchanged during the stage of cold precipitation, but was found to undergo change during the spray drying stage of both the processes studied. Based upon the experience of various unit operations involved, recovery of proteins and quality of product obtained, there seems to be a good potential for the application of these processes on industrial scale.

Whey proteins represent a nutritionally superior group of milk proteins, possessing unique biological, and excellent functional characteristics from the view point of their application in food industry[1-5]. It has been tentatively estimated that about 133 million kg of whey is available annually as by product, representing about 0.92 million kg of whey proteins[6]. In view of the prevalence of protein malnutrition in India, recovery of proteins from available sources for human nutrition assumes a special significance. Most of the earlier processes for the recovery of proteins from whey involved, application of heat for precipitation, yielding a denatured product, which was considered quite unsuitable for application for use in the food industry[7]. For developing newer food applications, a number of methods have been developed involving "cold precipitation" technique that yield whey proteins in soluble form. A process patented by Gordon utilized polymeric phosphates for the recovery of whey proteins[8]. Various aspects relating to the pH, ionic concentration, temperature, etc., on the use of polymeric phosphates have been investigated[9-11]. "Ferripolyphesphate (FPP) a liquid complex of ferric ions and polyphosphates, having Fe and P ratio of 1:12 has been found by Jones *et al.*[12] to be quite effective for the isolation of proteins from whey. Studies carried out on different whey systems of buffalo and cow milks have shown that 95 per cent of the proteins could be precipitated by the application of FPP[13].

In India, buffalo milk constitutes the major portion of the milk processed by the organized sector[14]. This study was, therefore, undertaken to investigate the efficacy of sodium hexametaphosphate (SHMP and FPP for the isolation of proteins on pilot scale from various whey systems of buffalo milk.

Materials and Methods

Milk and whey samples: Samples of buffalo milk were collected from the herd of 'Murrah' buffaloes maintained at the National Dairy Research Institute, Karnal. Samples of different whey systems were obtained from the semi-commercial production of casein, cheddar cheese and *Paneer* at the Experimental Dairy, National Dairy Research Institute, Karnal, as described earlier[15].

Isolation of whey proteins using SHMP: The concepts developed during the laboratory investigation reported earlier were employed for the development of process described here[13]. A 800-l. batch of whey was taken in a stainless steel vat of 900 l. capacity. Required amount of SHMP, 0.7 per cent for casein and cheese wheys and 0.4 per cent for *Paneer* whey, as determined by earlier laboratory investigations for precipitating 75 per cent of whey proteins, was dissolved in 80 l. of water, and added to whey with uniform stirring. The pH of this mixture was adjusted to 2.5 using (1:4) hydrochloric acid. Precipitated proteins were recovered by clarifying under continuous flow in a Sharplex Super Centrifuge at 40,000 rpm, using the Deep Layer Clarifier bowl. Complexed HMP was removed as follows: precipitated proteins were dispersed in water to give about 30 per cent total solids (T.S) and 0.5 mole of calcium hydroxide per mole of phosphorus (present in proteins) was added at pH of 8.9, and the mixture warmed to 50°C. The

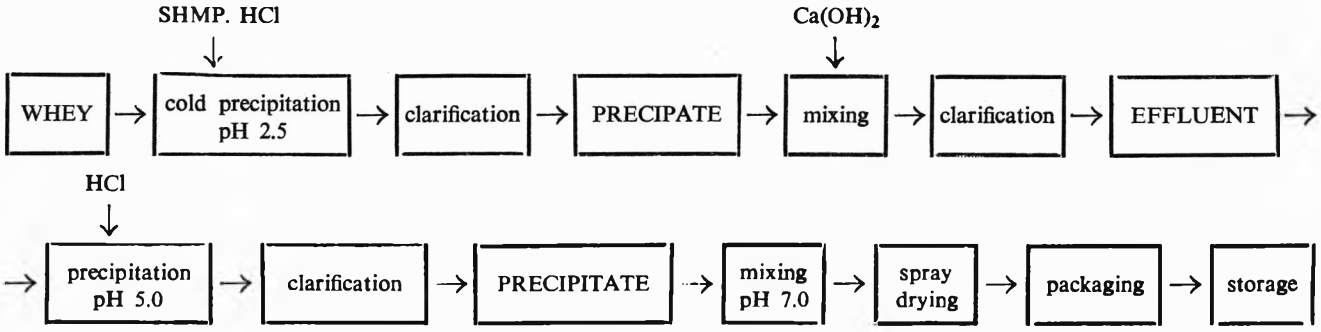

Fig. 1. Schematic diagram for the manufacture of WPI by employing SHMP for 'cold precipitation' of whey proteins.

precipitate of calcium-HMP so formed was removed by clarifying as described earlier. Proteins present in the effluent were precipitated once again at pH 5.0 and recovered by clarifying as described above. Thus, having removed most of the complexed HMP, proteins were dispersed in water to give about 30 per cent T.S. and pH of the suspention adjusted to 7.0 using 20 per cent ammonia solution. Whey proteins were finally spray dried in an Anhydro Spray Drier, by employing air at inlet temperature of 180°C and outlet temperature of 95°C. The rpm of the atomizer was controlled at 25,000±1,000. Spray dried product was packed in 1-kg polythene bags and stored at 10°C. This spray dried product has been referred as WPI.

Isolation of whey proteins using FPP: This process was developed on the basis of preliminary laboratory investigations reported earlier[13]. Seven hundred litres of whey were taken in a stainless steel vat of 900 l. capacity. To this was added a calculated amount of FPP so as to provide 0.048 M, 0.036 M and 0.030 M iron for the casein, rennet and *Paneer* whey systems, respectively (necessary for precipitating nearly 95 per cent proteins). The pH of mixture was adjusted to 3.5 with (1:4) hydrochloric acid. After allowing for 30 min, precipitated proteins were recovered by clarifying under continuous flow in a Sharples Super Centrifuge at 40,000 rpm, using the Deep Clarifier Bowl. Precipitated proteins were suspended in 70 l. acidified water (pH 3.5) and clarified once again, as described before, for removing residual salts and lactose. Washed proteins were suspended in water once again to give about 30 per cent total solids and pH adjusted to 7.0 using ammonia solution (20 per cent). This was dried in an Anhydro Spray Drier, employing air at inlet temperature of 180°C and outlet temperature of 95°C. The atomizer was controlled at 25,000±1,000 rpm. Spray dried product was packed in polythene bags of 1-kg capacity and stored at 12°C for further studies.

Analytical methods: Moisture and fat in various samples were estimated using the Mojonnier modification[16]. Ash was determined according to the method suggested by McKenzie[17]. Kjeldahl nitrogen was estimated according to the modified method of McKenzie[17]. Protein distribution was estimated according to the method of Nagasawa *et al*[18]. Electrophoresis was carried out by the Discontinuous Polyacryamide Gel Plate Electrophoresis (DPGE) technique[13]. Phosphorus was estimated according to the method of Meun and Smitch[19]. Lactose was estimated according to the method of Perry and Doan[20].

Results and Discussion

During the course of this investigation, suitability of the two processes for manufacture of WPI on pilot

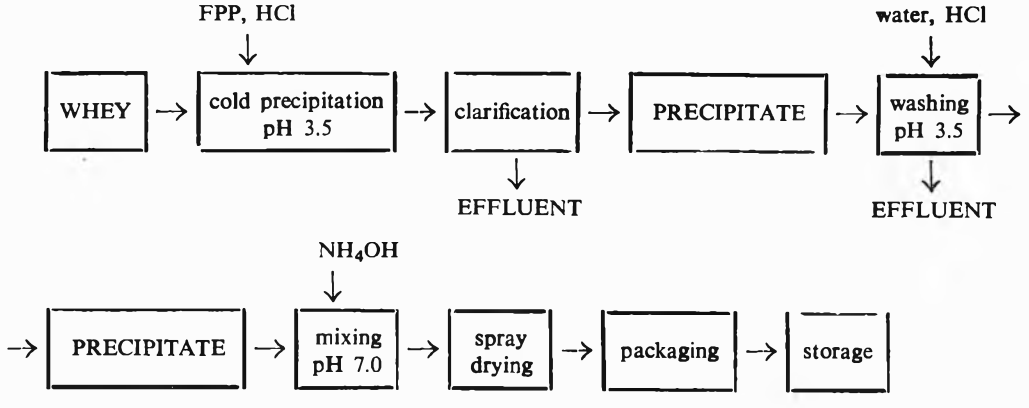

Fig. 2. Schematic diagram for the manufacture of WPI by employing FPP for the 'cold precipitation' of whey proteins.

TABLE 1. RECOVERY OF PROTEINS AT VARIOUS STAGES BY THE PROCESS EMPLOYING "SHMP" AND "FPP" FROM DIFFERENT WHEY SYSTEMS OF BUFFALO MILK ON PILOT SCALE

Manufacturing stage	Loss/recovery (%) of original protein of whey*					
	Casein whey		Rennet whey		*Paneer* whey	
	a	*b*	*a*	*b*	*a*	*b*
Loss in cold precipitation	27.17	6.58	26.29	6.85	25.84	6.25
Loss in HMP removal	6.35	—	6.16	—	5.89	—
Loss in clarification	2.12	2.28	1.87	2.06	1.91	2.64
Loss in spray drying	2.85	2.93	2.83	2.87	2.98	2.98
Total handling losses	38.49	11.79	37.25	11.78	36.62	11.87
Total recovery	61.51	88.21	62.75	88.22	63.38	88.13

*Expressed as the percentage of original protein content of the whey system used.
a = process involving SHMP; *b* = process involving FPP

scale (Fig. 1 and 2) was evaluated on the basis of recovery of proteins from different whey systems of buffalo milk, and quality of spray dried WPI obtained.

a) Comparative efficacy of SHMP and FPP for isolation of whey proteins: Handling loss at various stages of isolation and spray drying of the proteins was studied and results obtained are presented in Table 1. Recoveries of proteins by the process employing FPP were found to be higher (between 94.15 and 94.75 per cent) compared to SHMP (between 77.82 and 74.16 per cent). Electrophoretic studies of the supernatant obtained after "cold precipitation" stage under the influence of SHMP showed that a small proportion of alpha-lactalbumin and serum albumin remained in the supernatant, and could not be recovered. Further, loss of whey proteins occurred when attempt was made to remove the complexed hexametaphosphate thus lowering the overall recovery. However, when FPP was employed for "cold precipitation", almost all the fractions of whey proteins were precipitated. Losses during clarification and spray drying were found to be almost-

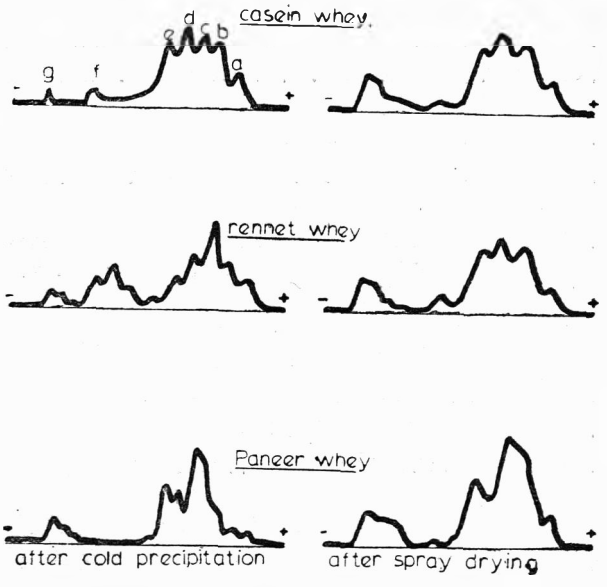

Fig. 3. Electrophoretic behaviour of whey protein components after the stages of 'cold precipitation' and spray drying in the process of their isolation by the application of SHMP.

a, b = beta-lactoglobulins; c, d = alpha-lactalbumins; e = serum albumin; f, g = globulins and lactoferrins.

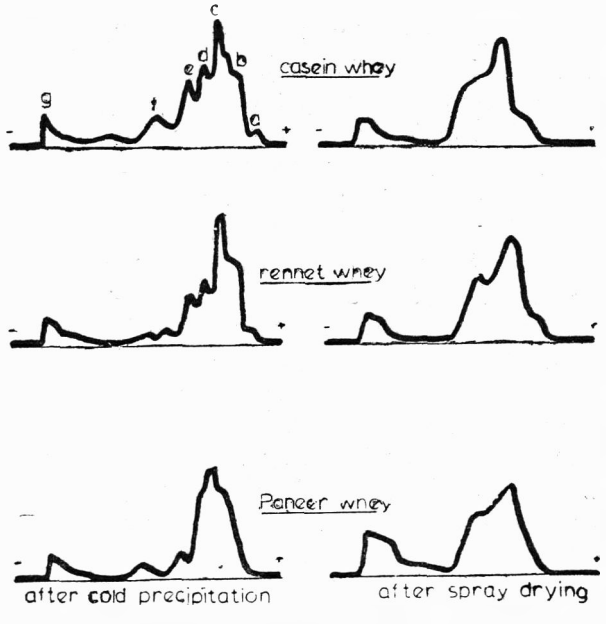

Fig. 4. Electrophoretic behaviour of whey protein components after the stages of 'cold precipitation' and spray drying in the process of their isolation by the application of 'FPP'.

a, b = beta-lactoglobulins; c, d = alpha-lactalbumins; e = serum albumin; f, g = globlins and lactofessins.

TABLE 2. COMPOSITION OF SPRAY DRIED WHEY PROTEIN ISOLATES RECOVERED BY "HEXAMETAPHOSPHATE" AND FPP COMPLEXING FROM BUFFALO MILK WHEY SYSTEMS ON PILOT SCALE

Constituent	Casein whey (%)		Rennet whey (%)		*Paneer* whey (%)	
	a	*b*	*a*	*b*	*a*	*b*
Protein	84.28	50.17	84.31	51.13	83.11	51.22
Fat	—	—	0.02	0.02	0.03	0.02
Lactose	0.01	0.01	0.01	0.01	0.01	0.01
Ash	12.70	46.79	12.63	45.85	13.85	45.73
Moisture	3.01	3.03	3.05	3.01	3.03	3.04
Phosphorus	4.43	27.38	4.96	27.93	4.87	29.07
Iron	1.02	15.41	1.02	13.82	0.82	11.64

a = spray dried WPI obtained by SHMP complexing.
b = spray dried WPI obtained by FPP complexing.

equal in all cases, irrespective of the nature of polyvalent ions used for the recovery of proteins.

b) Comparison of electrophoretic behaviour of WPI: The effect of "cold precipitation" under the spray drying on the denaturation of whey proteins was studied by their electrophoretic behaviour. It may be observed from Fig. 3 and 4 that electrophoretic mobilities of the whey protein components present in the precipitated proteins were quite comparable to the corresponding fractions of the original whey when either SHMP or FPP were employed for cold precipitation. It appears that during the stage of "cold precipitation", the electrophoretic behaviour of whey proteins is not modified. However, whey protein components exhibited a slightly different electrophoretic mobility and sharpness upon spray drying. In another investigation on the commercially available WPI prepared by SHMP complexing, loss of sharpness in the electrophoretic pattern was also observed[3]. It has been suggested by various investigators that the components of whey proteins undergo various degrees of aggregation and denaturation under the influence of heat, ionic concentration and pH[10,21,22]. The altered electrophoretic behaviour of whey proteins observed in case of WPI obtained by employing SHMP and FPP may therefore be attributed to the changes taking place during spray drying.

c) Composition of spray dried WPI: The composition of WPI obtained from different whey systems of buffalo milk by employing SHMP and FPP is given in Table 2. It may be observed that WPI obtained by SHMP complexing were richer in the protein content (83.11-84.31 per cent), compared to those obtained by FPP complexing (50.17-51.22 per cent). The former WPI were particularly low in ash (12.63-13.8 per cent), where as the other WPI were very high in the ash levels (45.73-46.79 per cent). In view of their high iron content, WPI obtained by FPP complexing appears to be useful ingredient for food formulations where concomitant fortification with protein and iron is needed.

Results obtained herein indicate the technical feasibility of employing SHMP and FPP for the isolation of proteins from different whey systems of buffalo milk, and spray drying on pilot scale.

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Isolation and Utilization of Proteins from Whey Systems of Buffalo Milk on Pilot Scale. II. Utilization of Whey Protein Isolates in Forumlated Dairy Products

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Spray dried "Whey protein isolates" (WPI) obtained by SHMP complexing could be utilized in ice cream manufacture for the improvement of "whipping ability" and resistance to "melt down". Such WPI was also found suitable for the manufacture of an infant food formula, having the same whey protein to casein ratio as in human milk. The PER and NPR values of this type of formulation were found to be 3.05 (casein 3.00) and 4.36 (casein 4.00) respectively. Incorporation of WPI obtained by FPP complexing in processed cheese led to some changes in firmness and elasticity but without affecting the flavour of the final product.

Desirability of isolating proteins from whey systems in a soluble and functional form for food applications has been pointed out in Part I of this communication. This investigation had the objective of evaluating the effect of utilization of spray dried WPI isolated by SHMP and FPP complexing on the rheolcgical and nutritional characteristics of a few formulated dairy products.

Materials and Methods

Utilization of WPI for ice cream manufacture: The composition of ice cream mixes used is given in Table 1. After mixing various ingredients (buffalo milk, cream, skim milk powder, WPI, and sodium alginate) in the desired proportion, the mix was forewarmed, homogenized, pasteurized, cooled and aged as suggested by Sommer[1]. Freezing was done in a batch freezer of 25 l. capacity in 10-l. lots. Liquid vanilla essence was added at the rate of 5 ml/10 l. directly in the freezer. After about 7 min when the mix has attained desired consistency, refrigeration was turned off and whipping carried out at air pressure of 15 psig. The time required to attain 100 per cent over run was noted. Ice cream was packaged in 130 ml cups and hardened overnight at −20°C before conducting further tests.

Utilization of WPI for infant food manufacture: A mix was formulated in which the ratio of whey proteins to casein was adjusted so as to simulate the protein profile of human milk, and at the same time meet the standards (other than vitamins) laid down by the Indian Standards Institution[2], as shown in Table 2. This composition was arrived at by using the following ingredients: concentrated skim milk (40 per cent total solids), concentrated whey (30 per cent total solids), cream (40 per cent fat), WPI (obtained by SHMP complexing) and sucrose. All the milk solids were derived from buffalo milk. This mix was forewarmed to 60°C, and homogenized at 175 kg/sq. cm. in first stage and 50 kg/sq.cm. in second stage. After pasteurization at 75°C for 10 min, it was spray

TABLE 1. COMPOSITION OF ICE CREAM MIXES

Ingredient	Control (%)	Exp I (%)	Exp II (%)
Fat	12.00	12.00	12.00
Serum solids from milk ingredients	11.00	10.00	9.00
from WPI	—	1.00	2.00
Sugar	15.00	15.00	15.00
Sodium alginate	0.25	0.10	0.10

TABLE 2. GROSS COMPOSITION OF THE INFANT FOOD FORMULA (PREPARED FROM BUFFALO MILK) HAVING MODIFIED WHEY PROTEIN TO CASEIN RATIO AND I.S.I. REQUIREMENTS FOR INFANT MILK FOODS

Constituent	Infant food	I.S.I. requirements
Whey proteins (%)	12.02	—
Casein (%)	8.06	—
Total proteins (%)	20.08	⩽20
Milk fat (%)	20.02	18.0–28.0
Lactose (%)	32.06	—
Sucrose (%)	18.03	—
Total carbohydrates (%)	50.09	⩽35
Ash (%)	6.64	Max 8.5
Moisture (%)	3.17	Max 3.5
Iron (mg/100 g)	4.01	⩽4.0

dried using the same condition for the manufacture of WPI as described in Part I. The final product was packaged under nitrogen in 500 g containers.

Utilization of WPI for processed cheese manufacture: Processed cheese was manufactured according to the recommendations of Meyer[3]. A blend of 20 kg Cheddar cheese (prepared from buffalo milk, according to the method of Burde and Srinivasan[4] was taken in following proportions: 1-2 months old: 25 per cent; 3-4 months old: 60 per cent; 5 months and more: 15 per cent. In the experimental lot, WPI obtained by FPP complexing was incorporated at the rate of 10 per cent on dry matter basis. Trisodium citrate and dihydrogen phosphate (1:1) were added to the cheese blend at the rate of 2.5 per cent of the weight of cheese. Adequate moisture was added so as to attain 46.5 per cent moisture in the final product. The mix was processed at 80°C/10 min and directly packaged into lacquered tins of 250–g capacity. After cooling to ambient temperature overnight, the product was stored at 12°±2°C, for 7 days, before testing for rheological properties.

Tests for ice cream: Method of Hansen and Black[5] for testing the foam stability was adapted for measuring the "meltdown" time of ice cream. Sample of ice cream was carefully removed by tearing off the sides of cup, and placed on a glass funnel of 10 cm diameter. The volume of melt that was collected in a measuring cylinder was noted at regular intervals of 5 min and expressed as a percentage of original volume of ice cream.

Tests for infant food: Gross composition was determined as described earlier for WPI. Lactose and sucrose were estimated according to the method of Perry and Doan[6]. PER value of the samples was determined by the method of Osborne and Mendel[7]. NPR value of the samples was estimated by method of Bender and Doell[8]. Determinations of PER and NPR values were carried out at the Indian Agricultural Research Institute, ICAR, New Delhi.

Tests for processed cheese: The firmness of processed cheese was determined by measuring the 'Penetration Value' as suggested by the American Oil Chemists' Society Tentative Method, Ce 16-60 (1964). 'Modulus of Elasticity' (EQ) was measured according to the method of Schwarz and Fischer[9]. Organoleptic evaluation of the samples was carried out by a panel of five judges formed from the departmental staff. Samples were tested as "acceptable" or "unacceptable".

Results and Discussion

Utilization of spray dried WPI was considered for the manufacture of ice cream, infant food and processed cheese. In view of the high ash content, use of WPI prepared by FPP complexing was not considered for infant food formulation.

Effect of using WPI in ice cream: Addition of the two types of WPI (prepared by complexing with SHMP and FPP) were found to improve the whipping ability and reduced time to attain 100 per cent over-run. Whipping ability of mix containing 2 per cent WPI was superior to that containing 1 per cent WPI. The values of rate of whipping overlapped for the two types of WPI and are presented in Fig 1.

Data obtained showed favourable effect of adding both types of WPI on the resistance of ice cream to "melt-down". It may be observed from Fig. 2 that with 2 per cent level of WPI, the resistance of resultant ice cream was superior to the ice cream with 1 per cent level of WPI. Furthermore, WPI obtained by SHMP complexing improved resistance to 'melt-down' as effectively as those obtained by FPP complexing. Earlier Lando and Dahle[10] have also shown that the melting resistance of ice cream could be improved by the addition of the whey proteins. It has been suggested that

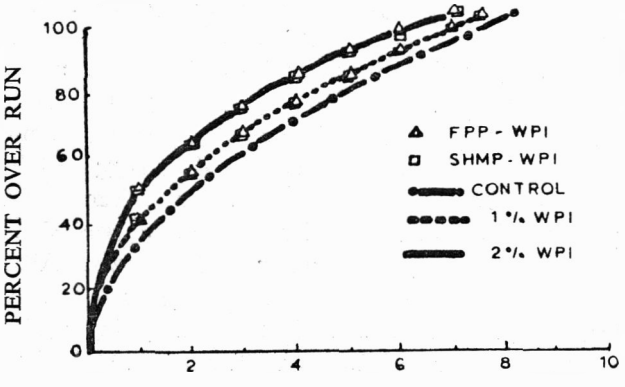

Fig. 1. Effect of addition of WPI on the whipping properties of ice cream mixes.

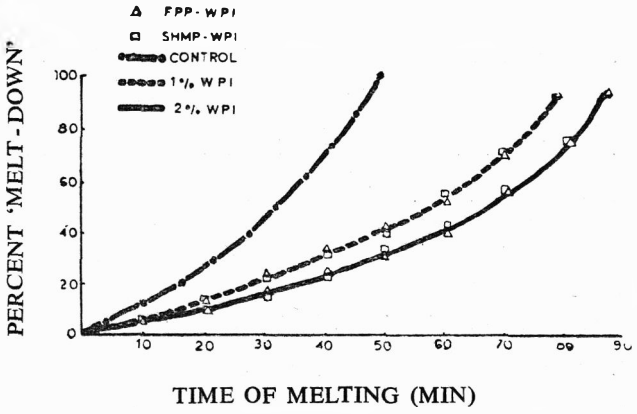

Fig. 2. Effect of addition of WPI on the resistance of ice cream to 'melt-down'

the problem of shrinkage in frozen desserts may be eliminated by the addition of WPI[11].

Effect of using WPI in infant food: Apart from the various differences in the make up of various constituents of buffalo and human milks, one major difference exists in the whey protein to casein ratio. Whey proteins constitute about 65 per cent of total proteins in human milk and about 20 per cent in the case of buffalo and cow milks[12]. Recent studies have shown that infants receiving low whey protein-high casein diets suffer from certain forms of physiological malfunctions which are manifested in the form of microcytosis, ferropenia[13], lower nitrogen retention[14], higher urinary osmillarity[15] and lower weight gain[16]. All of these reports signify the need to modify the protein make up of present day commercially available cow and buffalo milk based formulae for the nutrition of infants.[17].

In view of the above mentioned reports, the technical feasibility of modifying the whey protein to casein ratio by the incorporation of WPI for infant food manufacture was investigated. The composition of the spray dried product so obtained is given in Table 2. This product could be favourably compared to the buffalo whole milk powder in general appearance and was found to be organoleptically acceptable by a panel of five judges. The protein profile of this formulation was compared electrophoretically with that of human milk and three commercially marketed brands in infants foods in India (Fig. 3). It may be observed that in human milk, there was no evidence of the presence of beta-lactoglobulin, thus confirming earlier reports[18]. Two protein bands were observed in the alpha-lactalbumin region, and three in serum albumin region. In commercial samples, varying amounts of beta-lactoglobulin were observed, indicating different intensities of heat treatment during manufacture. Formula prepared by incorporation of WPI showed higher

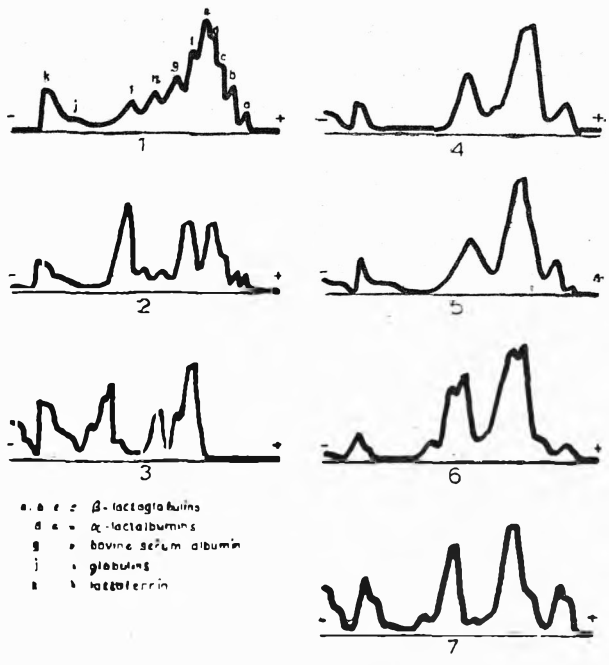

Fig. 3. Electrophoretic behaviour of whey protein components present in different types of milks and infant food formulations.

1. Buffalo milk; 2. Cow milk; 3. Human milk; 4, 5, 6. Commercial samples of infant food formulations; and 7. Formulation with modified whey protein to casein ratio from buffalo milk.

levels of lactoferrin and immunoglobulins thus resembling human milk more closely compared to the commercial samples.

The PER and NPR values of the formulation prepared during this investigation was found to be 3.05 (casein 3.00) and 4.63 (casein 4.00) respectively. It has been reported that feeding formula with modified whey protein to casein ratio give similar physiological response in infants as by feeding human milk[19].

Effect of using WPI in processed cheese: Addition of WPI at 10 per cent level (on dry matter basis) in processed cheese resulted in increasing the penetration value by about 16 per cent, thus reflecting reduction in

TABLE 3. DATA ON THE EFFECT OF ADDITION OF WPI OBTAINED BY USING 'FERRIPOLYPHOSPHATE COMPLEX' ON THE RATE OF PENETRATION, FIRMNESS AND MODULUS OF ELASTICITY OF PROCESSED CHEESE*

Rheological characteristics	Control	Experimental
Rate of penetration (0.1 mm/mm)	59.85±0.89	69.80±1.08
Modulus of elasticity (kg/sq. cm)	21.65±2.38	31.02±2.34

*Av from ten readings.

the firmness of final product (Table 3). The modulus of elasticity was found to increase by 43.3 per cent. However, subjective evluation of experimental product employing organoleptic tests (by a panel of five judges) revealed that the final product was acceptable on the basis of flavour. Other investigators have also recommended addition of whey proteins to processed cheese for increasing the bulk and nutritive value[20-22]. However, according to Krasheninin *et al*[23], this practice leads to reduction of firmness and hardness of the final product.

Results obtained during the course of this investigation indicated that spray dried WPI prepared by complexing with SHMP and FPP could be added to ice cream, infant food and processed cheese manufacture to enhance the desirable characteristics and nutritive value.

Acknowledgement

Authors express appreciation to the Director, Indian Agricultural Research Institute, ICAR, New Delhi for providing necessary facilities for the estimation of PER and NPR values. Assistance from Mr. J. V. Parikh for estimation of Penetration Value and modulus of elasticity is greatefully acknowledged. Thanks are also due to Prof. K. M. Shahani, Department of Food Science and Technology, University of Nebraska, Lincoln, USA for providing help in the preparation of this manuscript.

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Recovery of Cheese Whey Proteins Through Ultrafiltration

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A Process for recycling of whey proteins into cheese through ultrafiltration is proposed. Higher concentration of proteins in cheese whey was found to be necessary for effective recovery of proteins. At 70°C, protein recovery of 60% was obtained for a whey concentrate having 4.18% protein.

The proteins of cheese whey make up approximately 18 per cent of total proteins of milk. The nutritional value of whey proteins is evident from the fact that 14.5 g of α-lactalbumin per day would meet the amino acid requirements of an adult[1]. A process that can incorporate the whey proteins into the cheese itself is most relevant to the cheese plants. Such a process would improve the yield of cheese by 10 to 12 per cent and consequently is of practical value[2]. A two step membrane process is generally considered in the utilization and disposal of cheese whey. The first step of the process is ultrafiltration which reclaims the whey proteins and rejects lactose, salts and water. The second step–reverse osmosis, uses the deproteinized whey and separates from it the lactose which is primarily responsible for high biological oxygen demand (BOD) of cheese whey. The resulting permeate which has a BOD requirement of 1000 mg/l can be safely lead into riverse or recycled within the plant[3].

In this paper a process for recovery of whey proteins through ultrafiltration into cheese is proposed and the effect of whey protein concentration and processing temperature on the recovery of proteins is reported. The process proposed is illustrated in Fig. 1.

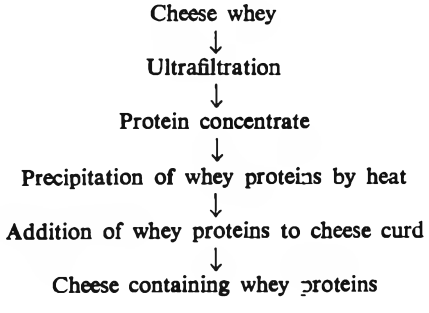

Fig. 1. Process for recycling whey proteins into cheese curd.

Materials and Methods

An ultrafiltration system was assembled with Calgon-Havens tubular membrane model 215 and Robbins and Mayer reverse osmosis pump model SRM-156-B-20. The membrane and pump were connected through piping and valves. The schematic diagram of the system is shown in Fig. 2. The whey to be concentrated was stored in the tank. The pump was started with the valves 1, 2, 3, 4, 5 and 8 open and 6 and 7 closed. With the pump running, the valves 2 and 3 were gradually closed to let the entire quantity of whey being handled to pass through the membrane. The system pressure was adjusted by regulating the valve 4. The membrane was operated at 50 psig. By keeping the valve 5 open and 6 closed, tap water was circulated through the heat exchanger. As the concentration progressed, samples of permeate were also collected. At the end of concentration operation, tap water was diverted into the tank by keeping the valve 5 closed and 6 open. Water was circulated once through to clean the system with valve 8 closed and 7 open. The circulation of water was continued until

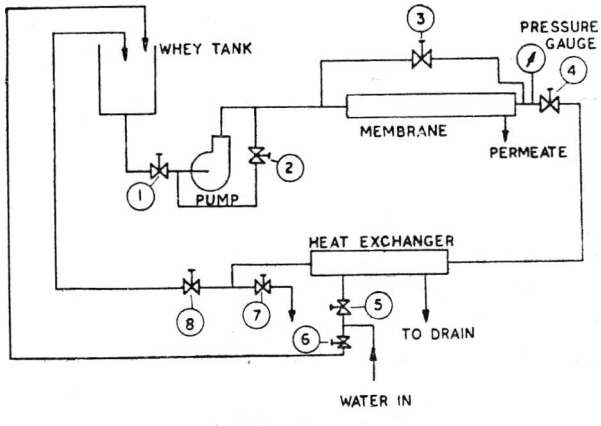

Fig. 2. Ultrafiltration system

clear water was noticed at the outlet of the membrane. An iodophor detergent sanitizer was added to the water in the tank and it was circulated through the system for about an hour. Finally, the system was drained. The protein content in the whey concentrate was estimated by Kjeldahl method. A constant temperature bath was employed for heating the whey concentrates. All the samples were held at the constant temperature for 15 min. A laboratory centrifuge was used for separating the precipitated proteins.

Results and Discussion

System performance: The performance of the ultrafiltration system is shown in Fig 3. It was observed that while the concentration of protein in whey increased steadily with time from 0.917 per cent in raw whey to 4.91 per cent, further concentration beyond this value become low. During the next 7.5 hr, the concentration of protein increased only to 5.01 per cent. The drop in permeation rate was due to the phenomenon of concentration, polarisation and fouling of the membrane.

Fig. 4 illustrates the relation between the percentage of protein in whey and that in permeate leaving the system. It was observed that increase in concentration of protein in whey results in increased loss of protein through the permeate. This observation was consistent with the another report[3].

Protein recovery from whey concentrates: The whey concentrates which were drawn at regular intervals were numbered 0, (raw whey), 1, 2, 3, 4 and 5 and the concentration of protein in these samples were 0.917, 2.74, 3.73, 4.18, 4.75 and 5.01 respectively.

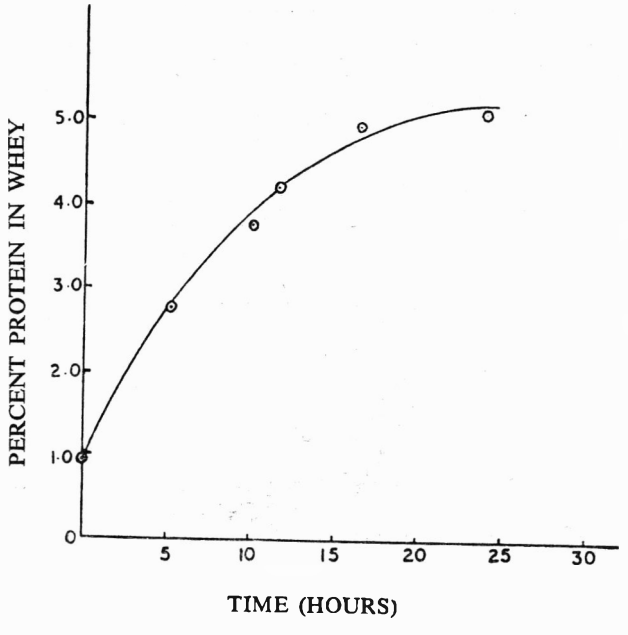

Fig. 3. Time taken by the system to reach various levels of concentration.

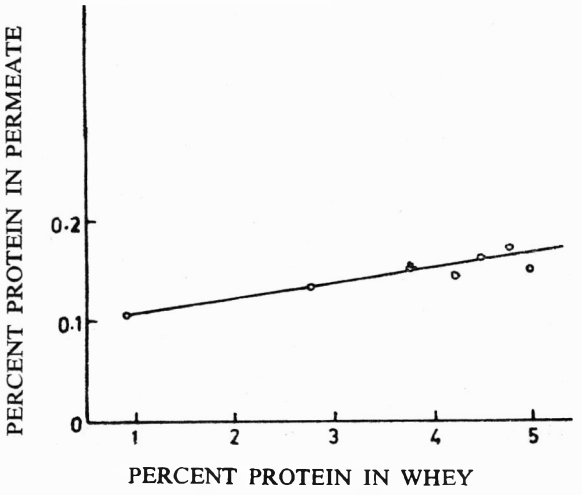

Fig. 4. Protein concentration in permeate at various levels of whey concentration.

Each of these concentrates was held at 40°, 50°, 60°, 70° and 80°C for 15 min in a constant temperature bath. The precipitation and settling of proteins were clearly visible. The concentrates were withdrawn from the bath and to complete the settling of proteins, they were centrifuged. The concentration of protein in the supernatants was estimated. With concentration of protein in the whey concentrate and its supernatant known, the

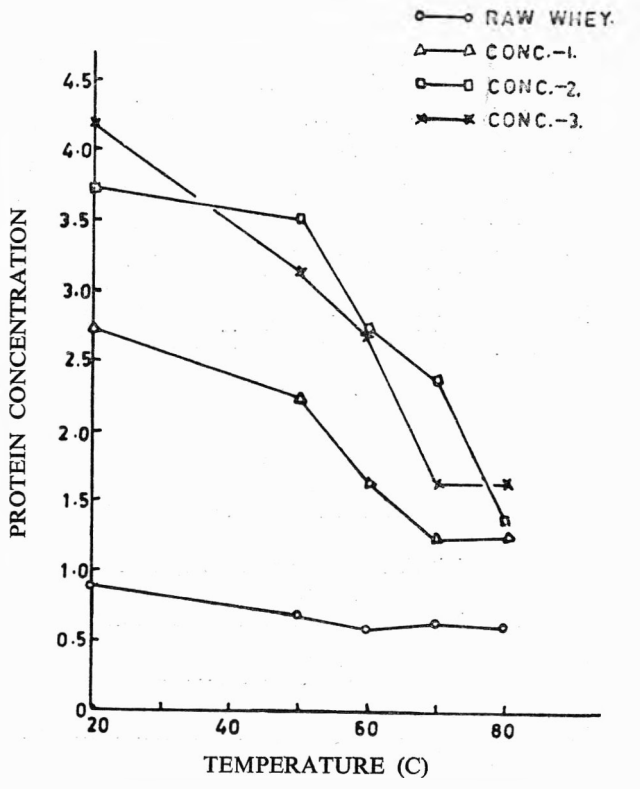

Fig. 5. Protein concentration in supernatants.

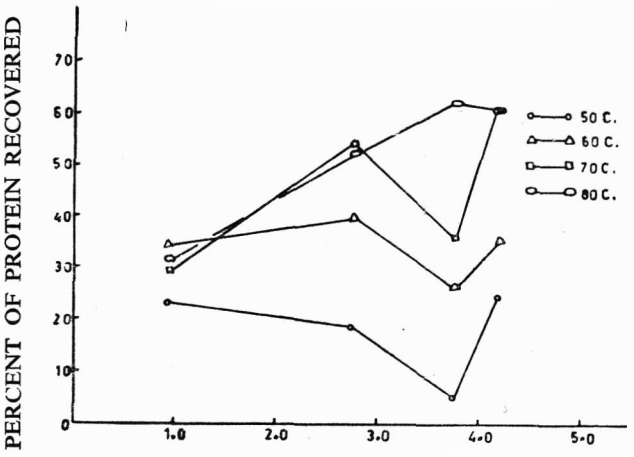

Fig. 6. Recovery of protein at varying concentrations.

percentage of protein precipitated at different levels of its concentration in whey was calculated.

The results obtained are illustrated in Fig. 5 and 6. Fig. 5 shows that for a given inital protein concentration, the amount of protein left in the supernatant decreases with increasing temperature. In other words, the amount of protein precipitated or recovered for a given level of concentration increased with increasing temperature. The concentration of protein in the supernatant of raw whey had not changed appreciably, whereas for the concentrated samples, the protein concentration in the supernatant rapidly decreased with increasing temperature. This clearly demonstrates the necessity of higher levels of protein concentration for effective recovery of proteins from the whey. Further, beyond 4.18 per cent of protein concentration in whey the recovery of proteins was not significant at temperatures above 70°C. Fig. 6 illustrates the relation between percent recovery of protein and concentration of protein in cheese whey at different temperatures. At temperatures 60°C and above the recovery of proteins increased with increasing concentration of protein in whey. However, the recovery dropped for all the temperatures studied except 80°C when the concentration of protein was 3.73 per cent. The recovery increased again beyond this level of protein concentration. This peculiar behaviour in solubility of whey proteins could be due to some complex protein interactions.

The samples having protein concentration of 4.75 per cent and above, gave gels on heating at 60°C. It may be recalled that the concentrate 3, which had 4.18 per cent protein had given a precipitate. This shows that gelation would begin to occur when the concentration of protein in whey lies between 4.18 and 4.75 per cent. With increasing concentration of protein in whey gelation was found to occur at lower temperatures.

It is thus concluded that proteins from cheese whey could be recovered through ultrafiltration and thermal processing of the concentrates so obtained. The percentage recovery of protein from whey concentrate having 4.18 per cent protein was found to be 60 per cent at 70°C.

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Modified High Temperature Short Time Cream Pasteurizer for Low Energy Consumption

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A Plate type high temperature short time cream pasteurizer previously being operated without regenerator section in a factory was modified to include regenerator with 70% regeneration efficiency. The design method and the performance of the designed pasteurizer are reported. Due to the saving in steam alone, the processing cost has been reduced by Rs. 210.00 per day.

The pasteurization of cream decidedly has desirable effects on the properties and shelf life of butter. Therefore, different time temperature combinations have been suggested[1,2] for pasteurization of cream. Of the different pasteurisation methods for cream, high temperatue short time (HTST) process has been reported to have many favourable points, and different temperatures have been suggested[2] varying from 82.2° to 95°C, followed by immediate cooling to 7.2°C or below.

It has long been established that the HTST pasteurization plants employing plate heat exchangers have many advantages over other heat exchangers. One biggest advantage is that the heat can be recovered efficiently and cheaply upto the tune of 90 per cent of the total requirement depending upon the viscosity of the product. That means 90 per cent of the total energy required for the pasteurization is saved. The management of a dairy plant owning a 60 tonnes per day capacity cream pasteurizer without having regeneration section, (Fig. 1) observed that the equipment was giving poor performance due to high energy consumption. It was estimated that the total steam consumed for cream pasteurization was 2.6 tonnes per day of which about 1.8 tonnes per day could be recovered by regeneration of only 70 per cent.

Therefore, the problem was to design HTST plate pasteurizer employing regeneration section, which would operate efficiently and economically and would cut down the energy consumption by about 70 per cent. The design method and the performance of the pasteurizer are reported here.

The pasteurizer was to pasteurize 4000 kg of cream per hour at 90°C for 15 sec immediately cooled by well water and finally chilled to 5°-6°C by chilled water at 2-4°C. The cooling water from cooling tower was available at 30-32°C. The cream containing 35-40 per cent fat was initially at 56-60°C temperature.

The pasteurizer consisted of four sections: regeneration, heating, cooling and chilling all employing plate heat exchangers (Fig. 2). In the design, following assumptions were made.

(*a*) The fluid existed only in the liquid phase within the exchanger. (*b*) The overall heat transfer coefficient was constant throughout the heat exchanger. (*c*) The flow and temperature transients across the plate were

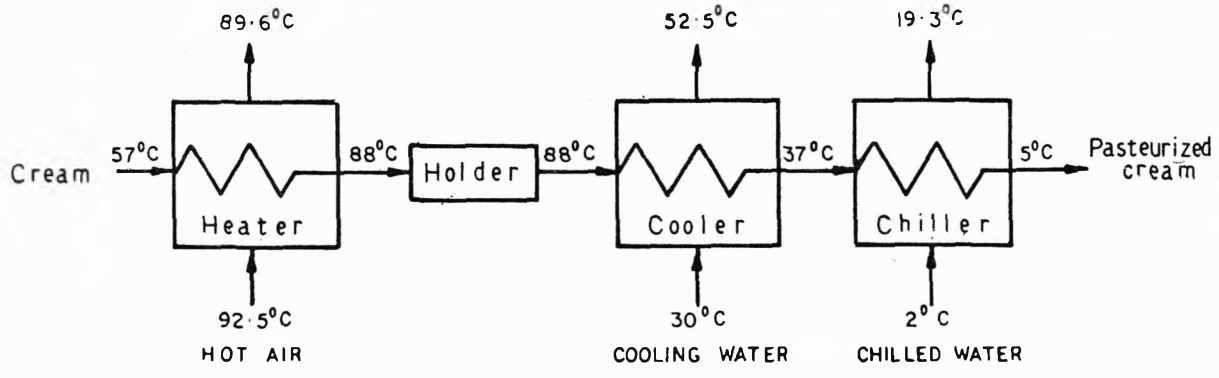

Fig. 1. Different sections of old cream pasteurizer

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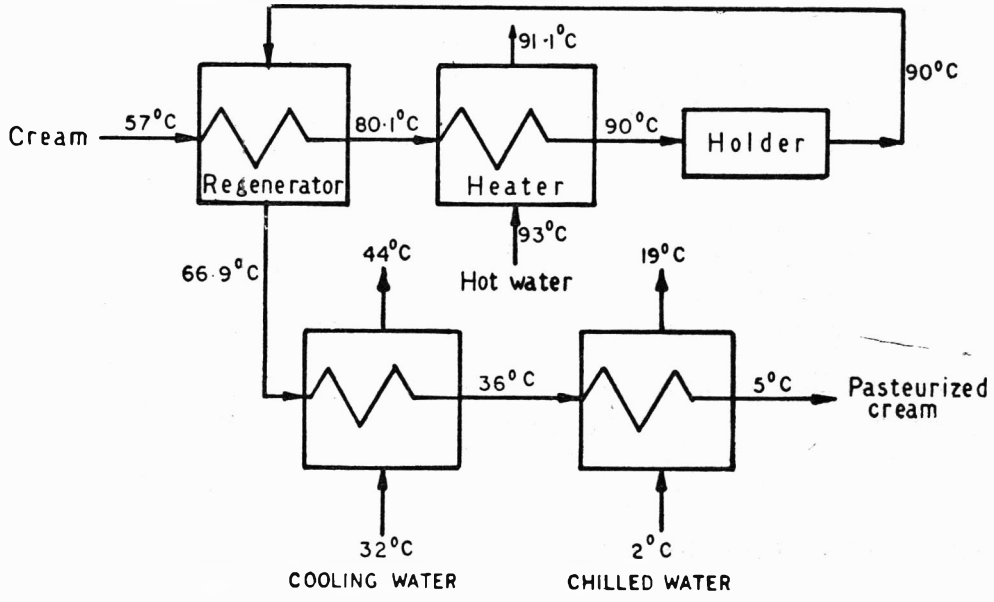

Fig. 2. Different sections in designed cream pasteurizer

negligible. (*d*) Air pockets did not form at or above the channel flow rate of 1000 kg cream per hour. (*e*) The heat losses to the surrounding areas were negligible.

Because of its high transfer efficiency, looped flow pattern as shown in Fig. 3 was used. However number of passes for both the sides were not the same in all the sections.

The basic equation for heat transfer from one object to another, $q=UA\ \triangle t$, was written for the present case in the following form:

$$q=U_{av}(A_p\ N)\ (F\triangle T) \qquad \ldots.(1)$$

where, q=rate of heat transfer, Kcal/hr.; U_{av}= average overall heat transfer coefficient, Kcal/hr m^2 °C; A_p=heat transfer surface area of one plate, m^2; N=number of plates; F=correction factor in $\triangle T$, for deviation from true countercurrent flow; $\triangle T$=log mean or average temperature difference between two fluids °C.

This equation was used to calculate N. The use of this equation however, required the calculation of q, U_{av} and $\triangle T$, as A_p was known and F could be read from curves[3].

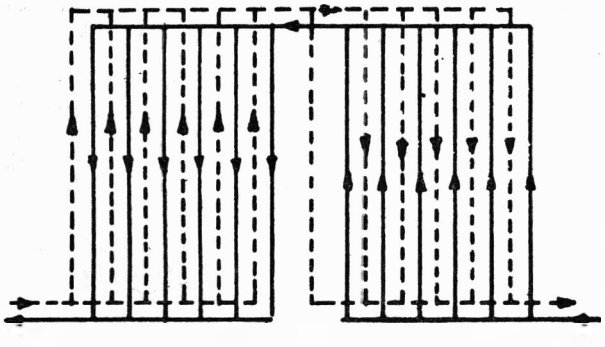

Fig. 3. Looped flow pattern 2 pass / 2 pass.

For true counter-current flow, it was equal to 1. The procedure used is summarized in the following steps.

1. The inlet and outlet temperatures of cream in all sections were determined knowing the regeneration efficiency and terminal temperature of cream.

2. Assuming the flow rates of heating water and cooling water, the outlet temperatures of both were calculated by using the heat balance equation:

$$mC_{pc}(t_1-t_2)=W\ C_{pw}\ (T_1-T_2) \qquad \ldots.(2)$$

where m=flow rate of cream; W=flow rate of medium (water); C_{pc}, C_{pw}=average specific heats of cream and medium respectively; (t_1-t_2)=temperature rise or drop of cream; (T_1-T_2)=temperature rise or drop of water.

In this equation only outlet temperature of water (in both heating and cooling) was unknown which was calculated.

3. Rate of heat transfer, q was calculated using the equation:

$$q=m\ C_{pc}\ (\triangle t) \qquad \ldots.(3)$$

4. The temperature difference, $\triangle T$, for heating and cooling sections was calculated by the following formula:

$$\triangle T=\frac{\triangle T_1-\triangle T_2}{ln\left(\frac{\triangle T_1}{\triangle T_2}\right)} \qquad \ldots.(4)$$

where, $\triangle T_1$=larger temperature difference between cream and water; $\triangle T_2$=smaller temperature difference between cream and water.

In regeneration section, cream flowed on both the sides with same flow rates and temperature drops. Therefore at both ends temperature difference was equal. The

$\triangle T$ in this case was simple temperature difference between the two side streams.

5. The overall heat transfer coefficient was calculated from the following equation neglecting the fouling factor on cream side:

$$U_{av} = \frac{1}{\frac{1}{h_c} + \frac{1}{h_h} + \frac{1}{h_f} + \frac{x}{k}} \quad \ldots(5)$$

where, h_c h_h=film heat transfer coefficients for cold side and hot side of the heat exchanger, respectively.

x=thickness of the plate; K=thermal conductivity of the plate; h_f = fouling coefficient on water side.

6. The film heat transfer coefficient for both the sides was calculated using the following equation recommended by Marriot[4] for most of the commercial plates:

$$\frac{h\,D_e}{k} = C\left(\frac{G\,D_e}{\mu}\right)^{0.65}\left(\frac{C_p\,\mu}{k}\right)^{0.4} \quad \ldots(6)$$

Here, C_p, k and μ = are specific heat, thermal conductivity and viscosity, respectively, of the liquid at its average temperature; D_e=equivalent diameter, twice the gap between 2 plates; G=mass flow velocity, which was calculated by dividing the total mass flow rate by number of passes and flow area of one pass. The value of constant C depends upon the design of plate and is therefore, different for different types of plates. Since the value of C for the plate used (Alfa-Laval type P-14 RB) was not known, it was determined by conducting experiments on an old cream pasteurizer. The value was found to be 0.125. Number of plates was calculated using the equation:

$$q = U_{av}(A_p\ N)\ F\triangle T$$

The details of the plates used are given in Table 1.

Literature values of the physical and thermal properties of cream and water were used; viscosity of cream was taken from Phipps[5], thermal conductivity from Fernandez-Martin[6] and density from Farrall[7]; the properties of water were taken from Perry *et al*[8]. These values were read at average temperature.

TABLE 1. DATA CONCERNING THE PLATES USED IN THE EXPERIMENT

Effective length of plate		= 0.8 m
Effective width of plate		= 0.32 m
Plate thickness	—	= 1.5×10^{-3}m
Effective gap between two plates		= 3.5×10^{-3}m
Effective heat transfer area of a plate		= 0.338 m^2
Net flow area between two plates		= $11.2\times10^{-4}m^2$
Equivalent diameter	—	= 7×10^{-3} m
Metal of construction	—	= Stainless steel
Thermal conductivity of plate	—	= 18 kcal/hr m^2 °C.

TABLE 2. CALCULATION RESULTS OF THE DESIGNED CREAM PASTEURIZER

Quantity calculated	Regenerator	Heater	Cooler
$\triangle T$, °C	9.9	6.2	13.7
F	1	0.92	1.00
q, kcal/hr	69300	30000	96100
h_c kcal/hr m^2 °C	1426.7	15.37	4383.1
n_h kcal/hr m^2 °C	1488.8	5931.1	1114.3
h_f kcal/hr m^2 °C	—	$(0.1\times10^{-4-1})$ water side	$(0.6\times10^{-4-1})$ water side
U_{av}, kcal/hr m^2 °C	686.9	1093.1	788.1
N	31	14	27
No. of passes	5 on each side	2 on cream side 1 on water side	4 on each side

The temperature conditions in the pasteurizer considering regeneration efficiency as 70 per cent are given in Fig. 2. In the regeneration section, the counter-current looped flow pattern was assumed to have 3 streams of cream per loop or pass on each side. In the heating section hot water flow rate was taken to be 3 times the flow rate of cream. In this section counter-current looped flow pattern with 3 passages per pass of cream and 6 passages per pass of hot water were assumed. In the cooling section the cooling water flow rate was taken as twice the flow rate of cream. Counter-current looped flow pattern with 4 passages per pass of both cream and cooling water was assumed in this section. Table 2 summarises the result of calculations.

In chilling section the terminal temperatures were the same as those in the chilling section of the old pasteurizer. Hence, there was no necessity of redesigning this section, and the number of plates used by the old pasteurizer in this section, i.e., 58, was taken in the new pasteurizer also.

The designed pasteurizer was tested for its performance. All the equipment of the system, viz. regenerator, heater, holding block, well water section, chilled water

TABLE 3. COMPARISON OF DESIGN AND OBSERVED TEMPERATURES OF CREAM IN THE DESIGNED CREAM PASTEURIZER

Section	Design temp of cream°C		Observed temp of cream°C	
	Inlet	outlet	inlet	outlet
Regenerator—down	57.0	80.1	57.0	81.0
Regenerator—up	90.0	66.9	90.5	66.0
Heating —	80.1	90.0	81.0	90.0
Cooling —	66.9	36.0	66.0	36.5
Chilling —	36.0	6.0	36.5	6.0

TABLE 4. PERFORMANCE OF OLD AND DESIGNED CREAM PASTEURIZERS

Particulars	Old pasteurizer	Designed pasteurizer			Saving by designed pasteurizer (%)
		Design value	Observed value	Deviation, (%)	
Specific steam consumption, kg steam/100 kg cream	4.320	1.325	1.344	1.460	69.12
Specific heat consumption, kcal/kg cream	24.21	7.42	7.53		
Specific heat load on cooling tower, kcal/kg cream	43.90	24.00	22.84	–6.58	48.02
Specific refrigeration load kcal/kg cream	32.00		30.5	—	4.69
Specific electrical energy consumption, kcal/kg cream	1.168		1.278		–8.6
Regeneration %		70.00	71.64	+2.29	38.48
Saving in total specific energy consumption, kcal/kg cream					

section, balance tanks for cream, chilled water, well water and hot water, pumps for cream, well water, chilled water and hot water, valves and pipings were all properly arranged similar to standard pasteurization plant. All connections were properly made, and the plant operated.

The performance of the designed and old pasteurizers was tested on the basis of specific steam consumption, specific heat load on the cooling tower, specific refrigeration load and specific electrical energy consumption. The specific steam consumption is defined here as kilogram of steam consumed for each kilogram of cream. Specific heat load on cooling tower is expressed as kcal/kg of cream and was calculated as follows:

$$q = \frac{m_w (t_o - t_i)}{m_c}$$

where, m_w is cooling water flow rate, kg/hr; t_o and t_i are respectively outlet and inlet temperatures of cooling water; and m_c is flow rate of cream kg/hr.

Specific refrigeration load is expressed as kcal abstracted from cream by refrigeration per hour. Specific electrical energy consumption is expressed as kcal/kg of cream. In this the electrical energy was converted into heat energy.

Results presented here are the average of three replications.

Test results on the performance of the designed pasteurizer gave excellent agreement with the design values. As shown in Table 3 the temperatures of cream at various points were very close to or same as the design temperatures. Table 4 compares the designed and the observed values of specific steam consumption, specific heat load on cooling tower and regeneration efficiency, and it is observed that the differences are negligible. Thus, the design method used here seems to be quite satisfactory and can be used without loss of accuracy.

The performance of the old and the designed cream pasteurizers is compared and the data are given in Table 2. It is observed that the designed pasteurizer affects saving in specific steam consumption by over 69 per cent, in specific heat load on cooling tower by about 48 per cent, in specific refrigeration load by about 5 per cent, and overall specific energy consumption by over 38 per cent. Specific electrical energy consumption is more by about 9 per cent.

Thus the designed pasteurizer is saving the factory energy by about 40 per cent of the previous consumption. The decreased steam consumption alone is saving the factory daily about Rs. 210, considering the cost of heat as Rs. 0.23 per 1000 kcal[9].

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Protein Concentrate from Waste Catfish and Its Quality Improvement by Enzyme

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An effort was made to utilize the catfish (*Silurus glanis*) for the production of a cheap and acceptable protein concentrate. After solvent extraction and other process treatments, the fish could be suitably converted to a product having dispersible and functional qualities of protein. Conventional processing was found to be improved by an initial steaming operation. Both steaming and addition of brine during blending showed beneficial effect on the colour of the product which also depends on the drying conditions. Mild hydrolysis of the product was necessary for desirable dispersion and foaming. This could be achieved by the proper control of some proteolytic enzymes. Treatment with acetic acid prior to proteolytic hydrolysis improved the swelling property of the product.

Silurus glanis, a catfish, available in large quantities in Southern Iraq is not liked by the people and hence goes waste. The fish could be processed for extraction of protein in a concentrated form which may find better and economic use.

The fish protein concentrate could become more nutritional and economically viable provided its method of extraction and process treatments are made suitable for the improvement in the functional quality of the final product. Available literature such as those of Anon[1], Cobb and Hyder[2] and Spinelli *et al*[3]. on the production of fish protein concentrate (FPC) show that the product from other fish does not attain desirable properties which warrant further studies to improve its dispersibility, swelling, foaming and rehydration capacity. Chemical methods have been applied for the improvement of these properties by Groninger[4], Hermansson *et al*[5]. and Koury and Spinelli[6], whereas Cheftel *et al*[7]., Groninger and Miller[8]. and Spinelli *et al*[9]. have tried to modify the product both by chemical and enzymatic treatments.

This study was aimed at developing a suitable process for the utilisation of catfish. It is also the intention to examine the effect of proteolytic enzymes on the product performance.

Materials and Methods

Fish protein concentrate was prepared from *S. glanis* (catfish) procured from fisherman's catches of the Shatt Al-Arab. The fish was kept in the deep freeze till used. Pepsin (porcine) and papain were obtained from Pfizer Chemicals Division, N. Y. Folin-Ciocaltau's phenol reagent from E. Merck, Germany. Reagent grade chemicals and solvents were used.

Sample preparation: Fish samples were prepared by washing outer slimes with 0.5 per cent salt solution. The fish flesh after removing bones and skin was blended either by addition of 2.5 per cent sodium chloride solution or after steaming under pressure for 5 min.

Analytical methods: Moisture, fat, protein and ash in fresh fish and finished products were determined by AOAC[10] procedures. Moisture was estimated in air-oven at 100-102°C till constant weight, fat by Soxhlet apparatus using petroleum ether for a circulating period of 12 hr, protein by micro-Kjeldahl method digesting 20 mg of sample in each estimation and ash in muffle furnace at 525°C till white and constant weight.

Enzyme assay: Enzyme activity was measured by taking 0.5 mg enzyme in 5 ml 1 per cent casein and allowing the reaction under specified conditions of time, temperature and pH. The enzyme was quickly inactivated by heating at 80°C for 15 min. Nitrogen in supernatant liquid separated from casein precipitated by the addition of 5 ml of 24 per cent trichloroacetic acid was determined by the method of Lowry *et al*[11]. and the enzyme units were calculated on the basis of tyrosine released.

Extraction: After blending, pH of fish samples was adjusted to its isoelectric point of about 4 and the extraction of protein was done with iso-propyl alcohol. Optimum conditions of temperature, time and the ratio

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between the fish and the solvent were determined by trial and error in batch methods.

Acetification: The FPC was treated with dilute acetic acid in the ratio of 1:10 (wt./vol) for about 30 min at room temperature.

Hydrolysis: Partial hydrolysis of protein concentrate was done by pepsin and papain using enzyme in the per centages of 0.01, 0.005 and 0.001 at a temperature of 35°C and pH 2.0 and 6.5 respectively for a definite time. The enzyme action was terminated in the same way as was done in enzyme assay. The degree of digestion was measured after estimating protein in the supernatant liquid[11]. After inactivation of enzyme and cooling, pH of the product was adjusted to 7. The extracted and hydrolysed protein isolates were dried at temperature of 85-90°C.

Dispersion: For determining the dispersibility, 1 g of the prepared product in 100 ml of water was mixed in a blender for 5 min and immediately transferred to a 250 ml graduated cylinder. The degree of dispersion in each run was noted by measuring the maximum settling.

Foaming: Foaming of both hydrolysed and untreated protein concentrate was tested. For the preparation of foam, 1 g of prepared and treated material was whipped in a Waring Blender for a definite period using 100 ml of water. Initial foam volume was determined by matching the level with that of the calibration already done on the blender glass. For determining the stability of foam, the whipped material after noting its initial volume was transferred to a graduated cylinder. The stability of foam at the room temperature was measured by noting the reduction in volume at regular intervals.

Results and Discussion

Washing with 0.5 per cent brine instead of water helped in removal of extraneous slimy materials. Bone and skin could be easily detached and separated by the operation of steaming the fish under low pressure for 5 min compared to that done by the addition of 2.5 per cent sodium chloride solution during blending. Blending with salt solution softened the structural rigidity of fish muscle. This was also observed by Chu and Piggot[12]. Steaming before blending or the addition of brine during blending improved the colour of the final product. Both steaming and salting might have inactivated biochemical changes in the fish.

For the removal of fat and deodorisation of protein concentrate, iso-propyl alcohol was found to be the most efficient solvent. Petroleum ether also extracted lipids and absorbed flavour, but the product was not satisfactory. Carbon tetrachloride did not extract oil completely, further it made the flesh a sticky mass. For optimum extraction, a ratio of fish flesh to iso-propyl alcohol (at 65°C for 20 min) was 1:4 (wt/vol). The same procedure when replicated at least three times resulted in almost complete removal of lipids. The extracted protein followed by water treatments three times in the similar way made the product free from solvent and its flavour. The composition of the fish and FPC is presented in Table 1.

TABLE 1. COMPOSITION OF FISH FLESH AND FPC

	Fresh fish (%) (wet wt basis)	FPC (%)
Moisture	77.70	3.74
Fat	4.38	traces
Protein	16.50	90.50
Ash	0.96	4.83

Specific activities of papain and pepsin used for the digestion of protein concentrate were found to be 15.8 and 24.5 units/mg enzyme respectively. They show different degrees of proteolysis on FPC.

Effect of temperature and pH on hydrolysis: The results on the digestion of FPC by changing temperature and pH are shown in Fig. 1 and 2.

It is observed that keeping other conditions constant the extent of hydrolysis does not increase beyond 35°C (Fig. 1). Fig. 2 shows that there is almost no change in the degree of digestion when pH is changed from 2 to 4 and 2 to 8 for pepsin and papain respectively.

Solubilisation of FPC by enzyme: The effects of both papain and pepsin on FPC are shown in Fig. 3. The

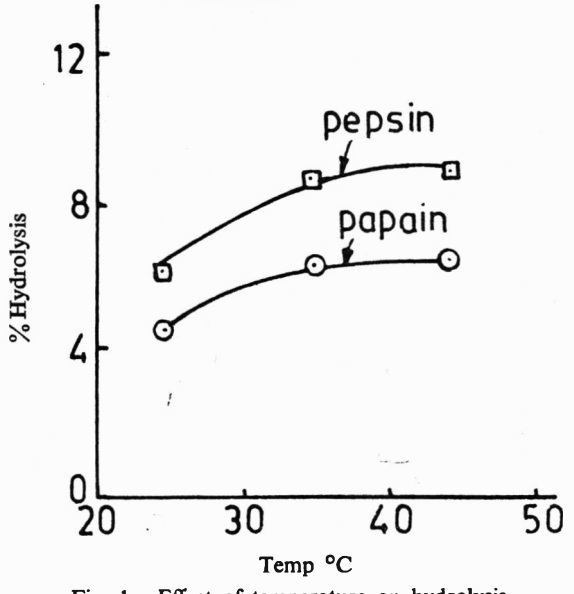

Fig. 1. Effect of temperature on hydrolysis

(FPC: H_2O = 10% (w/v); pH (pepsin) = 2.0, pH (papain) = 6.5; time = 2 hr and % enzyme = 0.005)

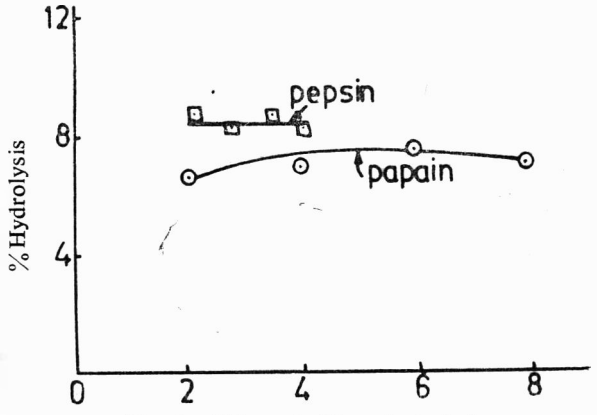

Fig. 2. Effect of pH on hydrolysis

(FPC: H_2O = 10% (w/v); temp. = 35°C; time = 2 hr; % enzyme = 0.005)

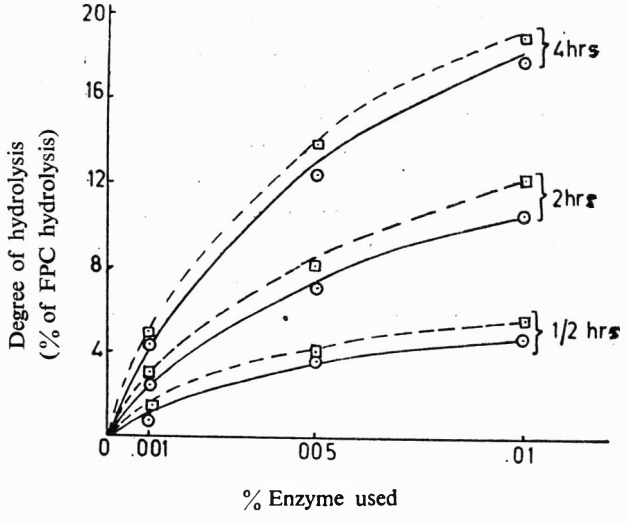

Fig. 3. Extent of enzyme hydrolysis
(pepsin (pH 2.0, temp. 35°C); papain (pH 6.5, temp. 35°C); FPC concentration = 10% (w/v))

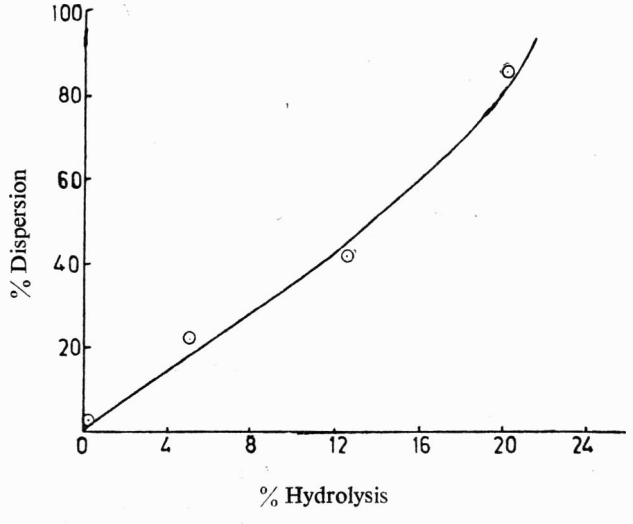

Fig. 4. Degree of dispersion.

TABLE 2. FOAMING OF HYDROLYSED FPC AND ITS STABILITY

Enzymes	Hydrolysis (%)	Initial foam vol (ml)	Foam reduction from initial vol (ml) after		
			½ hr	1 hr	2 hr
Pepsin	4.1	29	12	14	15
	9.0	37	13	16	17
	14.2	60	8	10	10
Papain	2.1	18	13	15	17
	6.7	35	14	16	18
	12.5	40	9	12	13

degree of hydrolysis was taken as the percentage of the amount of FPC hydrolysed.

With increasing concentration of enzymes, there is increase in soluble nitrogen. Though it is mild, more hydrolysis is observed with higher percentages of enzymes and longer period. The results agree with data reported by Hermansson *et al*[13]. Under same conditions of time, temperature and enzyme-protein ratio the hydrolysis brought about by pepsin is higher than that by papain. More hydrolysis can also be achieved with increase in time.

Dispersibility: Fig. 4 shows the degree of dispersion of hydrolysed FPC.

More dispersion is achieved with higher percentages of hydrolysis. The untreated product shows very little dispersion, whereas about 80 per cent dispersion is observed when the product is hydrolysed to 18.7 per cent. The swelling could be improved by treating the product with acetic acid. It is noticed that the dispersibility is nearly complete when the product is acetified and treated with enzyme for higher degrees of hydrolysis. A good dispersion is also achieved by whipping. With increase in whipping time, the product becomes more viscous and opaque in appearance. The dispersion is observed to be highest within 8 min of whipping.

Aeration and foam formation: The aeration capacity and foam stability in relation to the degree of protein hydrolysis are presented in Table 2.

Higher amount of foam is observed with greater degree of hydrolysis which also favours better stability of foam. The foam formation in untreated product is almost nil. The quantity of foam is increased with increase in whipping time, after 8-10 min maximum foam is attained. Maximum foam breakage occurs within 10 min of whipping which is almost stable after 30 min.

In addition to its attainment of good foaming and dispersion by acetification and enzyme treatment, the original gritty texture of FPC is also removed by

hydrolysis. The product shows no fishy flavour. From the view point of its quality and cost, the protein extract from the catfish appears to be promising as a food ingredient. Although there is a high flesh ratio in relation to its offal, the catfish is not consumed. On the other hand, the process indicates that the fish could be made into a proteinous food at lesser cost. Its use as a protein-fortifying agent in some formulated cereal foods is promising. Groninger and Miller[8] have studied the suitability of the application of FPC in foods like frozen dessert, scuffle and dessert toppings. The protein concentrate from catfish can be used in a similar way in different systems as one of the ingredients.

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Preservation of Indian Mackerel *(R. kanagurta)* by Gamma Irradiation

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The effect of ionization radiation on the storage life of Indian mackerel (*R. kanagurta*) was studied. Irradiation at 0.1 M rad extends the storage life of mackerel by about 7 to 8 days at 0-2°C over the control, whereas 0.15 M rad increases the storage life by about 14 days over the control under identical conditions of storage. Empirical relations have been worked out between organoleptic ratings, total volatile nitrogen and free fatty acid.

Ionization radiation is an important method for extending the storage life of fish[1,2] which is by nature very susceptible to spoilage even under refrigerated condition. Enumeration of total plate count, normally used as a parameter of spoilage in fresh food material may not be applicable directly in irradiated fish, particularly in fatty fish as oxidation of fat may make the fish unacceptable[3,4] even in spite of its low bacertiological count.

Bactericidal effect of gamma radiation has been utilized extensively for extending the storage life of fish by suppressing[5-7] the rapid spoilers. Usually radiation dose of 0.1M to 1.0M rad is being applied recently for extending the storage life of sea foods. However, radurised dose[8] of 0.1-0.2 M rad can be recommended provided the temperature of storage is maintained below 3.8°C.

Mackerel contributes about 20 per cent of the total marine landing in India but a good percentage is wasted due to improper handling and lack of proper refrigeration facility in our country. The present study was undertaken to correlate the most complex parameter like organoleptic rating with some objective tests into a mathematical term. However, there are such mathemetical correlations for lean fish but so far no attempt has been made with fatty fish.

Materials and Methods

Mackerel obtained from Bombay local markets was washed and sealed in polythene pouches which was subsequently irradiated in Co_{60}. Food Package Irradiator (Atomic Energy of Canada Ltd) at specified dose at the Biochemistry and Food Technology Division of

Bhabha Atomic Energy Research Centre, Trombay. Irradiated and control samples were packed separately in insulated ice boxes with fish to ice ratio of 1:3 and transported by rail to Calcutta. Samples received on the 3rd or 4th day after irradiation were held in ice (0-2°C) till the examinations and observations were complete.

Organoleptic ratings were carried out by a taste panel comprising of seven members according to Miyanchi *et al*[9]. Free fatty acid was estimated according to AOCS[10] method. Bacteriological count was carried out according to the method described by Bhadra *et al*[11]. Total volatile nitrogen (TVN) were estimated according to the method of Beatty and Gibbons[12] by using micro diffusion method of Conway[13].

Results and Discussion

Organoleptic score: Ionization radiation has been found to be effective in extending the storage life of mackerel over the control. However, this extension is dose dependent. Unirradiated mackerel under the specified condition of storage is organoleptically acceptable upto 7 days at 0-2°C. While the storage life of irradiated sample was found to be extended by about 7 and 14 days over the control at a dose level of 100 K-rad and 150 K-rad respectively. Organolpetic rating with days of storage at ice temperature is shown in Fig. 1.

Bacteriological count: The typical changes in bacteriological count of irradiated and control mackerel are shown in Fig. 2. By subjecting the fresh mackerel to ionization radiation at a dose level of 150 K-rad it is possible to reduce initial microbial load of the samples by a log cycle over the control. However, a dose level of 100 K-rad has not been found to be much effective in bringing down the initial microbial count of the samples. Generally at the time of spoilage the microbial load of the lean fish were between 10^6 and 10^7/g while in the irradiated samples[11] it was between 10^8 and 10^9/g. Here the microbial count of the control and irradiated samples

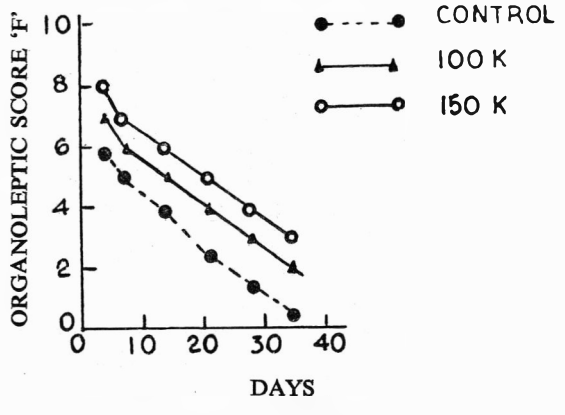

Fig. 1. Organoleptic score of radurised mackerel during ice storage.

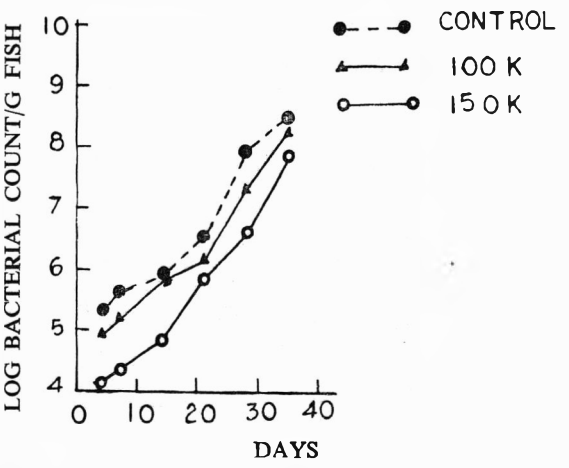

Fig. 2. Change in bacteriological count during ice storage.

were only 4.5×10^5 and 8.4×10^5, 7.1×10^5/g respectively when their organoleptic scores were 5 in all the cases. So, in case of mackerel it would be very difficult to assess its quality by mere bacteriological count as the oxidation of fat itself has rendered the fish unacceptable though the bacteriological count was appreciably low.

Changes in chemical indices: The typical changes in TVN in control and irradiated samples during storage at 0-2°C are shown in Fig. 3. When the TVN values are in the range of 16-24 mg per cent both irradiated and control samples were regarded as organoleptically unacceptable.

The changes in FFA contents of mackerel during storage are given in Fig. 4. Ionization radiation was found to be effective in controlling FFA values. When control samples were organoleptically unacceptable, FFA values were found to be higher (9-10 per cent oleic acid) than those of irradiated samples (7-8 per cent oleic acid) under identical condition of rejection, F=5.

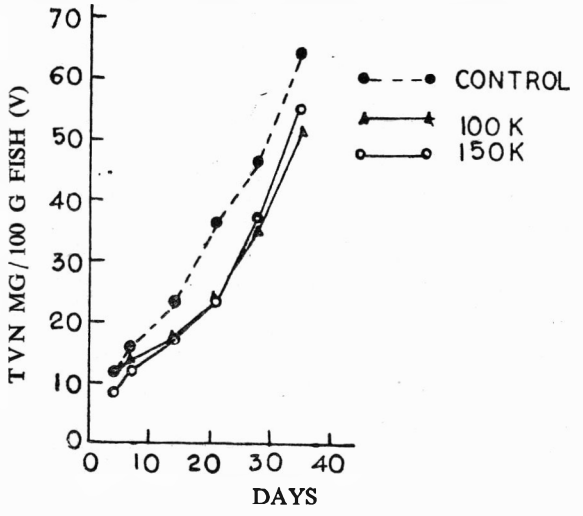

Fig. 3. Change in total volatile nitrogen during ice storage.

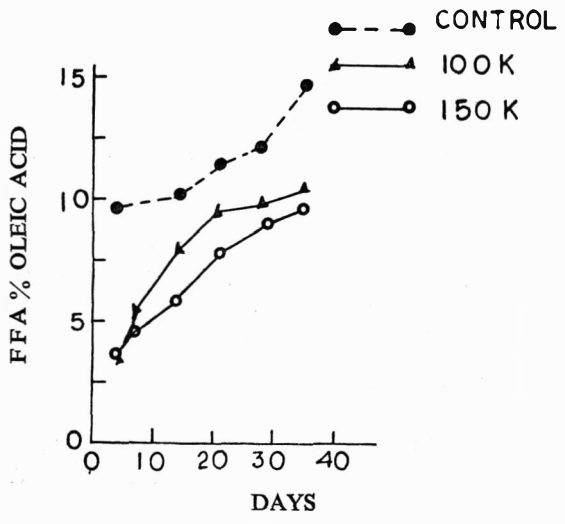

Fig. 4. Change in FFA during ice storage

Relation between organoleptic score and chemical indices: By simultaneous solving the equations the following relations were obtained.

$F+7.08 \log (1+V)+3.26 \log (1+f)=17.2$

$F+6.1 \log (1+V)+3.14 \log (1+f)=15.8$

$F+5.09 \log (1+V)+3.11 \log (1+f)=15.09$

Where,

V=total volatile nitrogen (mg per cent); f=free fatty acid (per cent oleic acid).

For control, 100 K-rad and 150 K-rad samples respectively.

With peroxide values, no such relation was obtained.

Acknowlegement

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The Application of Packaging Technology and Chemical Treatment for the Preservation of High Moisture Grains with Special Reference to Paddy*

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Packing of high moisture paddy with a suitable chemical treatment in containers that can act as air barriers was found to preserve the grains without spoilage. The treatment of moist paddy either with a combination of common salt 1 or 2% and sodium chromate 0.1% or calcium chloride alone at 1.5% and packaging in gunny with 150 g polyvinyl chloride (PVC) and 300 G high density polyethylene (HDPE) sacs as loose liners preserved the grains for 3-4 weeks. A mixture of camphor-citronella-chloroxylenol-carbon tetrachloride also protected the grains for 7 days in similar packaging from germination, mould growth and associated heating, discolouration and reduced the loss of dry matter. Among the 10 types of loose liners and laminated sacs tested, only gunny sac with a 150 G PVC and 300 G HDPE as loose liners was found to preserve the treated paddy.

The harvest of wet season paddy in Thanjavur district of Tamil Nadu state mostly coincides with rainy humid weather. The wet harvested paddy, difficult to dry in this situation, undergoes a variety of spoilage changes due to germination, mould growth, heating and discolouration, resulting in loss of dry matter, and reduction in quality and market value. The turning of moist paddy at frequent intervals in indoors and mixing of dry salt at 4-5 per cent are helpful to save the loss to some extent, but are of no avail when the inclement weather continues for a long period. The present paper reports the results of chemical treatment combined with packaging to preserve the high moisture paddy.

Materials and Methods

Moist paddy of 'ADT 31' and 'IR 20' varieties were used. Paddy was given the following treatments: (*i*) 1 or 2 per cent common salt (sodium chloride) with sodium chromate 0.1 per cent by weight of paddy, (*ii*) 1.5 per cent calcium chloride (2.25 per cent as commercial grade chemical containing 66-68 per cent $CaCl_2$), and (*iii*) 0.1 per cent (v/w) of a mixture of camphor (5 G), citronella (0.05 ml), chloroxylenol (0.05 ml) dissolved and made up to 100 ml with carbon tetrachloride. The common salt and the commercial sodium chromate were added to 2 per cent tap water by weight of the paddy and the slurry was thoroughly mixed with paddy. Calcium chloride was dissolved in a small quantity of hot water, the resulting solution was made up to a volume equivalent to 2 per cent by weight of paddy and mixed thoroughly with paddy. The treated paddy was filled in a B.Twill gunny with various liners. In the treatment with the mixture of camphor-citronella-chloroxylenol-carbon tetrachloride, the liquid was poured along with paddy while filling the containers. After filling, the innermost liner was tied first and the tip was retied after folding, then the next liner and so on and finally the gunny to prevent air entry.

Moist paddy was stored in galvanized iron (GI) tins of 7 kg capacity with an elevated one way outlet. An elongated 2" metal tube at the top of the container with a small hole in the stem 1 cm below the tip covered and tied with a tight baloon served as a one way exhaust. This acted like a one way valve similar to a valve tube and prevented entry of air. The joints and mouth of the container were also sealed with wax. Moist paddy treated and untreated and paddy soaked in 50 ppm solutions of streptomycin, streptomycin and penicillin and tetracycline for 16 hr were also stored in such tins and examined for spoilage changes.

In order to identify a sac container which can afford the advantages of air tight tins, a number of sacs with various liners (Table 1) were tested with paddy treated with the camphor-citronella-chloroxylenol-carbon tetrachloride mixture.

The dry matter loss was determined in 500 G paddy lots treated separately with the chemicals and taken in small knitted nylon bags. These bags were embedded

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inside the treated paddy packed in the gunnies under test and stacked. Samples were withdrawn at weekly intervals, steamed at 25 lb pressure for 5 min in an autoclave, air dried, dehusked in a laboratory model 'Satake' rubber roll sheller and then dried in an oven at 105°C for constant weights. The dry weights were taken and the per cent loss of dry matter was calculated.

The microbial load in paddy grains prior to and after storage was determined by employing the standard dilution plate technique. The total sugars were estimated in the alcohol extract of paddy samples following the method of Nelson[1].

Results and Discussion

It was observed that there was no germination and fungal growth when high moisture paddy was stored under air tight condition in tin containers. However, there was development of off-odour after storage for 3 days. On the other hand paddy soaked in 50 ppm solution of streptomycin alone or in combination with 50 ppm of penicillin and 50 ppm of tetracycline was preserved in fairly good condition and when stored similarly there was no development of off-smell. The arresting of off-odour development due to antibiotic treatment clearly revealed the role of bacteria in the smell development. It was also found that treatment of high moisture paddy with a mixture of common salt obtained from the market either at 1 or 2 per cent levels in combination with sodium chromate at 0.1 per cent level or calcium chlroide alone at 1.5 per cent level checked the development of off-odour. By these treatments the moist paddy was preserved well for 10 days in air tight tin containers. In the search for a chemical that can act in vapour phase it was found that a mixture of camphor-citronella-chloroxylenol-carbon tetrachloride at the rate of 1 ml/kg of paddy was effective for a period of 7 days.

The above results suggest that once the free exchange of air between grains and the external atmosphere is curtailed the germination and fungal growth are arrested. It is likely that due to the respiration of the grains the oxygen available inside would have been utilized creating a low oxygen tension which might be uncongenial for the development of germination and fungal growth. However, under such air tight conditions fermentative changes occur leading to the development of off-odour when moist paddy was stored under air tight conditions.

Among the various types of loose liners and laminated sacs tested for packing high moisture paddy (Table 1) it was observed that only a combination of B. Twill gunny/150 G PVC/300 G HDPE sacs as loose liners was

TABLE 1. PERFORMANCE OF VARIOUS SAC CONTAINERS IN PROTECTING HIGH MOISTURE IR 20 PADDY (30.0% MOISTURE) WITH VAPOUR PHASE CHEMICAL FOR A PERIOD OF 7 DAYS

Specification of container	*Observation*
1. B. Twill gunny+HDPE (300 G) loose liner (natural colour, transparent)	No germination; stray fungal growth; no off-odour.
2. B. Twill gunny+PVC (150 G) loose liner (natural colour)	Stray germination, caking due to fungal growth; no off-odour, slight heating.
3. B. Twill gunny+HDPE (300 G) outside+PVC (150 G) inside (natural colour+ natural colour transparent)	Stray germination; no fungus and no off-odour.
4. B. Twill gunny+HDPE (300 G) inside+PVC (150 G) outside	No germination; no fungal growth; no off-odour.
5. Bitumen laminated gunny bag (B. Twill gunny+bitumen+paper+bitumen+ 100 G HDPE Polyethylene)	Heavy germination; fungal growth and caking, musty smell.
6. „ +LDPE (150 G) liner stitched on sides and paper pasted along stitch lines.	Germination, fungal growth and caking, musty smell.
7. Bituminised seven ply bag with 100 G LDPE (B. Twill gunny+bitumen+paper+ bitumen+PVC+bitumen+100 G LDPE).	Heavy germination; fungal growth, musty smell and caking.
8. Polytape laminated woven sac.*	Very heavy germination; fungal growth and caking, musty smell.
9. Polytape laminated Woven sac*+HDPE (300 G) liner.	No germination; stray fungal growth and no caking, slight musty smell.
10. Sandwiched bag (Hoechst) polytape on both sides with a 100 G polyethylene in between.	Germination; fungal growth; musty smell.

*Polytape laminated with 100 G LDPE.

TABLE 2. RELATIVE EFFICACY OF DIFFERENT CHEMICALS IN PRESERVATION OF ADT 31 MOIST PADDY STORED IN GUNNY/PVC/POLYETHYLENE CONTAINERS (AFTER 21 DAYS OF PRESERVATION)

Treatment	Germination	Fungal growth	off-odour
Sodium chloride 1%+Sodium chromate 0.1%	Nil	Nil	Nil
Calcium chloride 1.5%	,,	,,	,,
Vapour*	,,	Very stray fungal growth	Light chemical smell
Untreated	,,	Nil	Intense off-odour
Untreated (stored in gunny alone)	Heavy germination	Fungal growth and caking	Nil

*Carbon tetrachloride 95 ml+camphor 5g+citronella 0.05 ml+chloroxylenol 0.05 ml made up to 100 ml and applied at 1 ml/kg.

effective in protecting the treated grains. In the B. Twill gunny with a 300 G HDPE alone as loose liner although germination and off-odour development were prevented, stray fungal growth was observed which might be due to partial permeation of air. In the case of 150 G PVC alone as a loose liner, stray germination and fungal growth was observed. In the loose liner, B. Twill gunny/300 G HDPE/150 G PVC (innermost) stray germination was noticed. Wherever PVC sac was in direct contact with the grains the sac showed development of faint black patches which might be due to the reaction of PVC with the moist paddy treated with the chemicals. On the other hand in the loose liner B. Twill gunny/150 G PVC/300 G HDPE there was no blackening of PVC sac. There was germination and fungal growth in the other types of bags tested. These spoilages were relatively more along the stitch lines which might have allowed free entry of air. However in polytape laminated woven sac with 300 G HDPE liner only a stray fungal growth was observed and germination was arrested.

In the scaled up experiment with bulk quantities of paddy treated with common salt 2 per cent and sodium chromate 0.1 per cent or calcium chloride 1.5 per cent and packed in gunny/150 G PVC/300 G HDPE loose liners, the moist paddy preserved well for a period of 21 days (Table 2). The spoilage due to germination, mould growth, heating and discolouration was eliminated completely and the loss of dry matter was also reduced considerably (Table 3). The dry matter loss was only 1-2 per cent in treated grains as against 9 per cent in moist paddy stored without any chemical treatment in the gunny sacs. The treatment of high moisture paddy with sodium chloride and sodium chromate decreased the microbial population in grains (Table 4). In paddy preserved in gunny/150 G PVC/300 G HDPE liners without any chemical treatment the fungal population declined considerably whereas the bacterial load increased considerably in the untreated paddy stored in the gunny sacs. Consequently the formation of free sugars was also at a higher level.

The preservative action of sodium chloride and calcium salts like propionates and acetates on moist paddy was demonstrated by Shivanna and Kutharathulla[2]. The withdrawal of water by the exosmotic action of salts, inhibition of microbial growth and inactivation of seed enzymes might be the mechanism of action of these salts. The preservative action of sodium chloride[2,3] and the enzyme inhibition by calcium ions were also well established[4,5]. Desikachar[4] also reported that calcium at higher concentrations inhibited the activity

TABLE 3. DRY MATTER LOSS IN IR 20 MOIST PADDY (28% MOISTURE) DURING PRESERVATION BY CHEMICAL TREATMENT AND PACKING FOR A PERIOD OF 7 DAYS

Treatment	Container	Brown rice yield (%)		Dry matter loss (%)
		Initial	After 7 days	
Control	gunny alone	77.58	70.60	8.99
Sodium chloride 1.0%+Sodium chromate 0.1%	gunny/PVC/HDPE	77.58	76.50	1.39
Calcium chloride 1.5%	,,	77.58	76.40	1.52

TABLE 4. CHANGES IN THE MICROBIAL LOAD AND TOTAL SUGARS CONTENT IN 'ADT 31' MOIST PADDY (28.9% MOISTURE) DURING PRESERVATION IN GUNNY/PVC/POLYETHYLENE LOOSE LINERS

Treatment	Container	Period of storage (Days)	Fungi (10^5/g)	Bacteria (10^6/g)	Total sugars (mg/g)
Nil	—	0	11.6	73.3	2.452
„	Gunny	15	16.9	33.9	3.342
„	Gunny/PVC/HDPE	15	12.3	82.8	3.041
Sodium chloride 2.0%+Sodium chromate 0.1%	„	15	5.7	56.1	2.930
Nil	Gunny	45	28.8	117.4	3.037
„	Gunny/PVC/HDPE	45	4.5	92.1	2.385
Sodium chloride 2.0%+Sodium chromate 0.1%	„	45	3.1	72.0	2.936

On dry basis.

of lipase. The inhibition of amylase of certain plant parasitic fungi was also reported[5].

The results of the present investigation indicate that the high moisture paddy of wet season harvest could be preserved in fairly good condition over a period of 3-4 weeks if the moist grains are packed in gunny/150 G PVC/300 G HDPE loose liners after treating the grains with a combination of common salt either 1 or 2 per cent along with sodium chromate 0.1 per cent or with 1.5 per cent calcium chloride. There is scope for extending a similar technology to other grains harvested under adverse weather conditions. The development of a cheap and durable composite sac that can act similar to the gunny/PVC/HDPE loose liner will be of immense use in preservation of moist grains.

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Studies on the Variability in the Quality of Market Paddy

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Fortynine samples of paddy comprising 'Basmati 370', (14) 'Palman 579', (16) 'IR 8' (8) and 'Jaya', (11) collected from different markets were tested for, physico-chemical, milling and cooking characteristics. High variability in refractions, head rice yield and broken rice were observed within and between varieties. The coarse varieties, 'IR 8' and 'Jaya' showed high breakage. The average values being 30.5 and 33.7 per cent compared with 23.3 and 18.0 per cent given by 'Basmati 370' and 'Palman 579' varieties.

Punjab is among the major surplus states in paddy production in India[1]. Among the number of varieties cultivated the coarse varieties have become popular because of their high yields. No information is available on the variability in quality of cultivated varieties. Sidhu *et al*[2]. made an integrated study of the quality of five varieties of paddy from an experimental farm. In this paper, results of a study on the variability in the quality characteristics of four varieties of paddy, viz., 'Basmati 370', 'IR 8', 'Jaya' and 'Palman 579', cultivated in different locations of Punjab are presented.

TABLE 1. VARIABILITY IN PHYSICAL AND MILLING CHARACTERISTICS OF DIFFERENT VARIETIES OF PADDY

Variety	Moisture (%) Mean±S.D.	Refractions (%) Mean±S.D.	1000-kernel wt. (g) Mean±S.D.	Length-breadth ratio Mean±S.D.	Brown rice (%) Mean±S.D.	Head rice (%) Mean±S.D.	Broken rice (%) Mean±S.D.
Basmati 370	13.9±1.8	2.4±1.5	22.1±1.2	4.5±0.3	77.7±0.9	76.9±6.8	23.3±7.0
Palman 579	13.7±1.4	2.4±1.2	21.3±0.7	4.4±0.1	77.8±0.9	82.0±7.1	18.0±7.1
IR 8	13.4±1.4	2.4±1.1	29.0±1.3	3.0±0.2	79.3±2.0	69.4±8.9	30.5±9.0
Jaya	13.9±1.9	2.8±1.3	27.9±1.2	3.0±0.1	78.4±0.9	66.9±12.5	33.7±9.2

S.D.—Standard Deviation.

Materials and Methods

Fourteen samples of 'Basmati 370', 16 of 'Palman 579', 8 of 'IR 8' and 11 of 'Jaya' varieties of paddy were collected from different market places in Punjab, from the 1977 crop. The high initial moisture content was brought down by drying under shade and then stored in air-tight containers and withdrawn for different tests.

Cleaning: The samples were cleaned in the laboratory pneumatic separator and the impurities (refractions) were removed and weighed.

Physical, chemical, milling and cooking evaluations: These were made according to the procedure used by Sidhu *et al*[2].

Starch-iodine blue value: This was determined by the method of Halick and Kenester[3].

Gruel solids: A measured aliquot (10 ml) of the gruel was dried on a water bath first and then in an air oven to constant weight.

Starch in the gruel: The starch in the gruel was determined according to the procedure described by Batcher *et al*[4]. and results expressed as transmission at 600 nm.

Results and Discussion

Moisture content: The initial moisture content (Table 1) of paddy ranged from 18.6 to 15.0 per cent; the coefficients of variation being 10.2 to 13.8 per cent among the four varieties.

Refractions: The impurities found are loose dust, soil clods, empty glumes and pieces of stones constituting a maximum of 6 per cent (Table 1). The coefficients of variation ranged from 46.8 to 59.6 per cent among the varieties.

Milling characteristics: Highest average yield of brown rice (79.3 per cent) was obtained with 'IR 8' and the lowest average (77.7 per cent) was given by 'Basmati 370' (Table 1). 'Palman 579' was resistant to milling breakage and took 27 sec to mill to 5 per cent polish. It also gave 82.0 per cent head rice, compared to 76.9, 69.4 and 66.9 per cent for 'Basmati 370', 'Jaya' and 'IR 8', respectively. Head rice yield showed greater variation with 'Jaya' and 'IR 8'. The coefficient of variation was also less for 'Basmati 370' and 'Palman 579' (8.9 and 8.6 per cent) compared with 'IR 8' (12.8 per cent) and 'Jaya' (18.6 per cent). The varieties 'IR 8' and

TABLE 2. VARIABILITY IN THE PHYSICO-CHEMICAL CHARACTERISTICS OF BROWN AND MILLED RICE OF DIFFERENT VARIETIES OF PADDY

Variety of rice	1000-kernel wt. (g) Mean±S.D.	Length-breadth ratio (1/b) Mean±S.D.	Protein (N×5.95) (%) Mean±S.D.	Fat (%) Mean±S.D.	Amylose (%) Mean±S.D.	Iodine blue value (O.D) Mean±S.D.
Basmati 370						
Brown	17.5±1.2	3.8±0.1	7.6±0.5	2.6 ±0.3	16.3±0.8	0.21±0.06
Milled	16.8±1.1	3.6±0.3	7.1±0.8	1.1 ±0.2	19.9±0.7	0.70±0.17
Palman 579						
Brown	16.8±0.6	3.4±0.2	7.5±0.7	2.5 ±0.3	16.9±0.9	0.29±0.13
Milled	16.5±0.6	3.3±0.2	6.9±0.9	1.0 ±0.20	20.3±1.0	0.92±0.19
IR 8						
Brown	23.6±2.2	2.6±0.01	7.7±0.6	2.3 ±0.2	16.9±2.7	0.26±0.06
Milled	22.4±1.9	2.5±0.1	7.1±0.6	0.70±0.10	21.1±1.1	1.01±0.23
Jaya						
Brown	22.6±1.0	2.6±0.1	7.5±0.6	2.1 ±0.2	1.67±0.8	0.24±0.04
Milled	21.7±1.3	2.6±0.2	7.0±0.4	0.73±0.20	21.4±1.3	0.88±0.10

TABLE 3. VARIABILITY IN THE COOKING QUALITY OF MILLED RICE OF DIFFERENT VARIETIES OF PADDY

Variety	Cooking time (min) Mean±S.D.	Water uptake (g/g) Mean±S.D.	Swelling ratio (v/v) Mean±S.D.	Elongation ratio (I/I) Mean±S.D.	Gruel solids (%) Mean±S.D.	Transmission (%) Mean±S.D.
Basmati 370	18.4±1.4	2.4±0.3	3.6±0.2	1.6±0.2	0.51±0.1	40.2± 7.0
Palman 579	19.4±2.3	2.3±0.2	3.6±0.2	1.5±0.2	0.54±0.2	33.5± 9.3
IR 8	21.0±2.0	2.1±0.1	3.3±0.2	1.6±0.04	0.38±0.2	56.4±11.2
Jaya	19.8±1.5	2.1±0.1	3.3±0.1	1.5±0.1	0.34±0.1	56.9± 8.3

'Jaya', were more susceptible to breakage than 'Palman 579' and 'Basmati 370'.

Physico-chemical characteristics: The average 1000-kernel weight of 'IR 8' paddy was higher than that of 'Jaya', 'Palman 579' and 'Basmati 370' (Table 1). The extent of variation within varieties was in the range of 3.2-5.4 per cent. The differences in the density and bulk density of paddy, brown and milled rice within varieties were inconspicuous.

The average length to breadth ratios (l/b) for paddy, brown and milled rice were generally higher for 'Basmati 370' and 'Palman 579' when compared to other two coarse varieties.

Amylose content of the brown rice ranged from 15.4 to 16.9 per cent among the varieties (Table 2). The values increased on milling and went upto 23.3 per cent. The range in iodine blue value (O..D.) was 0.13 to 0.82 for brown rice and 0.38 to 1.77 for the corresponding milled samples. The coefficients of variations for iodine blue values were quite high. Juliano *et al*[5]. observed that amylose content and iodine blue values were not proportional in samples with more than 30 per cent amylose. Protein content of brown rice ranged from 5.9 to 8.6 per cent which slightly decreased on milling (5.7 to 8.0 per cent). Similar trend was observed in the fat content of the varieties on milling.

Cooking characteristics: Cooking times varied among varietics and within the same variety among different samples as it ranged from 16 to 26 min (Table 3). Water-uptake, swelling and elongation ratios were generally higher for 'Basmati 370' and 'Palman 579' than those of 'Jaya' and 'IR 8'. The loss of starch in the gruels of coarse varieties ('Jaya' and 'IR 8') was higher than those of 'Basmati 370' and 'Palman 579'.

From these results, it is evident that there is high variation in the quality within and between varieties of paddy and rice. Thet Zin[6] pointed out that economic value of broken rice was one half to one third of the price fetched by intact kernels. The results of the present study and those of Sidhu *et al*[2]. established 'Palman 579' variety as being resistant to breakage during milling. If the factor of high yield of the coarse varieties of paddy such as 'IR 8' and 'Jaya' could be combined with those of 'Palman 579' kernels, it would go a long way to improve the potential of the high yielding coarse varieties presently under cultivation.

Acknowledgement

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Studies on the Growth of Baker's Yeast on Molasses Media

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Studies on the growth of Baker's yeast (*S. cerevisiae*) on molasses media are reported in a 200-l and 4000-l fermenter. The growth characteristics are analysed using a model suggested by Kono for microbial cell growth. The characteristic values for growth of Baker's yeast are predicted. Based on these values, the cell concentrations are calculated for a 10-l and 30,000-l fermenter. The model represents the growth of yeast satisfactorily for batch cultivation on all scales of operation.

The availability of large quantities of cane sugar molasses as a by-product from the sugar industry, coupled with the requirement of yeast for the many bakeries that have been set up during recent years in India, led to the use of molasses as the carbohydrate source for growing yeast. The process for yeast production using molasses has been standardised.

Study of growth characteristics are essential for evaluating the performance of fermenters. The kinetics of microbial growth has been studied by several workers[1] and growth rates have been expressed as a function of substrate concentration. However, they were found inadequate to explain the full course of microbial growth including the lag period. Based on the principles of chemical kinetics Kono[2] has derived growth rate equations for microbial growth introducing the concept of critical concentration and coefficient of consumption activity.

This paper presents data on growth of yeast and discusses the application of Kono's model for evaluating the characteristic values which are used for calculating the cell concentration for batch fermentation of yeast.

Material

Selected strains of *S. cerevisiae* were carefully grown from slant culture in stages, for inoculating the fermenters. Diluted, sterilised and clarified molasses (5 per cent sugars) was used for feeding at a uniform rate. Operating conditions were maintained identical for all the fermenters, (pH 4.5, temperature, 30 ± 1°C, and aeration at 1 volume per volume of broth/min.)

The 200-l fermenter used was a cylindrical stainless steel vessel with S. S. air spargers at the bottom. The 4000-l fermenter was a M. S. epoxy coated unit with S. S. air spargers at the bottom. Provision was made for circulating the broth through plate heat exchangers cooled with water at 20°C. The fermenters used were non-agitated type.

Fermentation was carried out for 17-20 hr and samples were drawn at regular intervals for estimating cell concentration by optical density measurements. The yeast cells were harvested in a nozzle separator.

Methods

The general form of equation of microbial cell growth has been expressed as follows[2]

$$\frac{dc_x}{d\theta} = K^{i}_{1} K j_2 C^{i}_{M} C j_S \text{ —— } K_3 C_R$$

where C_x = cell concentration
C_s = limiting substrate concentration
C_m = concentration of substrate M which react with limiting substrate and produce cell mass.

Defining the critical cell concentration C_{xe} as an equilibrium state between C_M, C_S and C_R.

$i = 1, j = 0$ at $C_x < C_{xe}$

$i = 0, j = 1$ at $C_x \geqslant C_{xe}$

In order to express C_M, C_R and C_S as a function of C_x, the coefficient of consumption activity ϕ has been defined as the fraction of cells which have ability to consume substrate and propogate themselves to all the cells at any arbitrary point in the time course of microbial cell growth.

The rate equation, therefore, simplifies to

$$\frac{dC_x}{d\theta} = K \phi C_x$$

The time course of microbial growth has been divided as 4 phases and the coefficient of consumption activity for each phase has been expressed as a function of cell concentration.

1. *Induction phase:* The consumption of substrate is zero (i.e. $\phi = 0$)

$$\frac{dC_x}{d\theta} = 0$$

$$C_x = C_{x0}$$

2. *Transient phase:* Consumption of substrate begins (i.e., ϕC_x)

$$\frac{dC_x}{d\theta} = K \phi C_x$$

3. *Exponential phase:* = Cell production with substrate consumption.

(i.e., $\phi = 1$)

$$\frac{dC_x}{d\theta} = KC_x$$

4. *Declining phase:* Cell production with substrate consumption and retarding of cell growth by secretions

i.e., $\phi(C_{xm} - C_{xc})$

$$\frac{dC_x}{d\theta} = K^1 (C_{xm} - C_{xc})$$

The rate equation and integral forms for all four phases are given in Table 1. In order to clarify the characteristics of the growth rate equation, the growth rate $\frac{dC_x}{d\theta}$ is plotted against cell concentration in Fig. 1. The growth rate equation in the exponential phase is represented by a straight line (oab) whose extension passes through zero and whose slope is equal to the coefficient of growth rate K. The equation for the declining phase is represented by a straight line (be) whose extension passes through $e\,(C_{xm})$ and whose slope

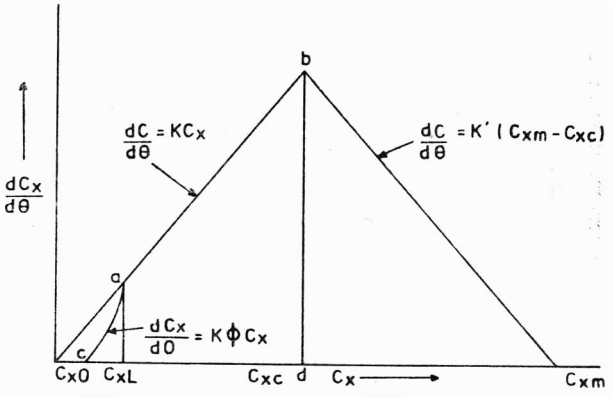

Fig. 1. Graphical representation of growth rate equation.

is K^1. The cell concentration at a point *b* of contact of these two straight lines is the critical cell concentration $d(C_{xc})$. The growth rate equation in transient phase is represented by a curved line ca which contacts abscissa at, $c(C_{xo})$ and contacts the straight line of the exponential phase at a (C_x).

Accordingly, the characteristic values representing the induction phase and transient phase are the final time of induction phase θ_0 and final time of transient phase θ_L. The characteristic values of exponential and declining phase are the coefficient of growth rate K, critical cell concentration C_{xc} and theoretical maximum cell concentration C_{xm}.

Results and Discussion

A time course of *S. cerevisiae* fermentation in a batch 200-l fermenter is shown in Fig. 2. The fermentation was carried out for 19 hr at a temperature 30°C and pH 4.5. Growth rate was studied by analysing the cell concentration at regular intervals of times by optical density measurements. Growth rate $\frac{dC_x}{d\theta}$ g/l/hr is plotted against

TABLE 1. GROWTH RATE EQUATION AND INTEGRAL FORM IN EACH PHASE OF MICROBIAL CELL GROWTH

Phases	Growth rate equation	Integral form
1. Induction	$\frac{dC_x}{d\theta} = 0$	$C_x = C_{x_0}$
2. Transient	$\frac{dC_x}{d\theta} = K \phi C_x$	$C_x = C_{x_0} + (C_{x_L} - C_{x_0}) \left(\frac{\theta - \theta_0}{\theta_L - \theta_0} \right)^2$
3. Exponential	$\frac{dC_x}{d\theta} = KC_x$	$C_x = C_{x_c} e^{k(\theta - \theta_c)}$
4. Declining	$\frac{dC_x}{d\theta} = K'(C_{x_m} - C_{x_c}) = KC_{x_c} \left(\frac{C_{x_m} - C_x}{C_{x_m} - C_{x_c}} \right)$	$C_x = C_{x_m} - (C_{x_m} - C_{x_c}) e^a$ $a = -K \frac{C_{x_c}}{C_{x_m} - C_{x_c}} (\theta - \theta_c)$

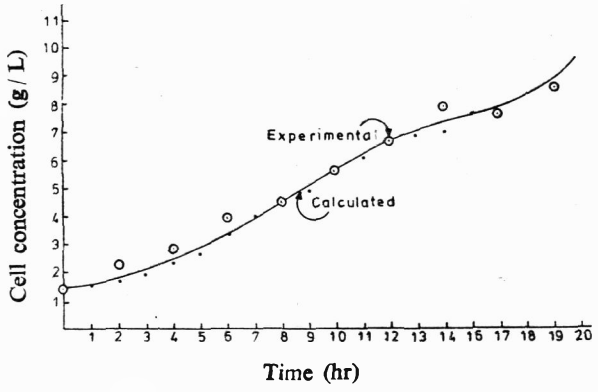

Fig. 2. Yeast concentration vs. time in 200-litre fermenter.

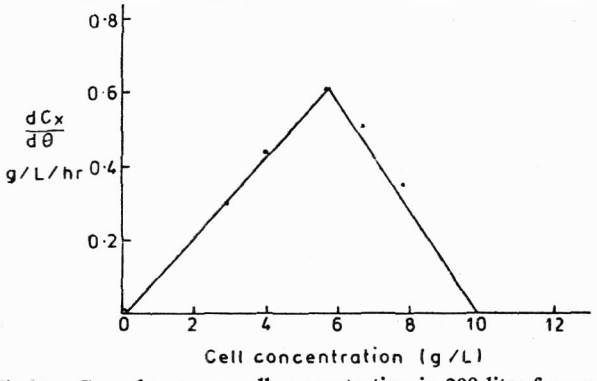

Fig. 3. Growth rate vs. cell concentration in 200-litre fermenter.

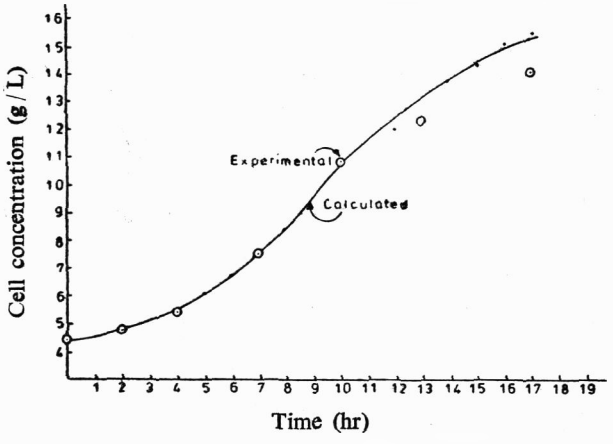

Fig. 4. Yeast concentration vs. time in 4000-litre fermenter.

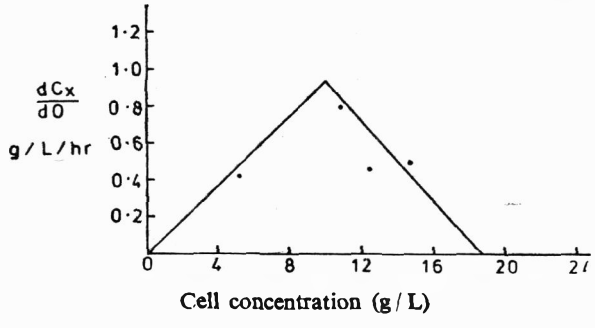

Fig. 5. Growth rate vs. concentration in 4000-litre fermenter.

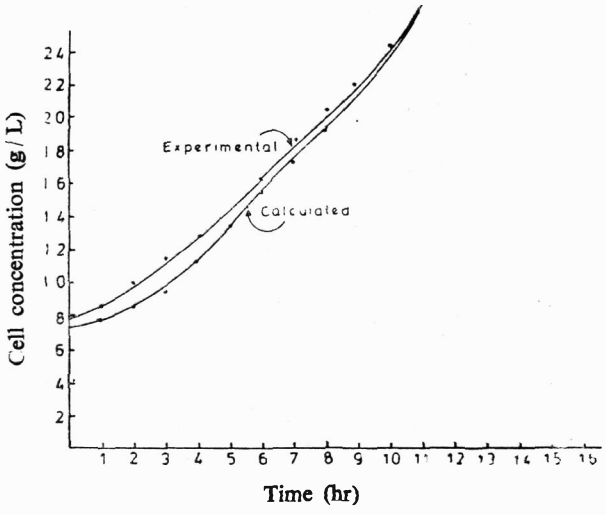

Fig. 6. Yeast concentration vs, time 30,000-litre fermenter (White, 1954.

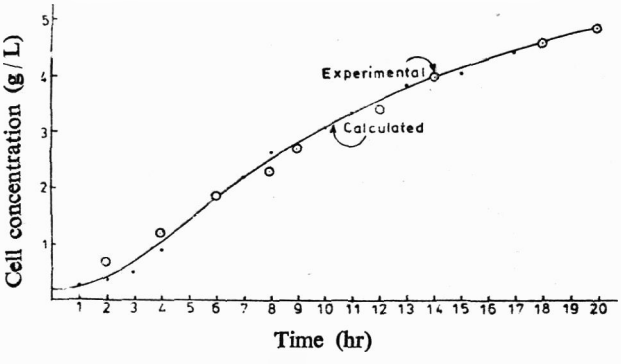

Fig. 7. Yeast concentration vs. time in 10-litre fermenter Patrakulpong.

cell concentration C_x $\frac{g}{l}$ in Fig. 3. The characteristic values of this fermentation is obtained from Fig. 3 and are shown in Table 2. Cell concentration can be calculated as follows.

$K = 0.11$

$C_{xc} = 5.8$ g/l.

$C_{xm} = 9.9$ g/l.

$\theta_c = 10.0$ hr

$\theta_l = 7$ hr

Exponential phase : $C_x = 5.8\ C^{0.11\,(\theta-10)}$

The cell concentration at 8th hr (0=8)

TABLE 2. CHARACTERISTIC VALUES FOR FERMENTERS

Fermenter capacity (l)	θ_L	K	C_{x_c}	C_{x_m}
200	7	0.11	5.8	9.9
4000	7	0.11	10.0	18.06

$$C_x = 5.8\ C^{0.11\,(8\text{-}10)}$$

The calculated value of cell concentration=4.65 g/l.
Experimental value of cell concentration=4.5 g/l.
Declining phase $= C_{xc} = 9.9\text{-}4.1C^{-a}$

$$a = 0.11 \times \frac{5.8}{4.1} \times (18\text{-}10) = 1.245$$

$$C_{xc} = 9.9\text{-}4.1\ e^{-1.245}$$

The calculated value of the cell concentration is 8.72 g/l. as compared to the experimental value of cell concentration 8.60 g/l.

Similar results on the 4000-l. fermenter are shown in Fig. 4 & 5.

Based on the growth rate equation given in Table 1. the values of cell concentration are calculated and are compared with experimental values in Fig. 2 and 4. The calculated and experimental values are in good agreement.

It can therefore, be concluded that the model represents the yeast growth satisfactorily and characteristic values can be used for calculating cell concentrations for similar fermenters for yeast growth on molasses.

To test the applicability of the model for industrial and laboratory scale fermenters, the cell concentration is compared with published data on 30,000-l, fermentation by White[3] and 10-l. fermentation by Prima Patarakulpong[4] in Fig. 6 and 7.

It can be seen that the model represents the data satisfactorily for growth of yeast in molasses media in batch fermenters, on all scales of operation.

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Nomenclature

C_M : Concentration of substance M which reacts with substrate and produces mass.

C_R : Concentration of secretion R which accumulates in broth and suppresses growth.

C_S : Concentration of limiting substrate

C_x : Cell concentration

C_{x_o} : Initial cell concentration

C_{x_L} : Cell concentration at the boundary between transient and exponential and declining phase.

C_{x_c} : Critical cell concentration, concentration at boundary between exponential and declining phase.

C_{x_m} : Theoretical maximum cell concentration

θ_L : Time at the boundary between transient and exponential phase.

θ_c : Critical time

K : Coefficient of growth rate

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RESEARCH NOTES

DISTRIBUTION OF LIPASE IN DIFFERENT PARTS OF COCONUT (*COCOS NUCIFERA*)

A study of the distribution of lipase activity in different parts of coconut (*Cocos nucifera*) showed that the cuticle had the highest specific activity whereas the endosperm and the layer immediately below the cuticle had about a twentieth and a sixth of the activity in the cuticle. When coconut was subjected to steaming for 15 min at 5 psi, nearly 70 per cent of the lipase was inactivated. On subjecting to desiccation at 60°C for 4 hr, an additional loss of 25 per cent of activity was observed. Thus desiccated coconut so prepared had a residual lipase activity amounting to about 6 per cent of the activity in fresh coconut. No lipoxygenase activity could be detected in tender or mature coconut endosperm or in the cuticle.

In connection with studies on the storage behaviour of desiccated coconut[1], it became necessary to look into the activities of enzymes involved in the deteriorative changes of lipids. Apart from a report by Sadasivan[2] on the qualitative detection of lipase in coconut germ, there is no published data in this regard. A study was therefore undertaken to find out (*a*) whether any lipoxygenase activity is present in coconut, and (*b*) the pattern of distribution of lipase activity in different parts of the coconut and changes in lipase activity at different stages in the preparation of desiccated coconut.

Tender or mature coconuts were purchased locally. A sample was obtained by pooling from at least two coconuts. For purposes of this study, different parts of the coconut kernel are designated as: (*i*) cuticle or testa—the outermost reddish brown layer, (*ii*) parings—the white layer (*s*) immediately below the cuticle and (*iii*) endosperm—the remaining portion of the kernel. The different parts were obtained by scraping with a stainless steel knife and cross contamination was avoided by careful hand-picking.

Lipoxygenase was determined spectrophotometrically according to Bounet and Crouzet[3] using linoleate as substrate; assays were done at pH 6.5 and 9.0 Lipase activity was assayed by using β-naphthyl laurate[4] (Method A) or coconut oil as substrate[5] (Method B); the oil was emulsified with a suspension of gum acacia using a sonifier. Enzyme extracts were prepared by homogenisation of the tissue with 3-6 volumes of 0.05 M phosphate buffer, pH 7.4 in a Sorvall Omnimixer at maximum speed for 1-2 min passing the homogenate through a stainless steel strainer, centrifugation in the cold (0-4°C) at 10,000×g for 20 min and collecting the supernatant after removal of the solidified fat, if any. With β-naphthyl laurate as substrate, incubations were for one hour, whereas with coconut oil, samples were incubated for five hours. Protein in the endosperm and paring was determined by the biuret procedure[6] using Bovine serum albumin as standard. Since pigments in the cuticle interfered in the determination of protein, trichloracetic acid precipitation of the protein followed by colour development with the biuret reagent was resorted to in this case. Lipase activity is reported as micromoles β-naphthol or fatty acid liberated per hr per mg protein.

Desiccated coconut was prepared as described previously[1]. At different stages of desiccation viz. (*i*) after shredding through the Fryma mill, (*ii*) after steaming for 15 min at 5 psi, and (*iii*) after drying for 4 hr at 60°C in

TABLE 1. LIPASE ACTIVITY IN DIFFERENT PARTS OF COCONUT

Component, g/100 g fresh kernel		Protein mg/g component	Lipase activity			
			Method A	Method B	Total activity in component*	% total activity**
Cuticle	2	6.16	0.135	3.92	48.29	7.5
Paring	5	37.50	0.021	0.23	43.13	6.7
Endosperm	93	32.98	0.037	0.18	552.09	85.8

Values are averages of three independent determinations
Method A; μ moles β-naphthol liberated/hr/mg protein
Method B; μ moles fatty acid liberated/hr/mg protein

*Specific activity by method B × mg protein/g component × component per 100 g coconut kernel.
**_Total activity in component_ × 100 / Total activity in cuticle + paring + endosperm.

TABLE 2. LIPASE ACTIVITY AT DIFFERENT STAGES DURING THE PREPARATION OF DESICCATED COCONUT

Sample	Lipase activity (Method-B) μ moles fatty acid liberated/hr/mg protein	% inactivation
Fresh coconut	0.16	—
Shreds	0.16	0
Steamed at 5 psi for 15 min	0.05	69
Desiccated coconut	0.01	94

*Values are averages of three independent determinations.

a throughflow drier, samples were collected for determining lipase activity using coconut oil as substrate.

Though lipoxygenase activity has been reported in a wide variety of plant species[7,8], no such activity was detectable in tender or mature coconut endosperm or in the cuticle even though calcium was present in the assay medium.

Lipase activity was observed in the three parts of coconut studied. The specific activity with the natural substrate was much higher than with β-naphthyl laurate. However, the pattern of distribution of lipase was similar with either substrate (Table 1). The highest activity was found in the cuticle whereas the activity was much less in the paring and endosperm fractions. Comparison with the distribution of lipase activity in the paddy grain[9] indicated similarities with the present data viz. high activity in the bran/germ portion and very low activity in the endosperm portion of the grain. Calculation of total lipase activity of the cuticle, paring and endosperm fractions in the proportion in which they are present in coconut indicated that a large part of the lipase activity was located in the endosperm fraction while the paring and cuticle had equal activity and together amounted to about 13 per cent of the total (Table 1).

Changes in lipase activity at different stages of desiccation are shown in Table 2. The shredding step did not affect lipase activity. Steaming caused 69 per cent inactivation. A further loss of another 25 per cent enzyme activity was noticed at the end of dehydration. Yet the remaining 6 per cent lipase activity would lead to lipolysis and consequent rancidity if conditions are favourable. Inactivation of about 70 per cent lipase activity by steaming is indicative of the heat resistant nature of the enzyme. This observation is similar to what has been reported for rice bran lipase[10]. Also, if the conditions of steaming were to be more drastic, obtaining a snow-white product with minimum cooked flavour would not be possible. So the process for making desiccated coconut as recommended by Rajasekharan *et al*[11]. and used here is a compromise between complete inactivation of lipase and the need to get an acceptable product. While these conditions of steaming are adequate to obtain a micobiologically safe product, the disadvantage arising out of residual lipase activity in the desiccated coconut has to be overcome by suitable storage conditions.

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STABILITY OF GROUNDNUT OIL DURING CONTINUOUS DEEP-FAT FRYING AT PLANT LEVEL

Changes in physico-chemical characteristics and fatty acid composition of groundnut oil were determined during continuous frying of "Sev" or Murukku (an extruded, fried, ready-to-eat food) from a blend of corn-soya-milk powder in a 30-tonne plant at fast and slow rates of oil turnover. Significant quality changes were observed only when the oil turnover was slow.

Sev or *Muruku* (Bengal gram dhal dough extruded and fried in oil) is manufactured in a 30-tonne continuous deep-fat frying plant established by CARE and the Government of Andhra Pradesh, and then distributed to 3,00,000 school-going children (6-11 years of age) under the Mid-day Meals Programme. Corn-soya-

milk powder and refined groundnut oil used in its preparation are supplied by the USA through CARE.

Though some investigations have been carried out abroad[1,2], hardly any information is available in the country on the stability of the fryer oil during continuous plant scale frying. From the viewpoint of childrens' nutrition and health, it is necessary to examine the stability of refined groundnut oil as the fryer oil. As foods absorb oil during frying, a portion of the oil is continuously removed from the frying kettle; to compensate for this, fresh oil is added continuously. This constant addition and removal of oil, known as "turnover", will influence the quality of the fryer oil. The present work deals with the physico-chemical changes in fryer oil heated to 180°C by continuous circulation in a heat exchanger, at slow and fast turnover rates.

Two sets of frying runs were carried out, one with slow turnover and the other with fast turnover of fryer oil, each with an initial charge of 2.5 tonnes of oil. An equal amount was added in the course of 13 hr in the slow turnover run, and 8 hr in the fast turnover run; this means that about 8 and 12 per cent respectively of the initial charge was added every hour to compensate for the oil absorbed by the fried food. Samples were drawn at intervals and analysed[3] for free fatty acid content, iodine value, peroxide value and refractive index. The oil samples were also examined[4] for the presence of oxidised glycerides by thin-layer chromatography on a 0.25-mm layer of silica gel G (E. Merck) coated on 20 × 20 cm glass plates using a mixture of benzene and diethyl ether (99:1). Methyl esters were prepared from oil samples by transesterification with methanol using sodium hydroxide as catalyst and analysed on a Toshniwal gas-chromatograph with a flame ionisation detector. A stainless steel column (2.40 m × 3.2 mm) packed with 10 per cent of EGSS-X (Applied Science Laboratories, State College, PA, USA) coated on Gas Chrom Q (80-100 mesh) and maintained at 200°C was used. The flow rate of carrier gas (nitrogen) was 45 ml/min.

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF FRYER OIL DURING SLOW OIL TURNOVER

Frying time (hr)	Free fatty acid (%)	Iodine value	Peroxide value	Refractive index at 40°C
0	0.5	95.0	0.7	1.4647
8	0.6	95.2	1.0	1.4651
25	1.1	95.6	3.8	1.4651
33	1.3	95.1	3.1	1.4652
50	2.0	95.3	2.5	1.4649
58	2.3	94.3	2.8	1.4645
75	3.2	95.2	3.2	1.4646
83	3.5	95.5	3.5	1.4650

TABLE 2. PHYSICO-CHEMICAL CHARACTERISTICS OF FRYER OIL DURING FAST OIL TURNOVER

Frying time (hr)	Free fatty acid (%)	Iodine value	Peroxide value	Refractive index at 40°C
0	0.6	95.0	8.0	1.4634
65	0.8	94.7	4.1	1.4636
156	0.7	95.3	4.5	1.4636
178	0.8	95.1	4.5	1.4636
367	0.7	95.2	2.7	1.4638
500	0.8	96.0	2.9	1.4628

The changes in the physico-chemical characteristics of fryer oil during slow and fast turnover of oil are recorded in Tables 1 and 2. Significant changes in the characteristics of the fryer oil were observed only when the oil turnover was slow (100 per cent in 13 hr) but not when the turnover was fast (100 per cent in 8 hr). During slow turnover, the free fatty acid content increased from 0.5 to 3.5 per cent in 83 hr and the yellow colour of the original oil turned brown after 75 hr and very dark after 83 hr; during the fast turnover run, the increase in free fatty acid content and deterioration in colour were negligible. Peroxide value also increased from 0.7 to 3.5 in 83 hr during slow turnover. In the high turnover run, the peroxide value (which was a little high initially) decreased perhaps through thermal decomposition but the free fatty acid content did not increase appreciably. No significant alterations in iodine value and refractive index were observed in both runs.

Thin-layer chromatography indicated the presence of oxidized glycerides in the fryer oil samples (75 and 83 hr) of the slow turnover run but not in those of the fast turnover run. To confirm that there is no reduction of unsaturated fatty acids by way of oxidation in the fryer oil of the fast turnover run, fatty acid composition was determined by gas-liquid chromatography (GLC). No

TABLE 3. UNSATURATED FATTY ACID COMPOSITION OF GROUNDNUT OIL BEFORE AND AFTER FRYING

Fatty acid	Fresh Oil (%)	After 500 hr frying* (%)
Oleic	47.7	45.1
Linoleic	29.4	30.3

*100 per cent turnover in 8 hr.

significant differences were observed in the contents of oleic and linoleic acids of the starting sample and of the sample drawn after 500 hr (Table 3).

Thus, the rate of oil turnover affects the quality of frying oil. From the viewpoint both of the quality or fryer oil and the economics of production, the rate of oil turnover should be optimised for a given capacity of the frying kettle.

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LEACHING OF PHENOLIC COMPOUNDS DURING SOAKING OF PADDY

Phenolic compounds, presumably arising from the free phenol reserve of paddy grains as well as synthesised afresh in paddy during soaking, was found to leach out into soak water. An increase in this constituent was observed when paddy, brown rice, milled rice, bran and husk were soaked for 3 days in cold water.

In the production of parboiled rice by the traditional process of parboiling, paddy grains are soaked in water consecutively for 2-3 days during which time considerable loss in dry matter occurs resulting in the reduced out turn of rice[1-2]. It was reported that sugars leach out from grains during soaking[3]. In the present study the excretion of phenolic compounds into the soak water is reported.

Paddy grains of 'ADT 8' variety harvested during January-February 1977 (Samba season) was used. Paddy was shelled in a laboratory sheller and milled (5-6 per cent polish) in a McGill miller No. 1. Paddy, brown rice and milled rice (120 g) were soaked in distilled water (180 ml) in a 250 ml measuring cylinder. The total phenol content in the soak water was estimated at intervals using Folin—Ciocalteau reagent[4]. In another experiment about 3 g of paddy, brown or milled rice was tied in cheese cloth and embedded in their respective bulk quantities soaked in measuring cylinder. At intervals samples were withdrawn, extracted after draining the water, with hot 80 per cent ethanol and the phenol content in the alcohol extract was estimated[4,5]. The changes in the content of phenolic compounds in husk and bran due to soaking were also determined. In another experiment, raw and 3-day soaked paddy were dried and milled to 1,3 and 5 per cent milling in a McGill miller No. 1. Phenol content was estimated in the bran.

Table 1. Release of phenols from 'ADT 8' paddy, brown rice and milled rice into soak water

Grain	Phenols* released at indicated soaking time (hr)				
	0	12	24	48	72
Paddy	0.006	0.034	0.038	0.055	0.074
Brown rice	0.008	0.061	0.090	0.157	0.235
Milled rice	0.016	0.054	0.079	0.188	0.241

*mg/ml in phloroglucinol equivalents

The soak water from paddy, brown rice and milled rice was found to contain phenolic compounds (Table1). The presence of phenolic compounds within a short period of soaking suggests the excretion of phenols from grains into the soak water. The phenol content of the soak water increased with the increase in soaking time and the increase was relatively higher in milled and brown rice than in paddy. Soaking of paddy, brown rice and milled rice also augmented the level of this constituent in the grain and in general an increase was observed with the increase in soaking time (Table 2). The total phenol content was relatively higher in the first 1 per cent polish of raw rice than in 3 and 5 per cent

Table 2. Changes in the content of phenols in 'ADT 8' paddy, brown rice and milled rice during soaking

Grain	Phenol* content at indicated soaking time (hr)				
	0	12	24	48	72
Paddy	0.408	0.475	0.435	0.472	0.478
Brown rice	0.276	0.312	0.341	0.331	0.322
Milled rice	0.148	0.150	0.244	0.255	0.418

*mg/g on dry basis in phloroglucinol equivalents

TABLE 3. PHENOL CONTENT IN DIFFERENT LAYERS OF BRAN FROM RAW AND 3 DAYS SOAKED 'ADT 8' PADDY

Condition	Phenols* (mg/g on dry basis) at different polishing (%)		
	1%	3%	5%
Before soaking	1.407	1.028	0.911
After soaking	2.954	2.119	2.490

*Phloroglucinol equivalents.

polish suggesting the increased concentration of phenolic compounds in the peripheral layers of brown rice (Table 3). Consequent on soaking there was an increase in this constituent in different layers of bran and the rate of increase was relatively higher in the interior layers than in peripheral layers. This suggests that the synthesis and formation of phenols in the interior of the soaked grains might be greater than in the peripheral layers. An increase in the phenol content was also observed in husk and bran soaked for 72 hr (Table 4). The excretion of phenols from paddy into soak water was much lower than that of brown and milled rice even though higher quantities of phenol were found in paddy. It is possible that the intact testa and pericarp might act as barriers for the permeation of phenols from whole paddy grains whereas in brown and milled rice these barriers are either damaged by abrasive action in shelling or removed completely while polishing.

The accumulation of phenolic compounds in soak water might arise due to the excretion of phenolic compounds from the free phenol pool of the grains. Phenols were found to be present in the cell vacuoles of plant cells[6]. Suzuki[7] reported the presence of phenolic compounds in root, stem and leaves of rice plant. Their presence in paddy grains has not so far been reported although Jayachandran Nair and Sridhar[8] reported the presence of phenolic acids in rice husk. Since free sugars and amino acids were also found in soak water[9] the formation of phenols from the sugars via-shikimate[10] and aromatic amino acids[10] by the microbes developing in soak water might also contribute to the increase. The increase in the soaked grains might also be due to fresh synthesis of phenols besides release from conjugated phenolic glucosides by β-glucosidase. Paddy grains possess a β-glucosidase system which may[11] remain active in spite of submergence in water as the grains remain viable even after 3 days of soaking. The formation of higher levels of phenolic compounds in soaked paddy grains and subsequent leaching out of this constituent in soak water might contribute partly to the soaking loss encountered in parboiling.

TABLE 4. CHANGES IN PHENOL CONTENT IN HUSK AND BRAN PRIOR TO AND AFTER SOAKING FOR 72 HR

Particulars	Phenols* (mg/g on dry basis)	
	Before soaking	After soaking
Husk	1.284	1.361
Bran	2.722	3.792

*Phloroglucinol equivalents.

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BOOK REVIEWS

The Composition of Foods: Fourth revised edition, by A. A. Paul and D.A.T. Southgate, Her Majesty's Stationery Office, Elsevier/North-Holland Biomedical Press, London, 1978.

This revised edition of McCance and Widdowsons' "The composition of Foods" has additional features providing analytical data on fatty acids, cholesterol, dietary fibre and zinc. The authors have outlined briefly the procedure employed for selection of foods, arrange ment of tables and modes of expression and determina tion of constituents. The analytical data are provided for cereals and cereal products, milk and milk products, eggs, fats and oils, meat and meat products, fish and fish products, vegetables, fruits, nut, sugars and confectionaries, beverages, sauces and condiment etc. in that order. Composition of a limited range of cooked dishes are also included.

Extensive data on proximate composition, vitamin and mineral content are provided for over 900 items. The items are number coded and in Section II and III data on amino acid composition and fatty acid content are listed for a number of the items. Rationale for expressing retinol equivalents of foods providing carotene is of particular interest. The FAO/WHO recommendation in computing the retinol equivalents is preferred. Key to the use of amino acid and fatty acid composition of ingredients for proper computation and examples given for calculating their contribution for cooked foods are useful.

As may be expected this revised edition is of considerable use to nutritionists and dietitians. However, from the point of relevance of this book to dietary patterns in India analyfical data particularly on legumes, dahls and legume cereal based diets are lacking. Furthermore, data on the contribution of spices, condiments and adjuvants are very limited. Perhaps this approach of the authors could serve as a base for collecting and systemising such data on nutrient composition of our staple foods and an abundant variety of receipes.

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Advances in Biochemical Engineering—New Substrates: Edited by T. K. Ghose, A. Fiechter and N. Blakebrough, Springer-Verlag, Berlin Heidelberg, New York, Vol. 6. 1977, Price: $ 24.90

The book covers four reviews of current interest to the industrial microbiology. Of these, the chapters on *Thiobacillus ferroxidans*, metabolism of methanol by yeasts and role of phosphates in regulation of antibiotic synthesis would be of particular interest to the students of microbiology. There is also a chapter on cellulase biosynthesis which covers extensively the cellulose-cellulase system. However, since the advent of energy crisis, one finds very often reviews on renewable resources like cellulase in the literature. This series itself has carried reviews on this system at least three times before and hence this topic tends to be repetitious.

Role of *Thiobacillus ferroxidans* in hydrometallurgical processes have been reviewed by A. E. Torma (New Mexico Institute of Mining & Technology, USA). This area of microbial technology is gaining importance in biohydrometallurgical extraction processes. These microbial systems are also of interest in ecosystems because of their fairly widespread occurrence in nature. Many aspects of the bacterium like morphology, physiology, kinetics of growth, and environmental influences (e. g. pH, temperature, composition of leach residue, organic nutrients etc.) on its growth and survival have been presented, perhaps for the first time, in form of a comprehensive review. Though microbial leaching techniques are employed primarily for large scale recovery of uranium and copper, possible applications for recovery of other metals such as cobalt, zinc, lead etc. had also been discussed.

Use of methanol as a source of carbon in preference to n-paraffins and methane for the production of biomass (single cell proteins) has received a considerable attention in recent years. The author, Prof. Sahm has included various metabolic aspects of methanol by yeasts, the intracellular localisation of enzymes of dissimilatory pathway of methanol and its regulatory aspects. Possible use of methanol as a carbon source for cell biomass and for production of some of the secondary metabolites, like amino acids, organic acids and purines have also been discussed.

Biosynthesis of secondary metabolites is inhibited by the presence of phosphates at a higher concentration. And yet the phosphate is an essential constituent of the nutrient medium of practically all the microbialsystems of industrial importance. Dr J. F. Martin has examined various theories in support of the regulatory role of phosphate ions on the biosynthesis of antibiotics and suggests that though it is difficult to generalise the

regulatory role of the phosphates, it would appear that the phosphates would control antibiotic synthesis through a common effector which is probably ATP. However, the final answer as to the actual involvement of ATP as an effector in case of antibiotic synthesising enzymes has yet to be determined. The review, though confined to a highly specific problem, should be of interest to many studying regulatory role of phosphates on growth and metabolite synthesis.

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Advances in Biochemical Engineering-Biotechnology: Ed. by T. K. Ghose, A. Fiechter, N. Blakebrough; Springer-Verlag, Berlain-Heidelberg, New York; Vol. 7; 1977; Price: $ 26.70

The book is well edited. The chapter on bubble column bioreactors is very well written and gives in a very concise form the characterisation and modelling of the bubble column. The choice and analysis of the equations and the logic of the deductions are well made. The way to have small bubble size without high rate of energy dissipation (i.e. high E/V_2) by preventing the bubbles to coalesce by additives, is industrially important.

The generalizations of the gas hold up and Fraude Number using different nozzles is useful. The deductions of bubble sworm velocity, size and stability as a function of Sauter mean diameter would be useful in designing aerobic fermentation reactors.

The influence of the distributor on the specific interfacial area, and the high interfacial areas obtainable by nozzles compared to porous plates is useful. The mass transfer coefficient data of oxygen under different system $K_La=0\ 0.0023 \left(\frac{wsa}{dB}\right) 1.58$ is valuable and indicates the importance of nozzles over plates.

The treatment of the multistage system is also exhaustive. The possibility of increasing the mass transfer coefficient by suitable multistage system is useful.

That mechanically agitated bioreactors are much less efficient than the self aspirating bioreactor or with injector ejector nozzles, and the data on mass transfer coefficients of different types of bioreactors are of immense value for designing efficient bubble column bioreactors for aerobic processes.

In the chapter of complimentary approach to scale up, the point that optimization of the entire operations on a fermentation is more important from overall economy than suboptimization of individual process has been stressed.

Systems equation for time cycles of operation in fermentation, e.g. sterilization, aeration, are given, but has not included the more efficient aerators like nozzles, perforated plates etc.

Process optimization has been exemplified by a case study. When recovery of the product is included the optimization process would be much more complicated. For efficient recovery it may be necessary that the fermentation has to be continued to completion, then the logic of optimization on the fermentation cycle cannot be applied as shown. In such cases the method will have some limitations.

The treatment of the theory is elementary. The method of measuring the gross redox potential of the system, without a consideration of the actual biochemical reactions which are the controlling parameters seem to be superficial, and cannot lead to any basic understanding of the process.

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Advances in Biochemical Engineering-Microbial Processes: Edited by T. K. Ghose, A. Fiechter and N. Blakebrough; Springer-Verlag, Berlin Heidelberg, New York, Vol. 9, 1978, Price: $ 29.

This volume reviews four topics of current interest under a common title *Microbial Processes.* Two of these deal with anerobic fermentations concerned with continuous cultivation of microorganisms in industrial alcohol processes (V. L. Yarovenko, Moscow) and microbial production of hydrogen (J. E. Zajic, *et al.* Canada). Other two articles are more of basic nature dealing with mechanism of liquid hydrocarbon up-take by microorganisms (Y. Muira, Japan) and *in vitro* synthesis of enzymes (T. Enatsu and A. Shimyo, Japan.)

The article by Yarovenko discusses the theory and practice of continuous fermentation for industrial production of alcohol. The fermentation system described by the author is adaptable to other types of anerobic fermentations particularly butanol and acetone. It makes use of conventional fermentors arranged sequentially which permits, apart from long residence times for culture broth usually required for such processes, continuous sterilisation of each of the vessels without interrupting fermentation at any stage of operation. In such a system, fermentation rates, concentration of

cells, and residence time of the culture broth are regulated by controlled addition of nutrients, and seed culture through interconnected tubes from the moment of "first fermentor charge-up to the filling of whole battery". Volume of the fluid in each fermentor is maintained by an "outflow" into the next fermentor and so on. Disadvantage of the system is that residence time of the culture fluid in each of the fermentors varies from 120 hours in the first to 24 hours in the last fermentor on the battery. Detailed kinetics of the system described by the author as "battery cycle system" with respect to bulk transport, effects of yeast cell concentration and "preventive" sterilisation of equipment to maintain continued asepsis during the fermentation process have been discussed.

Review by Zajic *et al.* on 'Microbial Production of Hydrogen' is quite comprehensive and covers the description of various microbial strains producing hydrogen as an end-product of metabolism, their nutritional requirements and enzyme systems involved in biogenesis of hydrogen in bacteria. Hydrogen gas is considered as an "ideal fuel, not only as an alternative energy source but also as a highly efficient energy carrier", and when burned in pure oxygen, only end-product is water. Other gaseous pollutants are non-existent. Among several available processes for production of hydrogen from water, electrolytic hydrogen has been industrially produced, but only in areas where electricity is cheap. On the other hand, production of hydrogen through anerobic digestion of cellulosic and other carbohydrate material appears to be quite feasible in the long run, particularly if it is coupled with waste treatment plants designed for biogas production. In fact, hydrogen is a component of biogas in small amounts and an essential precursor to methane. This is therefore, a timely review for those interested in biogas research particularly with a view to improve its energy value.

Remaining two articles are concerned with Mechanism of 'Liquid Hydrocarbon Uptake by Microorganisms' (Y. Muira) and a review on '*In Vitro* Synthesis of Enzymes; Physiological Aspects of Microbial Enzyme Production' (T. Enatsu and A. Shimyo). First of these, looks into the kinetics of uptake of liquid hydrocarbon by yeast, *Candida intermedia* and is essentially a survey of the author's work on the subject.

The second article is a review of the present knowledge about molecular mechanisms involved in *in vitro* biosynthesis of microbial enzymes with special reference to hydrolytic enzymes which have industrial applications.

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Table Grapes and Refrigeration: International Institute of Refrigeration 177. bd. Malesherbes, Paris-75015, 1978, pp, 248.

This book, exclusively on table grapes, includes reports of Commissions I and II of the International Vine and Wine Office and Commission C 2 of the International Institute of Refrigeration which met jointly during October, 12-14, 1977, at the Ministry of Agriculture in Paris. In all 28 reports were presented by specialists from 14 different Countries, of which 21 are in French and 7 in English. Each paper includes a summary either in English or French and is accompanied by the discussions that followed the presentation of each paper. General discussion is presented at the end of the book.

The subject matter is covered in four sections.

Section one on product characteristics includes quality standards, tests and maturity indices for table grapes; behaviour of conventional grape varieties; characteristics and development of secretions during ripening of grapes; some new grape varieties and their keeping qualities under refrigeration; and the economic feasibility of refrigerated storage (6 reports).

Section two on pre-harvest processes includes treatment of grapes against gray rot in *Botrytis cinerea;* use of growth regulators; aspects of pesticide residues on grapes; vineyard irrigation practices; pre-harvest interventions; and fertiliser application during grape cultivation (6 reports).

The third section on processes between harvest and storage covers packing and packaging of table grapes and precooling in relation to market quality (3 reports).

The fourth section on storage and transport includes storage characteristics of some Rumanian table grape varieties; observations on the filmy skins of *Vitis vinifera* held under cold storage; correlation between biochemical characteristics and storage of table grape varieties; long and short term storage of grapes; feasibility of low pressure storage; storage trials; table grapes and refrigeration, modified atmospheres with special reference to the influence of SO_2, preservation by means of "generating bags", refrigerated storage, new plastic diffusers of SO_2 for the cold storage, practical applications for the long term preservation and transport of table grapes (13 reports).

This document, the first published by the International Institute of Refrigeration on table grapes and refrigeration, will serve as a good guide to researchers and users engaged in the preservation of table grapes during the period from harvest to final consumption.

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

Energy Balance and Obesity in Man: by J. S. Garrow, published by Elsevier/North-Holland Biomedical, Press, Review Department P.O. Box No. 1527, Amsterdam, The Netherlands, 1978, pp, 224+*xii*, Price: $ 53.50.

Generally, later editions of a book tend to have many additions and few deletions. The second edition of Garrow's book is, therefore, a delight—thinner than the original, but no less informative. Such of the topics where not much new information is available have been discussed in an appropriately limited manner, leaving the reader to refer back to the first edition. Thus, the book has not become voluminous and the first edition does not become outdated.

The introductory chapter is concise and clear. The chapter on energy intake is considerably revised. The discussion on the role of the hypothalamus in appetite control, which was presented at length in the earlier edition, is almost deleted. Recent research indicates that this may not be important in man. George Cahill's lecture (referred to on page 56) stressing the importance of the discriminatory powers of the brain over its mere physiological control also appears to have been a determining factor for this omission.

The innumerable difficulties in measuring energy expenditure and stores and the fallacies in interpretation of data are discussed in detail in the subsequent chapters. Three factors are identified as important in stabilising energy balance: A control on food intake, an increase in energy expenditure with an increase in energy storage and changes in metabolic rate following large changes in body weight.

The last chapter deals with treatment of obesity. Despite the claims of efficacy of various drugs, dieting still remains the best method of control; yet it is the most difficult. Garrow emphasizes the need to assess the immediate and long-term benefits of reducing weight for each patient and the causes for failure—to recognize, "when to try hard and when to give up". The futility of most drugs and surgical procedures is also discussed. This chapter offers many important tips to clinicians who are directly involved in dealing with obese patients.

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

J. V. Prabhakar
Signature of the Publisher

ASSOCIATION NEWS

SOUTHERN REGIONAL BRANCH

Report of the Annual General Body Meeting of the Branch held on 29th January 1979 at Hotel Dasaprakash, Madras.

The President welcomed the members for the 3rd General Body Meeting of the Branch. More than sixty members were present. The Hon. Secretary presented the Annual Report of the Branch for the year 1978 which was adopted by the House. The Hon. Treasurer presented the audited annual accounts for 1978 which was also later adopted by the House. The Office bearers for 1979 are as follows:

President
Shri S. Rajagopalan

Vice-President
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Shri M. Srikrishna

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Dr. A. Sreenivasan
Shri S. Subramanyan
Shri T. V. Subramanyan

The Annual General Body Meeting was followed by a keynote address by Dr. S. G. Sreekantaiah, Director, National Institute of Nutrition, Hyderabad. Dr. B. P. Baliga, President of the Association also addressed the members. The meeting ended with a dinner.

HYDERABAD CHAPTER

Report of the Annual General Body Meeting of the Chapter held on 10th February 1979.

The President of the Chapter welcomed the members and presented the Report for 1978. This was followed by the presentation of Report by the Hon. Secretary, and the audited statement of accounts by the Treasurer.

Following Office bearers have been unanimously elected for the year 1979.

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Shri M. Venkateshwara Rao

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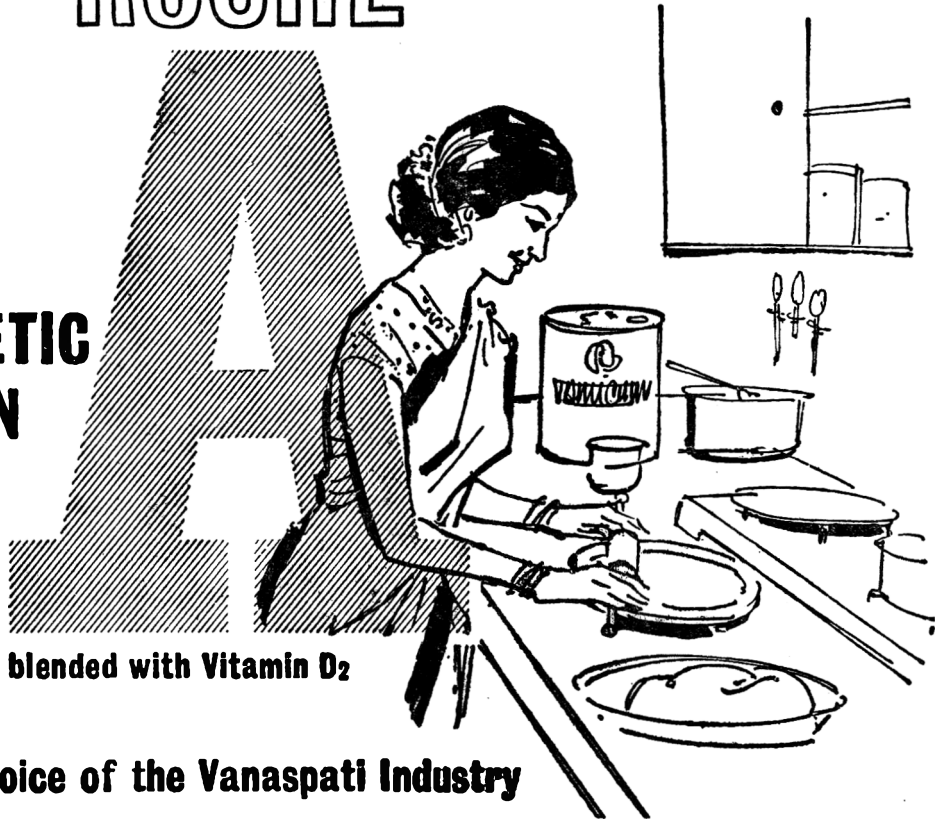

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2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.

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

4. **Abstract:** The abstract should indicate the scope of the work and the principal findings of the paper. It should not normally exceed 200 words. It should be in such a form that abstracting periodicals can readily use it.

5. **Tables:** Graphs as well as tables, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. Nil results should be indicated and distinguished clearly from absence of data.

6. **Illustrations:** Line drawings should be made with *Indian ink* on white drawing paper preferably art paper. The lettering should be in pencil. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size. Photographs must be on glossy paper and contrasty; *two copies* should be sent.

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

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

   In the text, the references should be included at the end of the article in serial order.

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

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

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

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

   (*d*) *Proceedings, Conferences and Symposia:* As in (*c*).

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

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