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ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS

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Affiliated to the Institute of Food Technologists, USA

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 2. To provide a forum for the exchange, discussion and dissemination of current developments in the field of Food Science and Technology.
 3. To promote the profession of Food Science and Technology.
- The ultimate object is to serve humanity through better food.

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CONTENTS

Research Papers

- Development of a Mathematical Model for Oil Expression from a Thin Bed of Rapeseeds Under Uniaxial Compressions** 1
Jaswant Singh and B. P. N. Singh
- Studies on Oxidative Stability of Crude and Processed Yellow Nutsedge Tuber and Almond Seed Oils** 8
A. O. Tairu, M. A. Omotoso, R. A. Oderinde and F. O. Bamiro
- Standardization of Manufacture of Kadhi Powder** 12
Bikash C. Ghosh and Satish Kulkarni
- Preparation and Evaluation of Soy Paneer** 15
Sangeeta Arora and B. K. Mital
- Effect of Modilase Enzyme on Characteristic Flavour of Buffalo Cheddar Cheese without and with *L. casei* at Different Ripening Temperatures** 18
Y. K. Jha and Singh
- Effect of Chilled Seawater Storage of White Sardine (*Kowala coval*) on its Canned Product Quality** 23
G. Jeyasekaran and K. V. Saralaya
- Flow Behaviour Properties of Rice Bran Protein Concentrate** 27
M. B. Bera and R. K. Mukherjee
- An Objective and Sensory Assessment of Cooking Quality of Some Rice Varieties Grown in Andhra Pradesh** 31
Sunita Kumari and Padmavathi
- Secretion and Composition of Bile in Rats Fed Diets Containing Spices** 35
K. Sambaiah and K. Srinivasan

Research Notes

- Influence of Packaging Systems on the Microbiological Quality of Processed Cheese During Storage** 39
G. K. Goyal and K. E. Babu
- Nickel in Vanaspati** 42
Satya Prakash and R. K. Sarin
- Enhanced α -Amylase Production on Wheat Bran Medium by *Bacillus subtilis*-159** 44
S. G. Gayal, V. G. Khandeparkar and D. V. Rege
- Mineral Requirements for Growth of *Volvariella volvacea* Mycelium by Submerged Fermentation** 46
Chaitali Das Gupta and S. K. Majumdar

Effect of Common Salt in Preventing Spoilage of Wet Paddy Under Inclement Weather Conditions	48
<i>V. Krishna Rao, T. Ratnasudhakar, K. Sreerama Murthy and P.V. Narayana Rao</i>	
Effect of Drying on Microbial Population of Wet Paddy Using Batch Type Dryer	50
<i>V. Krishna Rao, V. Joga Rao, T. Ratnasudhakar, K. Sreerama Murthy and P. V. Narayana Rao</i>	
Dehydration of Bitter Gourd (<i>Momordica charantia</i> Linn) Rings	52
<i>S. Siva Kumar, R. Kalra and Nirankar Nath</i>	
Preservation of Raw Mango Slices (Var <i>Neelum</i>) for Use in Pickle and Chutney	54
<i>Vijay Sethi</i>	
Chemical Quality of Some Marketed Indigenous Milk Products —	
I. Major Constituents and Mineral Composition of Paneer	57
<i>V. R. Boghra and O. N. Mathur</i>	
Chemical Quality of Some Marketed Indigenous Milk Products —	
II Mineral Composition of Khoa	59
<i>V. R. Boghra and O. N. Mathur</i>	
Free Fatty Acid Contents and Off-Flavour in Commercial Canned Mutton Curry	61
<i>T. S. Vasundhara, K. V. Kumudavally and B. Bhagirathy</i>	
Extraction of Juices from Peaches, Plums and Apricots by Pectinolytic Treatment	64
<i>V. K. Joshi, S. K. Chauhan and B. B. Lal</i>	
Effect of Dietary Tallow on Carcass Fat and Serum Cholesterol Profile in White Leghorn Cockrels	66
<i>V. G. Butala and S. Rajagopal</i>	
Book Reviews	68

Development of a Mathematical Model for Oil Expression from a Thin Bed of Rapeseeds Under Uniaxial Compression

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The mathematical model considered the radial movement of oil under vertical force application. It correlates oil expression with pressure, coefficients of consolidation and permeability, time of compression, area of cross section through which oil flow occurs and density of oil. Model adequately describes oil expression behaviour having close agreement with experimental values and holds for all values of time.

In India, about 90 per cent of the total production of oil-seeds is crushed for production of oil through mechanical expellers¹. Studies in the past on mechanical oil expression mostly related to the experimental investigations on influence of moisture, applied pressure and temperature on oil yield^{2,5}. Koo's⁶ equation on oil expression fails at larger values of pressing time resulting in infinite predicted yield. Sukumaran and Singh⁷ studied pressure induced oil expression from rapeseed and identified oil point and extrusion point. The polynomial models, of Singh *et al.*³ developed on the basis of experimental data on residual oil in cake, pressure and temperature, are valid only over the range of experimental conditions and do not provide any theoretical base. Mrema and McNulty⁸ considered flow of oil in the direction of applied force and applied the modified form of Terzaghi's equation for oil expression without considering the stage of saturation at which it should have been applied and also did not account for the bed resistance. But in the most practical situation of mechanical oil expression, such as screw expeller, the flow of oil is normal to the direction of applied force. Therefore, in the present study a mathematical model, with theoretical base involving the radial flow of expressed oil, has been developed and experimentally evaluated which would help a great to the researchers and designers to understand the process of oil expression and the mechanics better and design more efficient systems of expression.

Theoretical development: In the initial stage of compression, rearrangement of seeds causes decrease in the void volume of bed. As compression proceeds, individual seeds are squeezed into interparticle spaces with concomitant loss of their initial geometry. With further compression, a stage soon reaches when intercellular structure of seeds breaks releasing the oil⁹. Later Diekert and Diekert¹⁰ and Mrema

and McNulty¹¹ suggested that during expression, the porous nature of biological cell walls might be the channels through which oil flows out of the seed and saturates the bed. With sustained compression, a stage reaches when surrounding voids are filled with oil and the sample under compression represents a saturated two phase system of incompressible particles forming a cake matrix and incompressible oil contained in voids of that matrix. Under such a condition, the applied pressure is shared both by solid portion of deformed rapeseed and the oil as well. When oil pressure exceeds the pressure required to move the oil through tortuous passages of the cake body, the flow occurs causing decrease in residual oil and in turn enabling the solid structure of the cake matrix to share more of the total load.

The test cell-geometry: A typical test cell geometry consists of inner and outer walls of radii R_1 and R_2 respectively. The material is confined within the annular space of the test cell. The flow of oil during the process of compression is not impeded from sides. Earlier studies¹² on similar type of annular cell geometry had also indicated that perforations provided in the side walls neither offered any resistance to oil outflow nor allowed whole or deformed rapeseed to come out of the cell during compression process prior to extrusion point. Mrema and McNulty⁸, reported that porous disc at base of the cylinder would offer resistance particularly when porosity reduces during compression. Therefore, radial flow of oil from confined bed of rapeseed has been considered in the present study.

The bed: A 10 mm thin layer of rapeseed (weighing about 12.1 g) in the test cell has been considered as the thin bed. With such a thickness of rapeseeds, stress and density gradients do not exist under uniaxial compression¹²⁻¹⁵.

Development of governing equation: For ease in developing the mathematical model, following assumptions were made.

(i) The bed is saturated; (ii) oil expressed is equal to the volume displaced; (iii) the cake matrix which forms the solid phase consists of incompressible solid particles; (iv) the liquid phase consists of incompressible oil; (v) the characteristic parameters of oil flow are constant and (vi) for flow of oil through the porous media of cake, Darcy law is valid. After certain stage of further compression, the pressure attains its present value. This stage occurs much after the oil point stage. Corresponding to this particular stage when preset value of pressure is obtained, the time of compression is defined as the operational time $t=0$, deformation as δ_0 and quantity of oil expressed as Q_0 . Obviously, prior to this stage the compression is dynamic. With further compression for any value of time t , the deformation that occurs is denoted by δ , and the oil expressed by Q . Initial bed height H is then reduced to $(H-\delta)$ at $t=0$ and $(H-\delta_0)$ at any time $t=t$ with the constant bed width of $R_2-R_1=L$. As oil flows out, pore volume decreases such that initial void ratio e_0 at $t=0$ decreases to value 'e' at $t=t$. Thus if δ_0 and δ be the deformation corresponding to e_0 and 'e' then coefficient of compressibility, 'C' could be written as

$$C = \frac{\pi (R_2^2 - R_1^2) \rho_p (\delta - \delta_0) (100 - M)}{m_s \partial_p} \dots\dots\dots 1$$

Where, ρ_p = particle density; M = moisture content (% wb) and m_s = weight of solid free of moisture and oil. And decrease in volume of voids per unit weight of initial volume could be expressed as

$$\Delta V = \frac{\pi (R_2^2 - R_1^2) \rho_p (\delta - \delta_0)}{m_s (1 + e_0)} \dots\dots\dots 2$$

Which on substitution of the value of e_0 from its basic definition and that of $\delta - \delta_0$ ^{14,15} could be written as

$$\Delta V = C_v \partial_p \dots\dots\dots 3$$

Where C_v = coefficient of volume decrease which is equal

$$\text{to } C_v = \frac{C m_s}{\pi (R_2^2 - R_1^2) \rho_p (100 - M) (H - \delta_0)} \dots\dots\dots 4$$

Now differentiating Eq 4 with respect to t we get

$$\frac{\partial V}{\partial t} = C_v \frac{\partial p}{\partial t} \dots\dots\dots 5$$

Which could be defined as rate of decrease of volume of voids. On occurrence of oil flow, the oil pressure 'p' gradually decreases which causes increase in effective expression pressure 'E' similar to the cases of soil consolidation¹⁶. Therefore, Eq 5 could also be written as

$$\frac{\partial V}{\partial t} = C_v \frac{\partial E}{\partial t} \dots\dots\dots 6$$

This implies that increase of effective expression pressure per unit of time involves a corresponding decrease in the volume V of rapeseed sample.

We consider a differential element ' $2\pi r dr dz$ '. The volume of oil which flows out of the element per unit of time must be equal to the sum of volume of oil which enters in and the volume of oil which is generated inside the element.

$$\text{i.e. } 2\pi r v dz + 2\pi \frac{\partial V}{\partial t} r dr dz - 2\pi (vr + \frac{\partial vr}{\partial r} dr) dz = 0 \dots\dots 7$$

Solving this we get $\frac{\partial V}{\partial t} = \frac{1}{r} \frac{\partial vr}{\partial r}$..8, which from Darcy's

law could also be written as

$$\frac{\partial V}{\partial t} = -k \left(\frac{\partial^2 E}{\partial r^2} + \frac{1}{r} \frac{\partial E}{\partial r} \right) \dots\dots\dots 9$$

Where k is coefficient of permeability. With the help of Eq 6 this could also be written as

$$\frac{\delta E}{\delta t} = C_v \left(\frac{\partial^2 E}{\partial r^2} + \frac{1}{r} \frac{\partial E}{\partial r} \right) \dots\dots\dots 10$$

Where, C_v = Coefficient of consolidation and is defined as

$$C_v = \frac{k}{(e - e_0) \partial_p}$$

Eq 10 is the basic differential equation which governs the process of oil expression under uniaxial compression of rapeseed bed for constant load. It represents the relationship between the change of expression pressure per unit of time and per unit of radial distance. It is also evident that coefficients of consolidation and permeability are interrelated.

In terms of non-dimensional variables, Eq 10 could be written as

$$\frac{\partial \eta}{\partial \theta} = \frac{\partial^2 \eta}{\partial \xi^2} + \frac{1}{\xi} \frac{\partial \eta}{\partial \xi} \dots\dots\dots 11$$

Where, $\eta = E/a$; $\xi = r/(R_2 - R_1)$; and $\theta = C_v t/L^2$

Solution of the equation: To get explicit expression, Eq 11 is solved with following initial and boundary conditions

— Initial condition; $\eta(\xi, 0) = 1$, Boundary conditions; $\eta(\xi, \theta) = 0$; & $\eta(\xi_2, \theta) = 0$ And, the solution¹⁷, could be

$$\eta = \pi \sum_{n=1}^{\infty} \left(\exp - \left(\frac{U_n \theta}{\alpha} \right) \right) \frac{J_0(\xi_1 \alpha_n) U_0(\xi \alpha_n)}{J_0(\xi_1 \alpha_n) + J_0(\xi_2 \alpha_n)} \dots\dots\dots 5$$

Where, $U_n = \alpha_n \xi_1$; $\xi_1 = r_1 / (R_2 - R_1)$; α_n = roots of Bessel's function;

and $U_0(\xi \alpha_n) = J_0(\xi \alpha_n) Y_0(\xi_2 \alpha_n) - J_0(\xi_2 \alpha_n) Y_0(\xi \alpha_n)$

Total flux: If q_1 and q_2 are the volumetric discharges of oil per unit time and per unit area through the inner and outer surfaces of the annular ring respectively, applying Darcy's law the total flux could be expressed as

$$q = q_1 + q_2 = \frac{\pi ka}{L} \sum_{n=1}^{\infty} \exp(-\alpha_n^2 \theta) F_n \dots\dots\dots 13$$

Where, $F_n = G_n |_{\xi=\xi_1} + G_n |_{\xi=\xi_2}$ and $G_n = \frac{J_0(\xi_1 \alpha_n) U_0(\xi \alpha_n)}{J_0(\xi_1 \alpha_n) + J_0(\xi_2 \alpha_n)}$ and: $U_0(\xi \alpha_n) = J_0(\xi_2 \alpha_n) Y_1(\xi \alpha_n) - J_1(\xi \alpha_n) Y_0(\xi_2 \alpha_n)$

Quantity of oil released

The quantity of oil released upto any time t which starts after achieving constant pressure can be expressed as

$$Q - Q_0 = Y = \frac{\pi ka}{L} \rho_o \int_0^\theta \sum_{n=1}^\infty (\exp(-\alpha_n^2 \theta)) \alpha_n F_n$$

A (θ) dθ 14

Since both oil and solid particles are incompressible volume of oil expressed must equal the volume displaced, Therefore,

$$Y = \pi \rho_o (R_2^2 - R_1^2) (\delta - \delta_o) \dots\dots\dots 15$$

Substituting δ = H - A (θ)/2 π (R₂ + R₁)

in A (θ) = 2 π (R₂ + R₁) (H - δ)

We get A (θ) = A₀ - $\frac{2\pi (R_2 + R_1)}{\pi \rho_o (R_2^2 - R_1^2)}$ Y 16

Hence, Y = $\frac{ka\pi\rho_o L}{C_o} \int_0^\theta \sum_{n=1}^\infty (\exp(-\alpha_n^2 \theta)) \alpha_n F_n$

(A₀ - $\frac{2Y}{\rho_o L}$) dθ 17

Differentiating w.r.t. θ and solving Eq 17, we get

OR $1_n (1 - \frac{2Y}{A_o \rho_o L}) = -D (\sum_{n=1}^\infty \frac{F_n}{\alpha_n} (1 - \exp(-\alpha_n^2 \frac{C_o t}{L^2})))$ 18

Eq 18 can also be expressed in a simplified form as

$$Y = \frac{1}{K_o} (1 - \exp(-D\phi)) \dots\dots\dots 19$$

Where, D = $\frac{2\pi ka}{C_o}$, 20; K_o = 2/A_o ρ_oL 21

and φ = $\sum_{n=1}^\infty \frac{F_n}{\alpha_n} (1 - \exp(-\alpha_n^2 \theta))$ 22

The expression at Eq 19 is the general solution for oil expression.

Properties of function φ: It could be seen that for θ > 0.02 only three terms of the series were adequate. Beyond θ = 0.10 only the first term of series was significant to evaluate φ. However, for θ ≤ 0.02 five or more terms need to be considered. This presented some difficulty because beyond first five terms values of α_n were not available.

As θ → 0, $\sum_{n=1}^\infty \frac{F_n}{\alpha_n} (\exp(-\alpha_n^2 \theta)) \rightarrow \sum_{n=1}^\infty \frac{F_n}{\alpha_n}$

And truncation errors become significant. In view of the foregoing, following approach is suggested for evaluation of φ for θ < 0.03.

First five values of α_n, F_n, F_n α_n and F_n/α_n are given in Table 1. The sum of first five terms has a value of 0.024. The terms of φ being exponential functions, power series expansion of each term leads to the following expression.

$$\phi = \sum_{n=1}^\infty \frac{F_n}{\alpha_n} - \sum_{n=1}^\infty \frac{F_n}{\alpha_n} \sum_{i=0}^\infty (-1)^i \frac{(\alpha_n^2 \theta)^i}{i} \dots\dots\dots 23$$

TABLE 1. VALUES OF α_n, F_n, F_n α_n AND F_n/α_n

n	α _n	F _n	F _n α _n	F _n /α _n
0	3.022	0.086	+0.259	0.028
1	6.053	-0.041	-0.250	-6.83 × 10 ⁻³
2	9.082	+0.027	+0.247	+2.996 × 10 ⁻³
3	12.111	-0.021	-0.250	-1.703 × 10 ⁻³
4	15.139	+0.017	+0.259	+1.132 × 10 ⁻³

Changing the order of summation and solving we get

$$\phi = \sum_{n=1}^\infty \frac{F_n}{\alpha_n} \sum_{i=0}^\infty (-1)^i a_i \frac{\theta^i}{i} \text{ where, each}$$

$$a_i = \sum_{n=1}^\infty F_n \alpha_n \cdot 2i - 1 \dots\dots\dots 24$$

If terms involving the third and higher degrees are discarded,

$$\sum_{n=1}^\infty \frac{F_n}{\alpha_n} \exp(-\alpha_n^2 \theta) = a_0 - a_1 \theta + a_2 \theta^2, \text{ thus clearly,}$$

$$a_0 = \sum_{n=1}^\infty \frac{F_n}{\alpha_n} \dots\dots\dots 25$$

The coefficients a₁ and a₂ can not be calculated from their analytical expressions because of large truncation errors involved. To get around this difficulty, a second degree equation was fitted between φ and θ. The resulting equation is : θ = 0.1566 φ - 0.2956 φ² 26

Fig 1 shows a plot of φ/θ vs θ. Points marked represent

values from Eq 22 while line drawn represents the Eq 26. Fig 2 shows a plot of φ for θ ≤ 0.10. Over this range, true values and those computed through differentiation of Eq 17 are the same for all practical purposes. However, the difference in φ values for θ ≤ 0.02 indicated the need for considering more than five terms which could not be considered for practical limitations. Therefore, for small values of time i.e. θ ≤ 0.10 Eq 26 is an adequate representation of φ. For large values of time i.e. θ ≥ 0.10.

$$\phi = 0.024 - 0.028 \exp(-9.1317 \theta) \dots\dots\dots 27$$

Forms suitable for evaluation of D and C_o : From Eq 26 and 27 it could also be seen that for small values of time

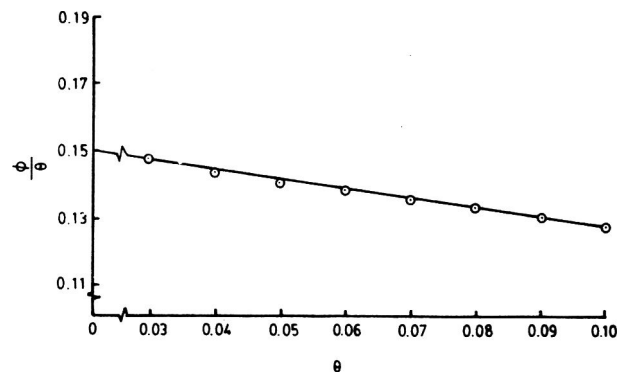


Fig.1. Relationship between φ/θ and θ

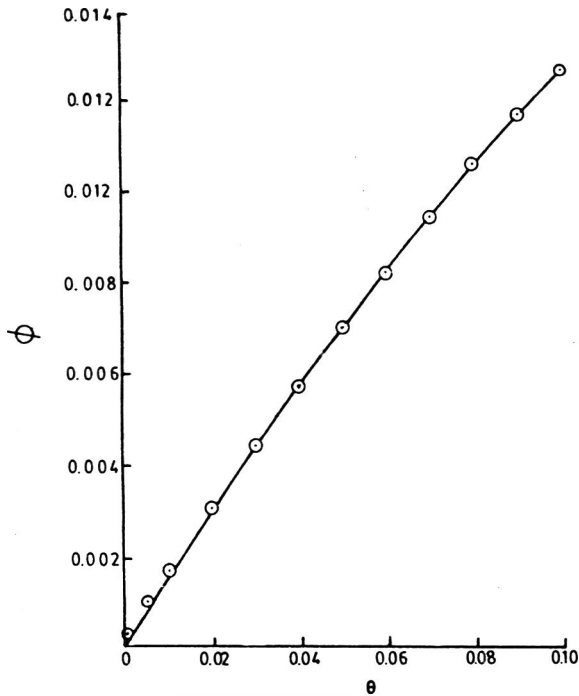


Fig.2. Relationship between ϕ and θ

$$\ln \frac{(1 - K_0 Y)}{t} = - \frac{DC_0}{L^2} (1566 - 0.2956 \frac{C_0}{L^2} t) \dots\dots 28$$

Eq 28 is particularly convenient for evaluation of D and C_0 which are generally not known, and have to be evaluated from experimental data. Now let $\ln(1 - K_0 Y) = Z$. Alternatively, When θ is large, it could be seen that for two successive equispaced values of θ or t, $Z_n = Z_{n+1} - 0.024 D (\lambda - 1) \dots 29$

Where, $\lambda = \exp(\theta_{n+1} - \theta_n) = \exp \frac{C_0}{L^2} (t_{n+1} - t_n)$

From linear regression of Eq 29 values of λ and D could be evaluated. Since $t_{n+1} - t_n$ is constant and L is known C_0 could be computed from λ .

Evaluation of the Model: In the Eq 18, parameters D and C_0 are not known. To determine their values, various approaches viz trial and error, least square, point of inflection method and least squares regression using Taylor Series expansion method were tried. Of these, least squares regression using Taylor Series expansion yielded the best fit. After determining the value of D and C_0 (Table 2) values of oil yield were predicted with the help of Eq 19.

For experimental evaluation of the model, a 10 mm uniform thick bed of rapeseed each weighing about 12.1 g, as mentioned earlier, was held in the test cell and compressed uniaxially at different rates of deformation (3.96 and 2.47 mm/s), moisture contents (about 5.7 and 9 per cent), static pressures (6.70, 10.70, 14.72, 16.60 and 18.70 MPa/m²) and pressing time (0, 15, 30, 60, 120, 180, 240, 300 and 360 s). Standard Carver Laboratory hydraulic press (25 T capacity) was employed for the compression of rapeseeds in

the test cell (Fig 3). After each experiment, through the mass balance weighing the samples before and after compression, the oil yield was determined. The values of oil yield were plotted against the time of compression corresponding to different moisture contents at particular values of pressure and rate of deformation. Sample plots are shown in Fig 4 and 5.

The predicted values through the model and the experimentally observed values were found to be in very close agreement of each other (Fig 4 and 5). Curves drawn through data points represent computed values which include the response of the model. Similar were the responses corresponding to different values of static pressure, time of pressing and various levels of moisture contents. The standard error (SE) and error sum of squares (ESS) between observed and predicted values of oil yield (oil expressed) were found to be in the range of 0.0105 to 0.1995 and 0.0008 to 0.2785 respectively (Table 3). On overall basis the model, thus, gave an adequate representation of oil expression.

It was also found that dry matters' particle density of rapeseed cake was not influenced by the moisture content, applied pressure and the time of being under that pressure. Particle density was constant with a mean value of 1.2186 g/cm³, and sample standard deviation of approximation of 0.1 per cent¹⁴. This established that the basic solid particles that make up the compressible rapeseed bed are incompressible.

A mathematical model to explain the oil expression process was developed and evaluated. Analysis showed that oil expression process could be described adequately through the model following Terzaghi's¹⁶ classical approach. The experimental and predicted values on oil yield were in very close agreement of each other. It holds good for any value of time. The basic solid particles that make up the compressible bed were incompressible.

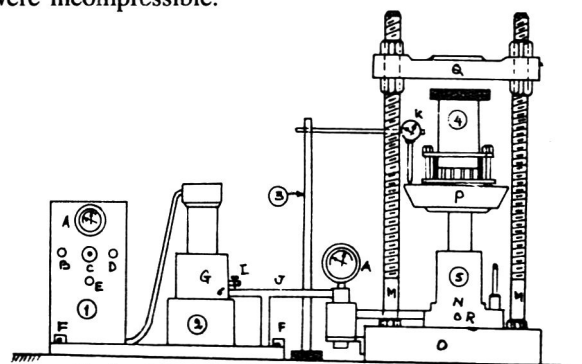


Fig.3. Schematic diagram of experimental set-up.

- | | |
|-------------------------------|---------------------------|
| 1 CARVER MOTORIZATION PACKAGE | 3 STAND |
| A PRESSURE SWITCH GAUGE | K COMPRESSION-INDICATOR |
| B ON-OFF SWITCH | 4 TEST CELL |
| C SPEED CONTROL | 5 CARVER LABORATORY PRESS |
| D JOC/AUTO CONTROL | L MANUAL LEVER |
| E STOP SWITCH | M SHAFT |
| F ON BUTTON | N HYDRAULIC UNIT OF PRESS |
| 2 HYDRAULIC PUMPING UNIT | O BASE |
| G TWO WAY CONTROL | P PLATEN |
| I SAFETY VALVE | Q HEAD |
| J HYDRAULIC LINE | R RELEASE VALVE |

TABLE 2. VALUES OF C_0 , D AND k OF THE MODEL AT DIFFERENT LEVELS OF PRESSURE AND MOISTURE CONTENT CORRESPONDING TO TWO RATES OF DEFORMATION

Sl. No.	Moisture (% w.b.)	Rate of deformation (mm/s)	Pressure (MPa/m ²)	C_0 (mm ² /s)	D	$k \times 10^{-4}$ (s/g/mm ³)
1	5	3.96	6.70	0.10	2.52	0.61
			10.70	0.22	8.95	2.83
			14.72	0.31	12.26	4.08
			16.60	0.38	13.94	5.04
			18.70	0.17	17.65	2.49
2	7	3.96	6.70	0.17	2.69	1.06
			10.70	0.18	8.23	2.13
			14.72	0.30	11.57	3.73
			16.60	0.23	11.91	2.55
			18.70	0.26	14.15	3.03
3	9	3.96	6.70	0.10	2.14	0.52
			10.70	0.15	8.55	1.89
			14.72	0.46	7.07	3.45
			16.60	0.40	7.96	2.98
			18.70	*	*	*
4	5	2.47**	6.70	0.87	4.11	0.83
			14.72	0.24	14.40	3.67
			18.70	0.20	20.43	3.46
5	7	2.47	6.70	0.15	4.26	1.47
			14.72	0.19	13.87	2.76
			18.70	0.23	15.44	3.02
6	9	2.47	6.70	0.18	1.77	0.76
			14.72	0.33	18.63	6.47
			16.60	0.51	13.39	6.44
			18.70	*	*	*

* Corresponding to 9% m.c. and 18.70 MPa/m² pressure, extrusion during experimental evaluation presented problems due to which no experimental value could be made available to compare.

** Since corresponding to one rate of deformation (3.96 mm/s) the prediction was quite close, for another lower (the minimum available on machine) rate of deformation (2.47 mm/s) the results were verified only with selective levels of pressure (in present case almost alternate ones).

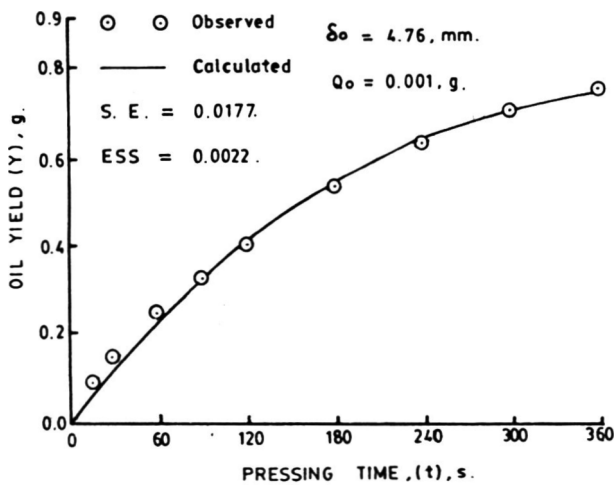


Fig.4. Effect of pressing time on oil yield [a = 6.70 MPa/m², M = 5% (w.b.), R = 3.96 mm/s]

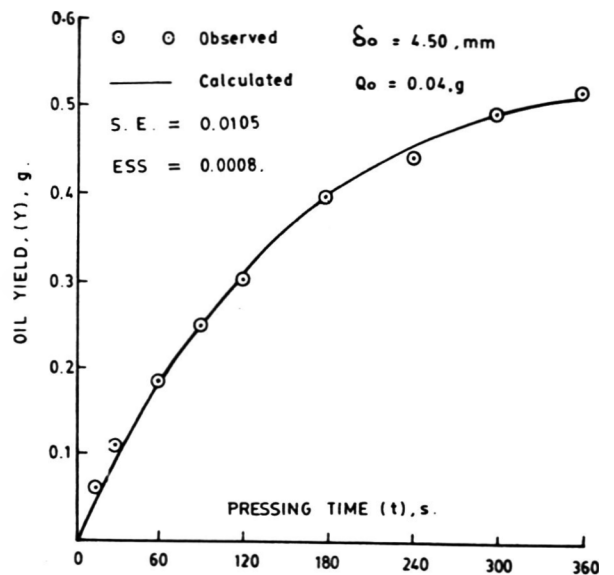


Fig.5. Effect of pressing time on oil yield [a = 6.70 MPa/m², M = 5% (w.b.), R = 2.47 mm/s]

TABLE 3. VALUES OF STANDARD ERROR (SE) AND ERROR SUM OF SQUARE (ESS) FOR THE PREDICTED VALUES THROUGH MODEL AND THE EXPERIMENTAL VALUES

Sl. No.	Moisture content (% w.b.)	Rate of deformation (mm/s)	Pressure (MP _a /m ²)	SE	ESS
1	5	3.96	6.70	0.0105	0.0008
			10.70	0.1358	0.1291
			14.72	0.1648	0.1901
			16.60	0.1902	0.2532
			18.70	0.1507	0.1589
2	7	3.96	6.70	0.0214	0.0032
			10.70	0.0975	0.0665
			14.72	0.1484	0.1541
			16.60	0.1529	0.1636
			18.70	0.1276	0.1148
3	9	3.96	6.70	0.0069	0.0003
			10.70	0.0745	0.0389
			14.72	0.1048	0.0768
			16.60	0.1454	0.1480
			18.70	*	*
1	5	2.47**	6.70	0.0177	0.0022
			14.72	0.1028	0.0739
			18.70	0.1416	0.1403
2	7	2.47	6.70	0.0160	0.0018
			14.72	0.1356	0.1287
			18.70	0.1425	0.1421
3	9	2.47	6.70	0.0173	0.0021
			14.72	0.1373	0.1319
			16.60	0.1995	0.2785
			18.70	*	*

* and ** same foot note of Table 2.

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Notations

- A = Base area, mm²
 A(0) = Area of cross section at t=0 through which oil flow occurs
 A(θ) = Area of cross section at any dimensionless time θ through which oil flow occurs
 a = Static pressure, MPa/m²
 C = Coefficient of compressibility, cm²/g
 C_v = Coefficient of consolidation, mm²/s
 D = Dimensionless parameter
 = $2\pi ka/C_0$
 e = Void ratio at any time, t
 e₀ = Void ratio at time, t=0
 E = Effective expression pressure, MPa/m²
 F_n = Functions relating Bessel's functions

- H = Initial bed height, mm
 J₀ = Bessel's function of first kind of order zero
 J_{1,2} = Bessel's function of first kind of order one and two
 K₀ = $2/A_1 \rho_0 L$
 k = Coefficient of permeability, s/g/mm³
 L = R₂ - R₁, mm
 M = Moisture content % (w.b.)
 P = Oil pressure, MPa/m²
 R = Rate of deformation, mm/s
 r = Radius, mm
 t = Time of pressure applications, s
 δ = Deformation measured downward from height H at any time t, mm
 δ_t = Deformation measured downward from height H at time t=0, mm
 ρ₀ = Density of oil, g/c.c.
 ρ_p = Particle density, g/c.c.
 ξ_p = Roots of Bessel's functions

$$\phi = \sum_{n=1}^{\infty} \frac{F_n}{\alpha_n} (1 - \exp(\alpha_n^2 \theta))$$

 φ = C₀ t/L²

Studies on Oxidative Stability of Crude and Processed Yellow Nutsedge Tuber and Almond Seed Oils

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Crude and partially processed oils of yellow nutsedge and almond were evaluated for their stability towards oxidative deterioration. Crude, processed and illuminated oil samples were withdrawn and active oxygen values determined. Plots of the log of active oxygen values versus heating hours had slopes which reflected the oxidative stabilities of the oils on storage. 'Kaduna-big' and 'Kano' variants of yellow nutsedge showed good stabilities while almond oil showed similar oxidative behaviour in its crude and processed forms.

Search for new and unconventional sources of good quality edible oils to meet the requirements of the ever growing population of tropical countries of the world is one of the dire needs of the present day. Potential edible oil sources when discovered, should be carefully developed, evaluated and its quality ascertained. In the development, evaluation and quality control of such edible oils, stability of the oils to oxidative deterioration is usually of primary importance, while additional information on refining and bleaching without lowering oxidative and flavour stabilities are also essential.

Three yellow nutsedge (*Cyperus esculentus*) tuber variants and almond (*P. amygdalus*) seed oils which are lesser-known abundant sources of nutritious edible vegetable oils are being investigated to ascertain their oxidative deterioration on storage when exposed to fluorescent light irradiation. Available information on the effects of light on processed, stored oils and fats has mainly been restricted to those of commercial importance, while little information is available on lesser-known edible oils that possess potential commercial capabilities.

Work in our laboratory¹ and elsewhere² have demonstrated that oils of yellow nutsedge tuber and almond are nutritious and could be of commercial value, though there has been no report of industrial application of the oils of yellow nutsedge and almond in most tropical and sub-tropical countries of the world, this might be due to insufficient information on the nutritive and keeping qualities of these oils. These oils are therefore being evaluated and information obtained which could serve as a basis for further development.

Materials and Methods

Fresh samples of yellow nutsedge tuber variants were procured from three major producing towns in Nigeria and the samples were identified as 'Kaduna-big', 'Kano' and 'Abuja'. Fresh and ripe fruits of almond were collected within the Chemistry Department, University of Ibadan. The fruits were cracked open to remove the soft oil-bearing seeds. Yellow nutsedge tubers and almond seeds were washed and rinsed in water before being air-dried and stored in the refrigerator.

Tubers of yellow nutsedge and seed of almond fruit were ground in a Wiley Mill to pass through a 6 mm screen and the oil extracted in a Soxhlet extractor with food grade n-hexane. Excess solvent was removed in a rotary evaporator and the oil dried to a constant weight. Oil obtained by this method is referred to as crude sample throughout this study.

Crude samples 200 ml of each oil, were placed in a glass bottle closed with a cellophane covered stopper and exposed to fluorescent irradiation in a "photo-oxidation box", constructed with three 30W daylight tube mounted inside of a 150 cm long, 50 cm wide and 50 cm height wooden box. Interior of the box was painted white to reflect light from backside of the fluorescent tube and to prevent loss of irradiation through absorption by the wooden materials of the box. Holes were perforated around the box for free circulation of air while the internal temperature was maintained at 30°C.

Refining of the oils was achieved by the use of 10 per cent sodium hydroxide while the bleaching method of Crossley and Pierce³ was used. Crude, refined and bleached oils were exposed to fluorescent light irradiation in the photo-oxidation box. Oxidation rate of the exposed samples was followed by measuring peroxide values (POV), according to AOCS⁴

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Official Method Cd 8-53 while Active Oxygen Method values (AOM) were obtained by Official Method Ca 5a-40. AOM's were run until the POV of 100 was reached and that time was taken as the end point and recorded in hour. Per cent free fatty acids was estimated by AOCS⁴ Official Method Cd 12-57 while fatty acid compositions were estimated by the Metcalfe *et al.*⁵ method using a Pye-Unicam 204 series gas liquid chromatography. Tocopherols were estimated using the method of Strum *et al.*⁶ while sensory evaluations for the oils were conducted using the procedure of Moser *et al.*⁷ A 10-point scoring system was the basis for judging quality and intensity of off-flavour, analysis of variance and F-tests were used to test sample means for differences. Elemental analyses were carried out with a Perkin-Elmer Model 2830 Atomic Absorption Spectrophotometer (AAS).

Results and Discussion

Statistical analysis of the data obtained for the changes in peroxide values and active oxygen values as affected by fluorescent light irradiation, exposure time/storage time and temperature indicated that each of these had a significant effect on peroxidation on the oils under test. As stability measurements on unheated oils (POV) do not indicate reliably the performance of the oils on heating⁸, all evaluations in this study will be based on the active oxygen values.

Shown in Table 1 are the physico-chemical properties of the oils under study. The oils are similar, principally in their contents of oleic and linoleic acids which are the basic precursors of oxidative rancidity, and they are also related in their iodine values, free fatty acids and peroxide values. These parameters are important in the control of peroxidation in oils and fats as they indicate the level of deterioration. 'Abuja' variety of yellow nutsedge tuber oil and almond seed oil are closely related while 'Kaduna-big' and 'Kano' variants of yellow nutsedge are also related, similar behaviour towards

oxidative deterioration are expected.

The slopes from a first order linear regression analysis, the standard deviation and the mean of heated oils after refining and bleaching are shown in Fig.1. This plot indicates the rate at which the oils lost oxidative stability upon heating at 97.8°C. In the ensuing evaluations, crude oil samples are to be considered as the baseline. For crude oils, 'Kaduna-big' and 'Kano' variants of yellow nutsedge have similar initial higher stabilities when compared to the 'Abuja' variant of yellow nutsedge and almond seed oil, but the crude 'Kaduna-big' (A, in Fig.1) and crude almond (A'' Fig.1) have similar slopes and therefore have similar rates of loss of oxidative stability. It should be noted that the relative positions of the lines in Fig. 1 reflect the overall stabilities of the oil samples.

Refining increased initial stability for all the oil samples with very little effect on the rate of loss of oxidative stability with heating (B, B' and B''' in Fig. 1) except in 'Abuja' variant where the rate of loss of stability is of the same magnitude with its crude. This might be accounted for by the comparatively marginal reduction in the level of tocopherol during refining in 'Abuja' variety which is about 16 per cent reduction (Table 2). Per cent loss of tocopherol in 'Kaduna-big' of yellow nutsedge was 24, that of 'Kano' was 28 and almond was 32.

Bleaching of the 'Kaduna-big' yellow nutsedge oil on activated alumina marginally increased initial stability when compared to refined samples. However, upon heating the bleached 'Kaduna-big' variant of yellow nutsedge showed only a slight change in the rate of loss of oxidative stability when compared to its crude sample (A in Fig. 1) and refined (B in Fig. 1) samples. The same trend was also observed for 'Kano' variant while 'Abuja' variant showed only some marginal changes. Bleached almond showed a distinct trend; initial stability decreased when compared to refined oil, but only slight change was observed upon heating in the rate of

TABLE 1. PHYSICO-CHEMICAL PROPERTIES AND FATTY ACID COMPOSITION OF YELLOW NUTSEGE VARIANTS AND ALMOND OILS

Parameters	Kaduna-big	Kano	Abuja	Almond
Iodine value*	80-83	80-83	83-85	93-97
Peroxide value (meq/kg)	5.3-6.9	5.0-7.5	5.4-8.2	7.1-8.5
Free fatty acids (as % oleic acid)	1.3-1.8	1.0-2.0	1.7-2.7	1.8-2.9
Active Oxygen Method (AOM, hr)	18.90	17.50	12.40	10.70
Fatty acids (%)				
C _{16:0}	10.0 ± 0.2	12.0 ± 0.2	7.1 ± 0.2	3.2 ± 0.1
C _{18:0}	1.4 ± 0.3	3.0 ± 0.3	0.7 ± 0.3	Trace
C _{18:1}	75.0 ± 0.1	70.0 ± 0.3	78.0 ± 0.1	77.0 ± 0.1
C _{18:2}	11.0 ± 0.3	14.0 ± 0.3	13.0 ± 0.3	18.0 ± 0.3
C _{18:3}	0.6 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	1.0 ± 0.3
C _{20:0}	0.3 ± 0.1	Trace	0.1 ± 0.1	Trace

Mean ± SD

*Range of values provided

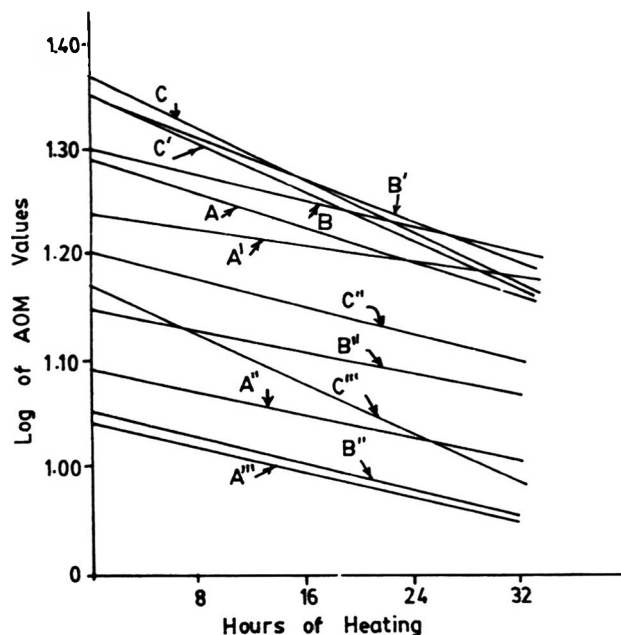


Fig. 1. Changes in active oxygen values with heating for crude and processed oils.

(Unprimed letters = Kaduna big; Primed letters = Kano variant, doubly primed letters = Abuja variant for yellow nutsedge and triply primed letters = almond oil.

loss of oxidative stability when compared to crude and refined almond samples (A''', B''' in Fig. 1).

Our observations from this study indicate that 'Kaduna-big' and 'Kano' variants of the yellow nutsedge have intrinsic stabilities that were not greatly affected by refining or bleaching despite the level of tocopherol reductions. Oxidative behaviours of the yellow nutsedge variants are similar with only 'Abuja' variant having close relationships with almond seed oil. Statistical analysis (Q-test) by a computer programme on the results obtained from the slopes of loss of oxidative stability indicate that only bleach almond seed oil sample was significantly different from the other eleven samples. Similarity in the oxidative behaviour of the samples could be attributed to its linoleic acid contents (Table 1).

Table 2 shows the tocopherol content of the crude, refined and bleached yellow nutsedge and almond oils with no added artificial antioxidant. List *et al.*⁹ had observed that the inherent stability of sunflower seed oil is determined by the

TABLE 2. PROCESSING EFFECTS ON TOCOPHEROL CONTENT OF YELLOW NUTSEGE AND ALMOND OILS

Processing	Tocopherol (μ g/g)			Almond
	Kaduna-big	Kano	Abuja	
Crude	815	761	746	725
Refined	619	641	536	492
Bleached	551	471	459	364

Average of six determinations

ratio of tocopherols to linoleic acid,⁸ this argument is extended to the oils being studied. The higher initial oxidative stability observed for 'Kaduna-big' and 'Kano' variants of yellow nutsedge could be attributed to the high ratios of tocopherol content to linoleic acid.

The contents of iron and copper in the test samples are given in Table 3. Level of copper in all the samples is lower than the mandatory 1 p.p.m. level¹⁰ which could cause deleterious effects in the oil samples. Refining process further more than three quarters of the original amount of copper present. Level of iron too was reduced by refining process. This bit of information is of good benefit in that it showed that shelf life of the oils could be improved through refining process which reduces the levels of iron and copper that have been shown to initiate peroxidation though metal contaminations from chemicals and machinery cannot be ruled out.

Sensory evaluation data obtained at 30°C are shown in Fig. 2 where noticeable downward trend in flavour scores with storage was observed for all the samples. However, 'Kaduna-big' of the yellow nutsedge showed the best flavour scores which is a reflection of its good oxidative stability and good shelf-life. 'Abuja' variant of yellow nutsedge showed its best stability during the first to the third weeks before deterioration set in, while 'Kano' variant of yellow nutsedge showed good flavour scores for the weeks one to five. Almond showed the least flavour score, the flavour decreased during the 6th week, gradually increased until the 8th week before decreasing like all other samples (Fig. 2).

From the results presented above, all the oil samples showed a considerable resistance to oxidation initiated by fluorescent light irradiation in their crude, refined and bleached forms. Of the yellow nutsedge variants, 'Kaduna-big' and 'Kano'

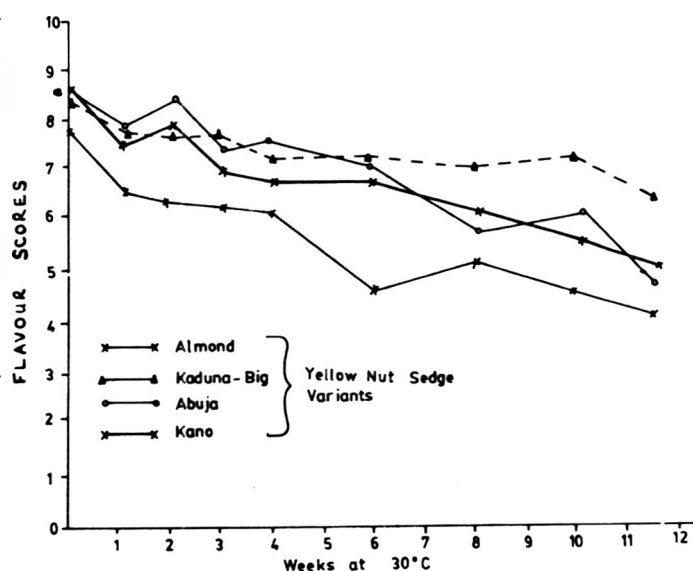


Fig. 2. Change of average flavour scores with storage time.

TABLE 3. CONCENTRATION OF IRON AND COPPER IN CRUDE AND PROCESSED OILS OF YELLOW NUTSEGE AND ALMOND

Processing	Kaduna-big		Kano		Abuja		Almond	
	Cu	Fe	Cu	Fe	Cu	Fe	Cu	Fe
Crude	0.17	2.81	0.12	2.95	0.08	3.61	0.21	3.00
Refined	0.04	0.70	0.03	0.74	0.02	0.90	0.05	0.75
Bleached	0.04	0.71	0.01	0.62	0.01	0.89	0.05	0.62

Average of three determinations in triplicate

stand out as the best. Despite the similarities in their contents of fatty acids, the yellow nutsedge oils were better in their oxidative stability than the almond seed oil. The proximate constitution of a yellow nutsedge tuber as earlier reported¹¹ showed that the tuber is very rich in carbohydrates, protein and oil. The production of oil/hectare of the yellow nutsedge has been worked out as 190 kg.

Yellow nutsedge (*Cyperus esculentus*) is regarded as one of the world's most difficult weeds, the tuber is however edible, and indeed, certain medicinal preparations have been reportedly prepared from the tuber with no indication of any toxic substances (personal communication). Production of large quantities of the tuber must especially 'Kaduna-big' and 'Kano' variants should be intensified to overcome shortage of edible oils in most tropical and sub-tropical parts of the world. Defatted meal can also be utilized in compounding animal feeds and in the production of composite flour.

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Standardization of Manufacture of Kadhi Powder

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Addition of 5% gram flour as thickening material, 0.03% turmeric powder as colouring and flavouring material and 0.5% salt were found to be suitable for the preparation of Kadhi. For the preparation of kadhi powder, a minimum fat of 0.8% in Dahi and total solids in the range of 15-18% in slurry were found to be optimum. Restricting the acidity in Dahi to 0.8—1.0% did not pose any serious problem during drying and also resulted in good quality product. Reconstitution of 1 part of powder with 4 parts of water resulted in a product comparable to control kadhi. The product kept good for nearly 3 months when packed in LDPE pouches at ambient temperature of storage.

Kadhi is a culinary item prepared in many parts of the country. It is prepared either from stirred curd (dahi) or sweet/sour butter milk. Commonly, gram flour is added as a thickening agent. Boiled or semi-boiled vegetables, spiced gram flour after deep-fat-frying (pakoras) or both are added as filling materials during or at the end of boiling. Kadhi is mildly acidic in character with typical cooked flavour after the addition of salt and spices along with little boiled oil. The milk solids content in kadhi varies from 6 to 8 per cent and other solids about 6-7 per cent¹. Since there is not much information available about its preparation, compositional characteristics and method of production in dried form, the present study was undertaken

Materials and Methods

Cow's skim milk (< 0.05 per cent fat) was obtained from the experimental dairy of the Institute. The average total solids (TS) content varied from 8.6 to 8.8 per cent. Commercially available gram flour, turmeric powder, salt and other spices procured from local market were used in kadhi preparation. The gram flour was used at 3, 5 and 7 per cent levels.

Cow's milk was standardised to 0.8 to 1.0 per cent fat by the addition of calculated quantity of cow's skimmed milk. The standardised milk was then heated to 72°C for 5 min, cooled (32°C) and inoculated with 2-3 per cent mixed culture of *S. lactis*, *S. thermophilus* and *S. cremoris*. It was then incubated at 30-32°C for 5-6 hr. The acidity of curd (dahi) at the end of fermentation varied from 0.9 to 1.3 per cent lactic acid. The curd was then stirred in a mixer to get uniform liquid. The gram flour at different levels mixed with equal quantity of water was added to the stirred dahi and was mixed thoroughly. Turmeric powder and salt were added. The mixture was then boiled for 20-25 min till the characteristic flavour was developed. About 5 min before the end of boiling, oil fried spices like mustard, cumin seeds, dried chilly powder and chopped onions were added for better taste. Kadhi so

obtained was used for sensory evaluation.

For the preparation of dried kadhi, the acidity during fermentation was controlled at different levels. Gram flour at 5 per cent level (wt/wt) was mixed with the dahi and was boiled for 10 min after the addition of turmeric powder and salt. The boiling resulted in slight increase in the TS. Since, during preliminary studies it was observed that the addition of spices before drying resulted in dried kadhi with a bitter taste, the same were not added before drying. This also enabled the end user to use the required quantity of spices at the time of kadhi reconstitution using the powder. The slurry was then cooled to about 60°C and dried on a double drum atmospheric drier. (Richmand Simon's double drum roller drier; made in England). The conditions of drying were; speed of rollers, 16-20 r.p.m.; steam pressure, 3.1-3.5 kg/sq cm; length and diameter, 45 cm each; gap between drums, 1.5 mm.

Boiling water was used for reconstitution of the powder. The reconstitution was done at 1:3, 1:4 and 1:5 levels of powder to water. Calculated amount of powder was slowly added into the hot water with vigorous agitation and the content was boiled for 3-5 min. The filling materials were added at the time of boiling as discussed earlier. The reconstituted kadhi was used for sensory evaluation.

Kadhi was evaluated by a panel of judges using 9-point Hedonic scale where a score of 9 corresponded to extremely liked and a score of 1 corresponded to extremely disliked. Titratable acidity (TA) of dahi was measured by IS method². The pH of the slurry was measured by Elico-digital pH meter. The moisture contents of slurry and kadhi powder were determined by drying in an oven² at 100±2°C. Fat content of the dried kadhi was estimated by gravimetric method using the Mojonnier Fat-extraction tube. The fat in milk was estimated by Gerber method². The total ash content of the powder was determined by IS method³. Dried kadhi was analysed for its protein content by the micro-kjeldahl

method³ using a multiplication factor of 6.25.

Results and Discussion

During the preliminary studies, gram flour was added at 5, 10 and 15 per cent levels. It was observed that at all the levels of addition, kadhi was very viscous except at 5 per cent. Hence, in further investigations, 3, 5 and 7 per cent gram flour was added to standardize the preparation. The sensory evaluation of the product made from these showed that addition of 7 per cent flour produced a slightly viscous product compared to other levels (Table 1). However, 3 and 5 per cent levels were rated highly acceptable. The mean overall acceptability score of 5 per cent level of gram flour was superior to the 3 per cent and hence in all further studies, gram flour was added at 5 per cent level.

Turmeric powder was used to give its typical colour and flavour to kadhi. A level of 0.03 per cent was found most suitable. The salt required varied from 0.5-1.0 per cent. However a level of 0.5 per cent was used for the preparation of slurry as this ensured the end user to add additional salt depending upon their requirements.

Partially cooked slurry was fed on a double drum drier at about 60°C. The compositional effect on drying of the slurry is presented in Table 2. It was observed during drying and sensory evaluation of the reconstituted product that a level of 0.8-1.0 per cent acidity to be the optimum level from the point of taste and convenience of drying. Further, it was observed that when the acidity of the fermented product was greater than 1.0 per cent, then the product was very sticky during drying posing serious problem. The product was also observed to become brown and caky when the acidity increased beyond 1.0 per cent level.

During preparation of dried kadhi, the acidity gets concentrated nearly 6 times with the final acidity of dried product reaching about 5-6 per cent lactic acid. When the acidity of the dahi is greater than 1.0 per cent, the solubility characteristics of the dehydrated product are affected seriously with more precipitation in the reconstituted product. When the acidity of dahi was lower than 0.8 per cent, the final taste of the reconstituted kadhi was mild and inferior. However,

TABLE 1. MEAN SENSORY SCORE OF KADHI PREPARED WITH DIFFERENT LEVELS OF ADDED GRAM FLOUR

Characteristics	Levels of gram flour (%) (wt/wt)		
	3	5	7
Body & texture	7.2 (5.5-9.0)	7.8 (7.0-9.0)	7.1 (6.0-9.0)
Flavour	7.0 (5.5-8.0)	7.9 (7.0-9.0)	8.1 (7.0-9.0)
Overall acceptability	7.7 (6.0-9.0)	8.0 (7.0-9.0)	7.4 (6.0-9.0)

Figures in parenthesis indicate the range.

TABLE 2. EFFECT OF COMPOSITIONAL CHARACTERISTICS ON SLURRY DRYING

Characteristics	Levels (%)	Remarks
Acidity	< 0.8	Product good; sourness is less after reconstitution of kadhi.
	0.8 to 1.0	Product good; Good powder with good flavour and overall acceptability.
	> 1.0	Drying is problematic. Sometimes product becomes brown and sticking on drum surface. Final product after reconstitution is sour.
TS	13-14	Thin film and burning of powder was observed. This can be controlled to some extent by adjusting steam pressure. The particle size was small and efficiency of drier was reduced. TS losses, increased.
	15-18	Ideal for drying. Good particle size. Product is good.
	> 18	Slurry is viscous; uneven distribution on the drum surface. Incomplete drying and caking.
Fat	< 0.8	Sticking and burning on the surface.
	> 0.8	Good for drying, good flavour characteristics in the reconstituted product.

The acidity and fat mentioned correspond to the base materials (dahi) used for drying.

lower acidity levels did not pose any drying problem. The pH of the slurry under optimum conditions of drying varied from 4.42 to 4.72.

It was observed (Table 2) that a TS level of 15-18 per cent in slurry was optimum for drying. When the TS was below 15 per cent, the kadhi powder was getting burnt. This could be reduced by reducing the steam pressure but the particle size of the final product was observed to be very fine resulting in higher losses in recovery. When the TS of slurry was beyond 18 per cent, it resulted in incomplete drying and caking during the manufacture. Further increase in the steam pressure upto 4.2 kg/sq cm did not improve the drying conditions. It was not possible with the drum drier used to increase the steam pressure beyond this level. At this concentration, the uniform distribution of the slurry on the drums was another problem. Hence, a TS level of 15-18 per cent in slurry was observed to be the optimum for drying.

A minimum fat level of 0.8 per cent (Table 2) in the dahi was observed to be essential for the preparation of kadhi in dehydrated form. When the fat content was below 0.8 per cent, it posed the problems of sticking and formation of dried film on the surfaces of the drum. This level of fat almost corresponds to the level of fat in butter milk which forms a common base material for the preparation of kadhi. Increasing the fat level upto 5 per cent did not pose any serious problem during the manufacture of the product. However, only a minimum level of fat was maintained for drying

TABLE 3. MEAN COMPOSITION OF KADHI POWDER

Constituents	Per cent
Moisture	7.81 ± 0.881
Fat	13.86 ± 1.904
Protein	30.57 ± 2.013
Ash	8.66 ± 0.625
Carbohydrates (by diff)	39.09 ± 3.581

Number of replicates was seven.

operation as the subsequent preparation involved the addition of fried oils, spices and vegetables. The effect of minimum fat on avoiding the burning and sticking of the particles could be due to the formation of protective fat surface around the casein/casein — protein complexes preventing the burning. The fat also contributed for the improved flavour characteristics. The mean composition of the product is given in Table 3.

It was observed during the studies that reconstitution of 1 part of dried kadhi with 4 parts of boiled water resulted in reconstituted product with good viscosity and overall acceptability characteristics (Table 4). However, based on the sensory evaluation scores, it is suggested that the level of reconstitution can vary depending upon individual taste requirements. After reconstitution, it was observed that it was essential to boil the reconstituted kadhi for 3-5 min to develop the desired cooked flavour in the product. Filler substances also could be added before boiling. It was observed that the best scores were obtained for dehydrated product with dilutions varying from 1:4 to 1:5. The sensory score of control

TABLE 4. SENSORY SCORE OF RECONSTITUTED KADHI

Characteristics	Ratio of dilution; (Powder:water)			
	1:3	1:4	1:5	Control
Body & texture	6.5 (5.5 — 7.5)	7.2 (6.5 — 8.5)	7.0 (6.5 — 8.5)	7.9 (7.5 — 9.0)
Flavour	7.1 (6.5 — 8.5)	7.7 (6.5 — 9.0)	7.6 (7.0 — 9.0)	8.0 (8.0 — 9.0)
Overall acceptability	6.7 (6.0 — 8.0)	7.6 (7.0 — 8.5)	7.5 (7.0 — 9.0)	8.2 (8.0 — 9.0)

Figures in parenthesis indicate the range.

kadhi was observed to be marginally superior to kadhi prepared with a reconstitution level of 1:4. The product was packed in LDPE bags and was stored at ambient temperature and was observed that under this condition it keeps good for nearly 3 months. There was slight browning beyond 3 months of storage. Flavour also deteriorated slightly during this period. Further studies relating to the effect of different packaging materials and storage conditions on the shelf life of the product are in progress.

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Preparation and Evaluation of Soy Paneer

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Soy paneer was prepared from soy milk and soy milk-skim milk blends and evaluated for chemical and microbiological quality. Skim milk addition to soy milk increased shear strength of the product and thus improved its texture. Use of soy milk - skim milk (80:20) yielded soy paneer with total solids similar to milk paneer. The product had a short shelf life at ambient temperature; however it could be extended to 7 days by storage at 5°C. The product was found acceptable on consumer preference trials.

Coagulation of soy milk by acids or salts yields a soft, white gelatinous mass. The product thus obtained has bland taste and unique body. It resembles milk paneer in appearance, colour, and texture. Thus, it can serve as an excellent substitute for milk paneer and can also be a cheaper source of quality proteins. Nasim *et al.*¹ developed a process for preparing soy paneer. They found that soy paneer on frying resembled milk paneer in colour and sponginess and was highly acceptable. However, they observed that unfried soy paneer had a fragile texture which posed problems in its handling. Vijayalakshmi and Vaidehi² prepared an acceptable paneer-like product by precipitating combination of soy milk and skim milk. The present investigation was undertaken to prepare soy paneer from soy milk and soy milk - skim milk blends and evaluate it for its chemical and microbiological quality and acceptability.

Materials and Methods

Soy dal obtained by dehulling dry, mature soybeans (variety 'Bragg') was used in this study. Fresh skim milk was obtained from Livestock Research Centre of the University.

Soy dal was soaked in tap water (1:3, w/w) for 16-18 hr at room temperature. The soak water was decanted and the dal was washed with fresh water. It was then ground for 3-5 min in a blender with hot water (85-90°C) using bean to water ratio of 1:9 (w/v). The resulting suspension was filtered through a double layer of cheese cloth. The soy milk thus obtained contained approximately 6.0 per cent total solids.

Soy paneer was prepared from soy milk alone and soy milk-skim blends of 90:10, 80:20, 70:30 and 60:40 ratios as per the procedure described by Nasim *et al.*¹ Soy milk alone and soy milk-skim milk blends were boiled for 10 min, cooled to 75°C and coagulated using appropriate amount of tartaric acid (2 per cent solution). The coagulum thus obtained was

pressed using 0.1 kg/cm² pressure, sliced into blocks of appropriate size and stored at 5°C.

Standard procedures were used to determine moisture, protein (N×6.25), ash and free fatty acid (FFA) contents³. Non-protein nitrogen was determined according to Bhatta and Finalaysen⁴ method using 10 per cent trichloroacetic acid. Titratable acidity was determined in meq. acid/g as described by Grover *et al.*⁵ For pH determinations, 10 g sample was triturated with 10 ml double glass distilled water and measurements were made using digital pH meter.

Shear strength was determined according to the procedure described by Nasim *et al.*¹ using shear test apparatus developed by Kulshreshtha *et al.*⁶

For microbial analyses, 11 g sample was withdrawn and macerated in a pestle and mortar aseptically. Appropriate dilutions were prepared using 2 per cent sodium citrate solution. Total count and psychrotrophic count were determined using plate count agar (PCA). PCA fortified with 10 per cent sterilized skim milk was used for enumerating proteolytic count. Violet red bile agar and acidified potato dextrose agar were used to determine coliform and yeast and mold counts, respectively. Duplicate plates were prepared and incubated at temperatures for the time recommended by American Public Health Association⁷ for each group of organisms.

The chemical and microbiological changes in soy paneer obtained from soy milk - skim milk (80:20) blend were studied at 5°C and 22°C storage temperatures.

Preliminary sensory evaluation trials showed that addition of skim milk to soy milk at a minimum level of 20 per cent yielded a product resembling milk paneer in appearance and textural characteristics. Therefore, soy milk - skim milk blend of 80:20 was used for consumer preference trials to determine acceptability of the product. One hundred twenty samples

of unfried soy paneer each weighing 200 g were distributed among the campus community at random. The consumers were directed to use the product in a manner similar to milk paneer and record their preference on Hedonic scale ranging from 1 to 9, where 1 represented dislike extremely and 9 represented like extremely⁸.

Results and Discussion

Soy dal used for preparation of soy milk contained (in per cent) moisture 11.05, protein 39.94, fat 18.31, ash 5.29 and carbohydrates (by difference) 25.41. Nasim *et al.*¹ reported that soy paneer prepared by using citric acid as coagulant exhibited fragile texture. They also observed that use of tartaric acid as coagulant yielded a product with higher shear strength than citric acid or lactic acid. Hence, tartaric acid was used as coagulant to overcome the problem of weak texture. Further, soy milk was boiled for 10 min as suggested by Kulkarni *et al.*⁹ to improve the texture.

Addition of skim milk to soy milk decreased yield but increased total solids, protein content and shear strength of soy paneer (Table 1). Similar observations have also been recorded earlier². The increase in total solids and protein upon addition of skim milk can be attributed to compactness imparted to the product by characteristic properties of casein.

Table 2 shows the proximate composition of soy paneer prepared from soy milk alone and soy milk - skim milk blend (80:20). Paneer prepared from soy milk alone contained (in per cent): moisture 74.85, protein 16.63, fat 4.26, ash 0.37 and carbohydrates (by difference) 3.89. Similar values for different soy paneer constituents obtained by acid coagulation have been reported earlier¹. Skim milk addition enhanced protein, ash and carbohydrate contents of the product. However, slight reduction in fat content of the product was observed. Milk paneer/channa contains approximately 30 per cent total solids. Use of soy milk - skim milk (80:20) blend yielded a product similar to regular paneer in total solids.

The biochemical changes during storage of soy paneer at 22°C and 5°C are shown in Table 3. The NPN content of soy paneer increased during storage at both the storage temperatures. However, the increase was more marked at

TABLE 2. PROXIMATE COMPOSITION OF PANEER OBTAINED FROM SOY MILK ALONE AND SOYMILK - SKIM MILK BLEND¹

Constituent (%)	Paneer	
	Soy milk	Soy milk-skim milk blend (80:20)
Moisture	74.85	69.46
Protein	16.63	20.98
Fat	4.26	3.77
Ash	0.37	0.78
Carbohydrates (ty diff)	3.89	5.01

¹ Average of three determinations.

22°C than at 5°C. The NPN content of fresh soy paneer was 1.38 per cent which increased to 2.77 per cent at 22°C after 2 days. In contrast, the NPN content was 2.62 per cent after 7 days at 5°C. Similar trend was observed with acid development. Freshly prepared soy paneer contained 80×10^6 and 88×10^6 meq acid/g at 22°C after 2 days and at 5°C after 2 days and at 5°C after 4 days, respectively. Corresponding changes in pH of soy paneer during storage at both the temperatures were observed. The FFA content of soy paneer increased during storage at both the temperatures. However, the increase was marginal as soy paneer contained only 3.77 per cent fat.

The microbial changes during storage of soy paneer at 22°C are shown in Table 4. A marked increase in total count, coliforms and proteolytic count was observed during storage at this temperature. In contrast, the increase in different microbial counts was less marked at 5°C storage (Fig. 1). Yeasts and molds were not detected in freshly prepared soy paneer. However, they appeared after 2 days of storage at 22°C but were not detected even after 7 days at 5°C. Grover *et al.*⁵ also did not record the presence of yeasts and molds in freshly prepared soybean curd. However, they detected them after 1 day at 30°C and after 3 days at 5°C storage.

Storage studies were conducted upto 2 days at 22°C and upto 7 days at 5°C as the product became unacceptable thereafter. The spoilage was characterized by development of off-flavour, sliminess and discolouration. These observations are

TABLE 1. EFFECT OF SKIM MILK ADDITION ON YIELD, TOTAL SOLIDS, PROTEIN AND SHEAR STRENGTH OF SOY PANEER¹

Soy milk: skim milk blend	Soy paneer			
	Yield* (g)	Total solids (%)	Protein (%)	Shear strength (dynes/cm ² × 10 ⁴)
Control	166	25.15	16.63	1.84
90:10	158	26.70	18.97	2.69
80:20	153	30.54	20.98	3.25
70:30	140	33.00	22.32	4.19
60:40	134	35.68	24.54	4.49

¹ Average of three determinations. *Per litre of soy milk alone and soy milk-skim milk blend.

TABLE 3. BIOCHEMICAL CHANGES DURING STORAGE OF SOY PANEER AT 22°C AND 5°C¹

Storage (days)	Non-protein N*		Acidity (meq acid/g×10 ⁻⁶)		pH		Free fatty acids [†]	
	22°C	5°C	22°C	5°C	22°C	5°C	22°	5°
0	1.38	1.38	80	80	5.35	5.35	0.14	0.14
1	1.74	1.41	86	80	5.28	5.35	0.18	0.14
2	2.77	1.63	88	83	5.15	5.30	0.26	0.14
3		1.74		87		5.28		0.16
4		1.86		87		5.23		0.17
5		1.96		97		5.18		0.17
6		2.31		97		5.18		0.19
7		2.62		101		5.13		0.23

¹ Average of three determinations; Soy paneer prepared from soy milk-skim milk blend (80:20) was used. *Non-protein nitrogen was determined as % of total nitrogen. †Free fatty acids were determined as % of oleic acid.

TABLE 4. MICROBIAL CHANGES IN SOY PANEER DURING STORAGE AT 22°C.

Storage (day)	Total count/g	Coliforms/g	Proteolytic count/g	Yeasts* and mold/g
0	1.21×10^4	1.11×10^3	2.10×10^3	ND
1	6.95×10^5	4.40×10^4	4.85×10^4	ND
2	3.95×10^6	1.25×10^6	1.02×10^6	3.85×10^3

ND = Not detected

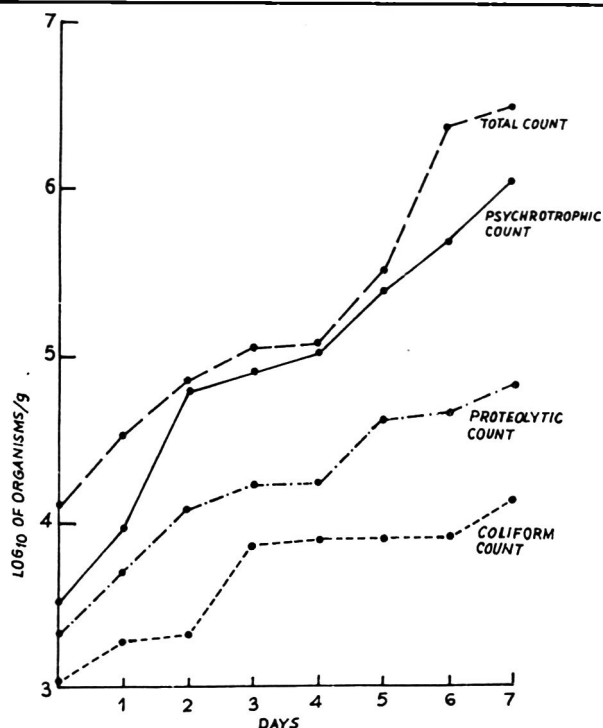


Fig.1. Microbial changes during storage of soy paneer at 5°C.

in agreement with earlier reports¹⁰. Wu and Salunkhe¹¹ found that soybean curd remained acceptable upto 7 days at 4.5°C. Therefore, they suggested microwave treatment to enhance shelf-life of soybean curd.

Consumer preference trials showed that the product was acceptable. The per cent distribution of responses was 12.50 liked extremely, 37.50 liked very much, 33.33 liked moderately and 10.83 liked slightly. The consumers recorded that soy paneer resembled milk paneer in appearance and

texture. However, approximately 4 per cent of the consumers experienced beany flavour when the product was consumed as paneer.

The results of this investigation show that addition of skim milk to soy milk improved the texture of soy paneer. The fresh product has a short shelf-life at ambient temperature. However, its shelf-life could be extended up to 7 days by storage at 5°C. The product was found acceptable on consumer preference trials.

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Effect of Modilase Enzyme on Characteristic Flavour of Buffalo Cheddar Cheese without and with *L. casei* at Different Ripening Temperatures

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Buffalo milk with C/F ratio 0.70 was used to manufacture Cheddar cheese using Modilase without and with *L. casei*. *L. casei* was added at 0.5 per cent to buffalo milk along with starter culture. The Cheddar cheese ripened at 8±1°C, 15°C for 3 weeks, 15°C for 6 weeks, 20°C for 3 weeks, and 20°C for 6 weeks then transferred to 8±1°C for further ripening were analysed for its sensory characteristic and bio-chemical changes. *L. casei* treated cheese samples ripened at 15°C for 6 weeks and 20°C for 6 weeks then transferred to 8°C showed characteristic cheesy flavour after four months of ripening in comparison to cheese samples made without *L. casei* produced cheesy flavour after 6 months of ripening at 8°C. Characteristic cheesy flavour was found to be related to glycolysis, proteolysis and lipolysis during ripening.

The accelerated ripening is of great economic importance to cheese industry. The extended ripening period involves increased cost due to storage and considerable loss in weight due to greater evaporation of moisture. It is known that buffalo cheese takes more time for ripening compared to cow's milk cheese¹. This delayed ripening and lack of full flavour in buffalo milk cheese appears to be due to marked qualitative and quantitative differences among the major constituents of buffalo and cow's milk. Enzyme preparations obtained from different molds have been reported to accelerate the ripening process without deteriorating the quality of cheese^{2,3}. *L. casei* has been shown to be implicated in cheddar flavour development^{4,5} due to its proteolytic and lipolytic activities^{6,7}. This study was therefore, undertaken to investigate in depth the possibility of accelerating the ripening process and provide a positive control over flavour development in buffalo cheddar cheese using *L. casei* and mould enzyme in combination with ripening temperatures.

Materials and Methods

Buffalo milk obtained from Experimental Dairy, National Dairy Research, Institute, Karnal, India was used in this investigation. Mother culture of L.F.-40 and *Lactobacillus casei* were obtained from Dairy Bacteriology Division, N.D.R.I., Karnal, India. Bulk starter culture was prepared using buffalo skim milk. The skim milk was autoclaved in conical flask at 15 p.s.i. for 15 min and cooled to 22 and 37.5°C. The activated mother culture of L.F.-40 and *L. casei*-300 were inoculated aseptically and flasks were

incubated at 22 and 37.5°C respectively for 14-15 hr. The bulk culture flasks were chilled and transferred to cold storage at 4°C till addition to the milk.

Modilase produced from *Mucor miehei* in liquid form was obtained from Christen Hansen's Laboratory Inc, Denmark.

The standardized buffalo milk (C/F ratio-0.70) was pasteurized at 53°C for 30 min, cooled to 28°C and then salt was added at 0.1 per cent. After 15 min, active starter culture of LF-40 and *L. casei* — 300 were added to milk at 2 and 0.5 per cent, respectively. Required amount of Modilase after dilution was added in order to set the milk in 50-55 min. The curd was cut, scalded, cheddared, milled, salted, hooped and pressed overnight. The cheese blocks were transferred to cold storage and paraffined after 2 days. These cheese blocks were ripened at 8, 15 and 15°C for 3 weeks, then transferred to 8°C: 15°C for 6 weeks, then transferred to 8°C: 20°C for 3 weeks then transferred to 8°C and 20°C for 6 weeks then transferred to 8°C. Samples of cheese were drawn at an interval of 2 months and evaluated for sensory characteristics and biochemical changes during ripening. Each treatment was repeated 3 times.

The cheese samples were evaluated for sensory quality attributes like flavour, body and texture and colour by a selected panel of judges using cheddar cheese score card⁸ at 2 months interval upto 10 months.

Cheddar cheese samples were analysed for chemical analysis at an interval of 2 months upto 10 months. The fat in milk was determined by Gerber method⁹ and in cheese by Mojonnier method¹⁰. The pH of the cheese was measured by

using Digital pH meter (Elico Pvt. Ltd. Hyderabad). Titratable acidity was determined by the A.O.A.C. method¹¹. The soluble protein and total free fatty acids were estimated by the method recommended by Kosikowaski¹² and Rama Murthy and Narayanan¹³.

Results and Discussion

Higher ripening temperatures have been reported to accelerate flavour development in cheese^{14,15}. It is apparent from Table 1 that cheese samples ripened at higher temperatures (15°C for 3 weeks then transferred to 8°C, 15°C for 6 weeks then transferred to 8°C, 15°C; 20°C for 3 weeks then transferred to 8°C and 20°C for 6 weeks then transferred to 8°C) showed normal flavour development after 4 months of ripening. Modilase cheese sample ripened at 15°C for 3 weeks then transferred to 8°C resulted in the best quality product. Cheese samples ripened at 15°C for 3 weeks then transferred to 8°C resulted in the best quality product. Cheese samples ripened at 15°C for 3 weeks then transferred to 8°C resulted in the best quality product. Cheese samples ripened completely at 15 and 20°C for 3 and 6 weeks then transferred to 8°C, though the flavour development was slightly faster

in the beginning, but it tended to develop off-flavours particularly proteolytic type. Moreover, higher temperatures tended to result in relatively drier products making the body comparatively hard.

Supplementation of milk with *L. casei* showed definite improvement in flavour in comparison to cheese made from Modilase alone (Table 1). The main criteria for this belief has been concurrent appearance of flavours and lactobacilli in cheese after 2-3 months of ripening⁴. The positive contribution of *L. casei* to cheese flavour formation in buffalo cheese may be due to its relatively higher proteolytic and lipolytic activities^{6,7,16}. The maximum score(40) was obtained in case of cheese ripened at 15°C for 3 weeks then transferred at 8°C after 6 months.

The body and texture of cheese ripened at all the temperatures were normal after 4 months. Addition of *L. casei* resulted in improvement of body and texture. Another notable feature was the complete disappearance of white granulation in cheese made with Modilase and *L. casei*. This textural defect was a persistent problem after 4 to 6 months of ripening in both cow and buffalo cheese made with calf rennet and regular starter culture. The colour of cheeses stored at

TABLE 1. EFFECT OF MODILASE AND *L. CASEI* ON SENSORY CHARACTERISTICS OF CHEDDAR CHEESE AT DIFFERENT RIPENING TEMPERATURES

Temp (°C)	Flavour at indicated ripening period (months)					Body and texture at indicated ripening period (months)				
	2	4	6	8	10	2	4	6	8	10
	Modilase									
8	35	36	37	36	36	25	26	26.5	27 Cr	26
15	35	36	36 Un	36 Pr	36 Pr	25	26	26	26	25
15:3	35	36.5	37.5 Ba	38 Ba	37 Pr	25	26	26	27	25
15:6	35	36	37 Ye	37 Pr	36.5 Pr	25	26	27 Gr	27 Gr	25
20:3	35	36.5	37.5 Ba	37	37 Pr	25	26	27	26.5	25.5
20:6	35	36.5	37.5	37	36	25	26	26	27	24
	Modilase <i>L. casei</i>									
8	35	37	37	37	36	25	26 Me	26.5	27	27
15	35	37	37	37.5	35	25	26	26.5	27	27
15:3	35	36	39 Ba	40 Ba	36	25	27	27.5	27.5	27
15:6	35	38	38 Pr	38	34	25	26	27	27.5	27
20:3	35	38	39	39.5	37	25	27 Me	27.5	27	28
20:6	36	37	39	39	36	25	26	26.5	27	25

Ba—Balanced; Pr—Proteolytic; Ye—Yeasty; Cr—Crumbly; Gr—Granules; Me—Mealy; Un—Unclean.

different ripening temperatures was almost similar in all the cases.

With respect to biochemical changes, it was observed that proper level of glycolysis, proteolysis and lipolysis was key to satisfactory flavour development. Fig 1 and 2 show that titratable acidity of cheese made with Modilase and *L. casei* was higher than cheese made only with Modilase. The rate of increase in titratable acidity was highest and lowest in cheese ripened at 15 and 8°C respectively. However, the former showed relatively faster increase after 6 months of storage (Fig.2). Modilase cheese sample followed the same pattern but at slower rate (Fig 1). The titratable acidity in cheese made from Modilase ranged from 0.62 to 1.34 per cent during 10 months of ripening (Fig 1). This compares well with cow cheese after 6 months of storage (0.70 to 1.16 per cent)¹⁷. Our findings are not fully in agreement with others that increase in titratable acidity in buffalo cheddar cheese is comparatively slower^{18,19}. Modilase and *L. casei* both increased the titratable acidity in cheese markedly. This could be attributed to synergistic effect of Modilase and *L. casei* at higher ripening temperatures.

The change in pH of cheese samples made from Modilase with and without *L. casei* followed more or less the same

pattern upto four months of ripening (Table 2). Modilase cheese sample showed sharp increase in pH values after 4 months of ripening in comparison to cheese sample made from Modilase with *L. casei*. The final pH ranged from 5.60 to 5.70, the highest being in case of cheese ripened at 15°C for 6 weeks then transferred to 8°C. This may mean that all the beneficial factors lead to a favourable level of lactic acid fermentation during 4 months which provides a suitable background for other biochemical changes like proteolysis and lipolysis.^{5,20}

Increase in soluble nitrogen was higher in cheese samples made from Modilase with *L. casei* in comparison to without *L. casei* at all ripening temperatures (Table 3). It is apparent that cheese samples showed steep increase in soluble protein during first 2 months, followed by decreased rate of increase upto the end of storage. The sample stored at 20°C for 6 weeks then transferred to 8°C showed a tendency of levelling-off after 6 months of storage. The cheese made from Modilase with and without *L. casei*, ripened at 15°C further increased soluble protein (8.27 and 7.69 per cent). Our results are in agreement with those of Kosikowski and Iwasaki²¹ that increase in soluble protein was proportional to that of enzyme (2.6 g/100 g curd) added to curd and soluble protein further

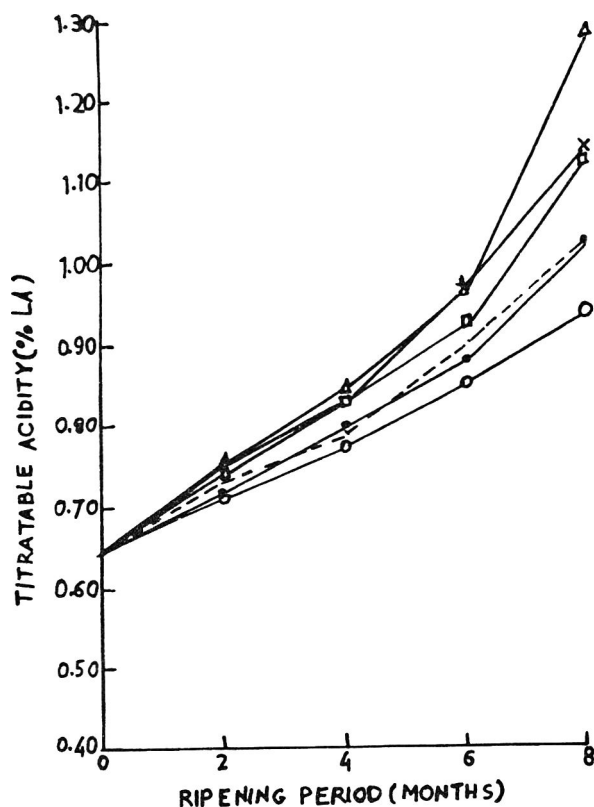


Fig.1. Effect of ripening temperatures on titratable acidity in cheddar cheese made from Modilase during ripening.

O—O 8+1°C; ●—● 15°C, 3 weeks;
 □—□ 15°C, 6 weeks; △—△ 15°C;
 ×—× 20°C, 3 weeks; ---- 20°C, 6 weeks.

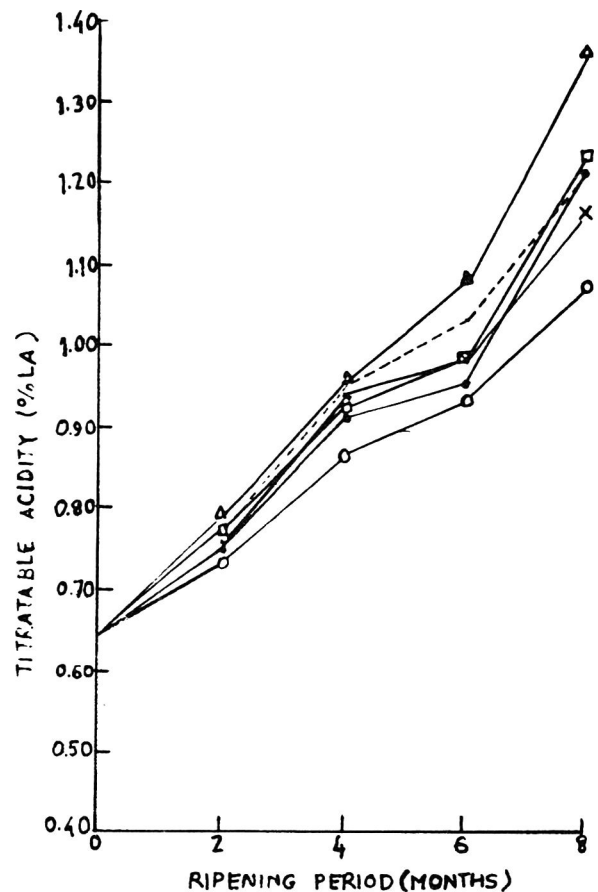


Fig.2. Effect of ripening temperatures on titratable acidity in cheddar cheese made from Modilase with *L. casei* during ripening. Legend as in Fig.1.

TABLE 2. EFFECT OF RIPENING TEMPERATURES ON pH OF CHEDDAR CHEESE MADE FROM MODILASE WITHOUT AND WITH *L.CASEI*.

Ripening		pH of Modilase addec. cheese at indicated ripening period (months)					pH of Modilase + <i>L. casei</i> added cheese at indicated ripening period (months)				
temp (°C)	Period (weeks)	0	2	4	6	8	0	2	4	6	8
8	32	5.15	5.22	5.18	5.37	5.50	5.20	5.25	5.15	5.33	5.52
15	3	5.15	5.22	5.15	5.42	5.44	5.20	5.29	5.17	5.38	5.48
15	6	5.15	5.30	5.22	5.45	5.60	5.20	5.30	5.22	5.32	5.43
15	32	5.15	5.25	5.15	5.45	5.55	5.20	5.32	5.25	5.35	5.35
20	3	6.15	5.27	5.15	5.47	5.40	5.20	5.26	5.25	5.43	5.50
20	6	5.15	5.27	5.20	5.42	5.47	5.20	5.29	5.20	5.36	5.42

All the cheese blocks were subsequently ripened at $8 \pm 1^\circ\text{C}$.

TABLE 3. EFFECT OF RIPENING TEMPERATURES ON SOULBLE PROTEIN OF CHEDDAR CHEESE MADE FROM MODILASE WITHOUT AND WITH *L. CASEI*

Ripening		Soluble protein of Modilase added cheese at indicated ripening period (months)					Soluble protein of Modilase + <i>L. casei</i> added cheese at indicated ripening period (months)				
temp (°C)	Period (weeks)	0	2	4	6	8	0	2	4	6	8
8	32	1.23	3.10	4.89	5.47	6.32	1.32	4.29	5.66	6.45	7.1
15	3	1.23	3.22	5.21	6.21	6.58	1.32	4.46	5.77	6.59	7.3
15	6	1.23	3.48	5.41	6.33	6.72	1.32	4.67	6.02	6.90	7.4
15	32	1.23	4.12	5.66	6.54	7.69	1.32	5.23	5.87	7.52	8.2
20	3	1.23	3.97	5.35	6.47	6.46	1.32	4.58	5.77	6.19	7.3
20	6	1.23	3.22	5.40	5.84	5.36	1.32	4.74	6.04	7.36	7.5

All the cheese blocks were subsequently ripened at $8 \pm 1^\circ\text{C}$.

TABLE 4. EFFECT OF RIPENING TEMPERATURES ON FREE FATTY ACIDS ($\mu\text{M/g}$) OF CHEDDAR CHEESE MADE FROM MODILASE WITHOUT AND WITH *L.CASEI*.

Ripening		F.F.A. of Modilase added cheese at indicated ripening period (months)					F.F.A. of Modilase + <i>L. casei</i> added cheese at indicated ripening period (months)				
temp (°C)	Period (weeks)	0	2	4	6	8	0	2	4	6	8
8	32	3.51	9.53	15.67	19.18	21.45	3.51	12.02	16.45	19.75	22.05
15	3	3.51	9.96	16.66	19.39	22.02	3.51	12.58	16.38	20.17	22.26
15	6	3.51	12.69	15.95	19.14	22.55	3.51	12.94	17.23	19.60	28.72
15	32	3.51	13.97	17.29	21.41	27.19	3.51	13.54	18.01	21.24	25.24
20	3	3.51	10.88	17.19	20.56	24.68	3.51	13.65	18.79	23.04	30.10
20	6	3.51	11.59	15.56	19.75	23.54	3.51	15.17	19.53	22.55	29.36

All the cheese blocks were subsequently ripened at $8 \pm 1^\circ\text{C}$.

increased from 5.70 to 9.40 per cent at higher ripening temperature (20 and 32°C).

The initial values of F.F.A. in cheese samples made from Modilase with and without *L. casei* were the same ($3.5 \mu\text{M/g}$ fat) whereas final values were 22.05 and $21.45 \mu\text{M/g}$ fat, respectively at 8°C (Table 4). The cheese made from Modilase with *L. casei* showed relatively faster increasing trend at different ripening temperatures. The cheese made from Modilase with *L. casei* and ripened at 20°C for 6 weeks then transferred to 8°C showed highest value ($29.36 \mu\text{M/g}$ fat). The use of *L. casei* had a definite stimulatory effect of F.F.A. formation. Our results are in agreement with those of Kosikowski and Iwasaki²¹, who showed that volatiles

F.F.A. increased at higher ripening temperatures. Nakae *et al.*²² reported that volatile F.F.A results from the action of intracellular lipases produced by lactobacilli.

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Effect of Chilled Seawater Storage of White Sardine (*Kowala coval*) on Its Canned Product Quality

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The keeping quality of canned white sardine (*Kowala coval*) can be improved by preserving the fish immediately after catch in chilled seawater before canning. Delayed icing caused considerable deterioration in quality and reduced the storage life of canned product. It was found that fish subjected to delayed icing became unacceptable after six days and those treated with immediate icing and chilled seawater were acceptable upto nine and eleven days respectively for canning.

Freshness has significant influence on the shelf-life of canned fish. The quality of the raw material as well as proper fish handling methods are of prime importance in getting standard canned products of satisfactory keeping quality. Chilled seawater preservation of fresh fish has proved to be useful in retaining the freshness of pelagic fishes¹. Some studies have been conducted on the effect of chilling in relation to quality of fish and their suitability for canning with respect to more important tropical marine species of India²⁻⁴. The present work was carried out to find the difference in quality and shelf-life of canned white sardine preserved in chilled seawater and in ice prior to canning. The effect of delayed icing was also studied.

Materials and Methods

Fresh white sardines obtained from a commercial fishing boat were divided into 3 lots. One lot was kept at ambient temperature ($28 \pm 2^\circ\text{C}$) for about six hours and then mixed with crushed ice in the ratio 1:1 (sample DI). The second lot of fish was directly mixed with ice in the ratio 1:1 (sample II). The third lot of fresh fish was immediately cooled in chilled seawater, with fish : ice : water ratio of 9:3:1 and agitated manually at intervals until the temperature equilibrium was attained and then held in the same medium (sample CSW). All the samples were stored in a chill room at 0 to 2°C until found unacceptable for canning purpose.

Fish were taken at intervals of two or three days from each of the three samples and canned as 'natural pack' (WSN) and 'oil pack' (WS3) using refined groundnut oil for the latter, following the procedure standardised by Jeyasekaran⁵. Moisture, crude protein, fat and ash of fresh raw material

were determined by AOAC methods⁶. Canned products prepared from the three samples were examined for general quality by can opening tests and for proximate composition and salt content by chemical analyses⁶. Organoleptic tests on canned products were conducted by a panel of 8 to 10 judges of fish processing faculty for evaluating sensory attributes on a numerical scoring system (9-10:excellent; 7-8:good; 5-6:acceptable; 3-4:poor and 1-2:very poor). The average scores were expressed as out of a possible maximum of 10.

Results and Discussion

The proximate composition (per cent) of the white sardine fish such as moisture, protein, fat and ash was observed to be 74.49, 23.19, 0.73 and 1.36, respectively. Only those cut-out characteristics of canned products which are most likely to be affected by fish handling methods are presented in Table 1. The drained weight of solids in canned fish mainly depends on the loss of moisture from fish during heat treatment. Joshi and Saralaya⁷ have shown in the case of oil sardines that fresh and fatty fish lose moisture to lesser extent than stale and lean fish, for the same pack weight, when heated in the cans. Therefore, lower drained weights may be expected in staler fish when used for canning. The other two important characteristics of canned products are turbidity of liquid and adhesion to can which are known to be related to fish quality and consequently on the handling method adopted. In the present study also, the turbidity and adhesion were present to a greater degree in products using fish subjected to DI method than to II or CSW method. The effect is more clearly seen in the 'natural pack' products than in the 'oil packs', because in the latter, the cook drip is removed by precooking

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TABLE 1. SELECTED CUT-OUT CHARACTERISTICS OF CANNED WHITE SARDINES SUBJECTED TO DIFFERENT HANDLING METHODS

Fish holding period (days)	Product code	Drained wt of solids (% net wt)			Exudate in liquid (% total liquid)		
		DI	II	CSW	DI	II	CSW
0	WSN	72.2	72.2	72.2	NAP	NAP	NAP
	WS3	68.2	68.2	68.2	23.8	23.8	23.8
2	WSN	70.3	69.2	69.9	NAP	NAP	NAP
	WS3	69.0	69.7	70.1	24.2	21.2	22.0
4	WSN	68.5	69.1	72.6	NAP	NAP	NAP
	WS3	66.0	65.4	67.8	21.4	22.4	21.8
6	WSN	65.5	68.9	67.3	NAP	NAP	NAP
	WS3	63.3	66.4	66.7	16.7	24.3	18.8
9	WSN	DIS	67.1	67.7	NAP	NAP	NAP
	WS3		63.6	65.4	DIS	20.3	16.3
11	WSN	DIS	DIS	68.7	NAP	NAP	NAP
	WS3			64.4	DIS	DIS	15.1

WSN = white sardine canned 'natural'; WS3 = white sardine in refined groundnut oil; NAP = Not applicable; DIS = Discontinued; All values are averages of three observations.

DI = Delayed icing; II; Directly mixed with ice; CSW : Chilled sea water.

before filling with oil, thus removing most of the fractions leading to turbidity and adhesion.

The difference between the influence of the fish handling methods on the quality of canned products are clearly seen from the combined sensory scores presented in Table 2. The combined scores (taking into consideration of all attributes but neglecting the age of the canned products) are above 5.5 for natural packs of fish prepared from DI sample held for 4 days, II sample for 6 days and CSW sample for 9 days. In the case of oil packed product, the score remained above 6, even when the fish used were of marginal quality, that is, 6, 9 and 11 days in DI, II and CSW methods respectively.

The proximate compositions of canned products made from fish samples did not show any definite trend except higher salt content in products made of staler fish (Table 3). The absorption of salt is more in natural packs than in oil packs. Higher fat content in oil packed fish is naturally due to

absorption of the filling medium.

Gradual lowering of quality in long term storage is due to deterioration of organoleptic attributes such as weakening of texture, fading of colour, loss of odour and flavour. This aspect has been studied in detail by Cecil and Woodroff⁸ in the case of several classes of canned foods. In the present experiment, the canned fish in natural pack (WSN) and oil pack (WS3) retained their quality very well for the first six months of storage, when the fish used were of prime freshness. However, when the fish packed were of poor quality, the canned products deteriorated at a quicker rate, especially in natural packs. The marginal quality fish canned as 'natural' scored poorly in sensory evaluation tests after a period of one or two months and the combined quality scores fell below the acceptability limit of 5.0, in several cases, as can be seen from the results presented in Table 4. It is evident that such fish of marginal quality cannot be used for

TABLE 2. INFLUENCE OF FISH HANDLING METHODS ON SENSORY SCORES OF CANNED WHITE SARDINES

Combined sensory scores reduced to maximum 10

Holding period (days)	DI		II		CSW	
	WSN	WS3	WSN	WS3	WSN	WS3
0	7.07	7.56	7.07	7.56	7.07	7.56
2	6.44	7.19	6.41	6.95	6.69	7.01
4	6.37	7.25	6.11	6.81	6.00	6.74
6	5.42	6.61	5.96	6.89	5.65	6.47
9	DIS	DIS	DIS	6.26	5.57	6.43
11	DIS	DIS	DIS	DIS	5.02	6.08

Values are combined scores of all attributes neglecting storage period of cans (average of 4 cans each); DIS = Discontinued.

DI, II, CSW are as in Table 1.

TABLE 3. CHEMICAL CHARACTERISTICS* OF CANNED WHITE SARDINES (NATURAL PACKS, WSN) PREPARED FROM FISH HANDLED DIFFERENTLY

Holding period (days)	DI					II					CSW				
	Mois-ture	Pro-tein	Fat	Ash	Salt	Mois-ture	Pro-tein	Fat	Ash	Salt	Mois-ture	Pro-tein	Fat	Ash	Salt
0	72.14	22.66	0.96	3.63	1.63	72.14	22.66	0.96	3.62	1.63	72.14	22.66	0.96	3.62	1.63
2	74.55	22.46	0.86	3.17	2.12	74.08	22.54	0.90	3.36	2.03	73.98	22.60	1.08	3.10	2.08
4	73.55	22.26	0.92	3.67	2.76	73.70	22.68	0.97	3.56	2.36	71.99	22.63	1.02	3.82	2.66
6	74.10	22.12	0.99	3.87	3.00	73.50	22.70	1.00	3.50	2.66	71.95	22.78	0.99	4.47	2.95
9	←	Discontinued		→		73.88	22.20	1.02	3.68	3.02	71.87	22.33	1.06	4.52	3.26
11	←	Discontinued		→		←	Discontinued		→		72.69	22.29	1.06	3.92	3.81

*All values are expressed as per cent of canned fish on wet weight basis;

DI, II, CSW are as in Table 1.

TABLE 4. INFLUENCE OF FISH HANDLING METHODS ON STORAGE STABILITY OF CANNED WHITE SARDINES

Combined sensory scores for all attributes reduced and expressed out of maximum 10

Handling method	Holding period (days)	WSN at indicated storage				WS3 at indicated storage period			
		0	1	3	6	0	1	3	6
DI	0	7.74	7.24	6.82	6.56	8.10	7.70	7.24	7.10
	2	7.00	6.68	5.96	6.12	7.68	7.02	6.70	7.22
	4	6.80	6.52	6.62	5.62	7.42	7.12	7.42	7.02
	6	5.62	5.68	5.66	4.94	6.44	6.50	7.00	6.54
II	0	7.74	7.24	6.82	6.56	8.10	7.70	7.24	7.10
	2	6.74	6.66	5.96	6.34	7.28	7.04	6.70	6.70
	4	6.40	6.00	6.03	5.80	6.96	6.20	7.60	6.44
	6	6.52	5.66	6.08	5.66	7.10	6.66	7.24	6.50
	9	5.94	5.40	5.12	5.52	6.20	6.06	6.06	6.78
CSW	0	7.74	7.24	6.82	6.56	8.10	7.70	7.24	7.10
	2	6.92	7.48	5.84	6.52	7.34	7.44	7.20	7.12
	4	6.66	5.40	5.90	5.98	7.02	6.32	6.72	6.80
	6	6.26	5.18	5.60	5.62	6.84	5.32	7.46	6.26
	9	6.12	5.54	5.12	5.66	6.44	6.16	6.36	6.90
	11	6.08	4.46	4.44	4.84	6.52	5.58	5.92	6.48

Symbols are the same as in previous Tables

DI, II, CSW are as in Table 1.

canning as natural packs whatever may be the method of fish handling. The results show that DI, II, and CSW fish may be acceptable for producing natural packs only upto 4, 6 and 9 days of holding, respectively. However, the oil packed products proved to be better throughout the study period and thus exhibiting greater storage stability.

In the case of oil packed sardines, it may be noticed that there was some improvement in quality scores during the initial couple of months. This is due to the mixing up of fish flavour with that of the filling medium (vegetable oil) during the initial storage period, generally known as 'ripening' or 'maturing' of the can contents, a feature common to several similar canned foods. The overall superiority of oil packs over natural packs may be attributed mainly to the removal of most of the components responsible for off-flavour from the fish

during precooking and to some extent due to the masking effect of the vegetable oil.

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Flow Behaviour Properties of Rice Bran Protein Concentrate

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Flow behaviour properties of rice bran protein concentrate dispersions were studied at three levels of pH (8.0, 9.0 and 10.5) and different proteins concentration (4-24 percent). An increase in the protein concentration and pH of the dispersion increased deviation from Newtonian behaviour. Flow behaviour index of protein dispersion was not appreciably reduced with increase in protein concentration and pH. However, the consistency index increased with protein concentration and pH. The yield value of full-fat rice bran protein concentrate dispersion increased as protein concentration and pH increased, while change in pH had hardly any effect on yield value of defatted rice bran protein concentrate. Dispersions of 4-10% protein concentration showed mixed type flow properties i.e., dialatent and pseudoplastic.

The knowledge of the flow properties and viscosity of protein dispersions are of practical significance in relation to the number of purposes, such as quality control¹, understanding the structure², process engineering application³, and correlation with sensory evaluation of food. Viscosity changes can be used to evaluate the thickening power of protein, a property of practical interest in fluid foods, soups beverages, batters, etc.

The power law model with or without a yield stress (Equations 1 and 2) has been employed extensively to describe the flow behaviour of viscous foods over wide range of shear rates^{4,5}.

$$T = KY^n \dots\dots\dots (1)$$

$$T - T_0 = KY^n \dots\dots\dots (2)$$

Where, T is shear stress (dynes/cm²), Y, the shear rate (s⁻¹), K, the consistency index (dynes sⁿ/cm²), n, the flow behaviour index. If the value of n < 1, the flow behaviour is known as pseudoplastic, similarly for Newtonian behaviour when n=1 then the consistency index, K is replaced by (μ) the viscosity. If the behaviour is non-Bingham plastic, the modification of the equation (1) includes another parameter, T₀, the yield stress (Equation 2). The Casson model⁶ (Equation 3) has been used for foods, particularly for estimating the yield stress:

$$T^{0.5} - K_0 = K_1 Y_0^{0.5} \dots\dots\dots (3)$$

The magnitude of K₀² has been used as the yield stress by a number of workers⁷⁻¹⁰. A number of studies have been published on the effect of various factors, i.e., solubility,

concentration, pH, salt, lipid, starch and temperature on rheology, viscosity and flow properties of soy protein, whey protein, groundnut protein and sunflower protein concentrate/isolate^{11,12}. However, literature on flow behaviour properties of rice bran protein concentrate is fragmentary. Bera and Mukherjee¹³ reported that rice bran protein concentrate could be used in the preparation of protein enriched food products. The present investigations were undertaken to make a systematic study on flow behaviour properties of rice bran protein concentrates with the following objectives (i) to study the effect of pH and protein concentration on flow behaviour properties of full-fat and defatted rice bran protein concentrate dispersions; (ii) to characterise type of fluid (dispersion) based on flow properties.

Materials and Methods

Rice bran protein concentrate i.e. full fat (FFB-PC) and defatted (DFB-PC) samples were prepared by following the methods described by Bera and Mukherjee¹³. Rice bran protein concentrates were reported to be more soluble above the neutral pH¹³. Hence, the protein dispersion of both FFB-PC and DFB-PC samples were adjusted to three levels of pH (8.0, 9.0 and 10.5). The pH of the dispersion was adjusted with 1N NaOH. The dispersion of samples at different concentrations (4-24 per cent) were made (weight/volume basis) by adding the sample to water with stirring at room temperature (22°C) The viscosity measurement of 25 ml dispersion was made in a Rheotest-2 (VEBMLW) coaxial narrow gap viscometer using S1 cylinder at an increasing shear rate. A holding time of one minute at each shear rate

was maintained to get an equivalent run for all systems under investigation. After one min, steady dial reading was noted and the flow curve of shear stress versus shear rate was obtained.

Results and Discussion

The flow curve of FFB-PC and DFB-PC dispersions in distilled water (pH 9.0) at various protein concentrations (4-24 per cent) is shown in Fig. 1 and 2. The data necessary to characterise the flow behaviour of protein dispersions by power law equation (Equation 2) at various concentrations and pH are mean of triplicate and presented in the Table 1. It is seen that with increasing concentration, there was

increasing deviation from Newtonian behaviour i.e., protein dispersion showed non-Newtonian behaviour ($K > 0$ and $n < 1$). DFB-PC sample (10 per cent) and FFB-PC sample (16 per cent) concentration had yield value (Table 1). The Casson plot gave good linearity (Fig.3), and when K_0^2 values were used in the double logarithmic plot, very good linearity was obtained (Fig.4). The flow behaviour index (n) of protein dispersions was not appreciably reduced with increasing protein concentration. However, the consistency index (K) increased with protein concentration. The values of ' K ' of FFB-PC were less compared to those for DFB-PC samples. Highest consistency index value of 2.6 dynes/cm² was obtained at 24 per cent dispersion of FFB-PC sample, whereas DFB-PC sample gave consistency index of 41.0 dynes/cm². Similarly, maximum yield value (T_0) of 56.25 dynes/cm² was observed at 24 percent dispersion of DFB-PC sample, while DFB-PC sample gave a value of (T_0) 529.0 dynes/m² (Table 1).

The FFB-PC and DFB-PC dispersions of different concentrations at pH 9.0 had flow behaviour index (n) in between 0.5 and 0.6 which is characteristic of pseudoplastic materials.

The effect of pH on power law constants of dispersions of rice bran protein concentrate is presented in Table 2. It is seen that at 10 percent concentration yield stress values were not present over the pH range of 8.0-9.0 but yield stress was observed at pH 10.5 even at 10 percent dispersion of DFB-PC sample. However, for dispersion of 20 per cent, yield stress values were present, which increased markedly as the pH increased (Table 2). It was observed that the yield values of FFB-PC sample increased, while change in pH had hardly any appreciable effect on yield stress of DFB-PC sample.

The apparent viscosity at various shear rates for different FFB-PC and DFB-PC dispersions were calculated and it has been observed that this property is highly concentration and pH dependent. At a fixed shear rate of 1312.0 S⁻¹, pH 9.0 and 24 per cent dispersion of DFB-PC showed an apparent

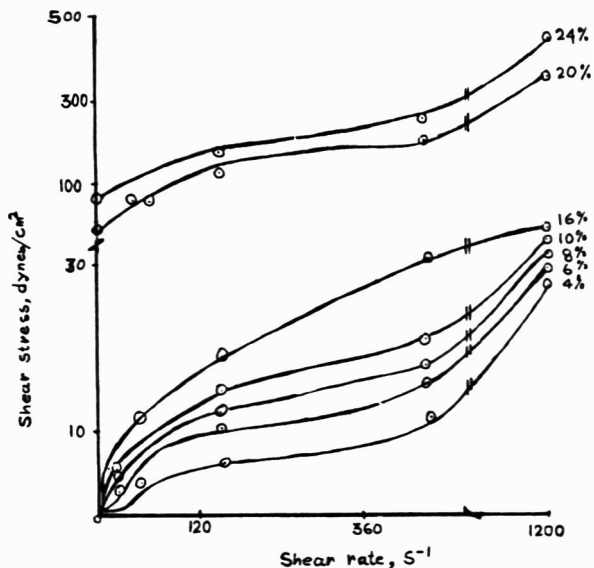


Fig.1. Flow curves of full-fat rice bran protein concentrate in various protein concentrations at pH 9.0.

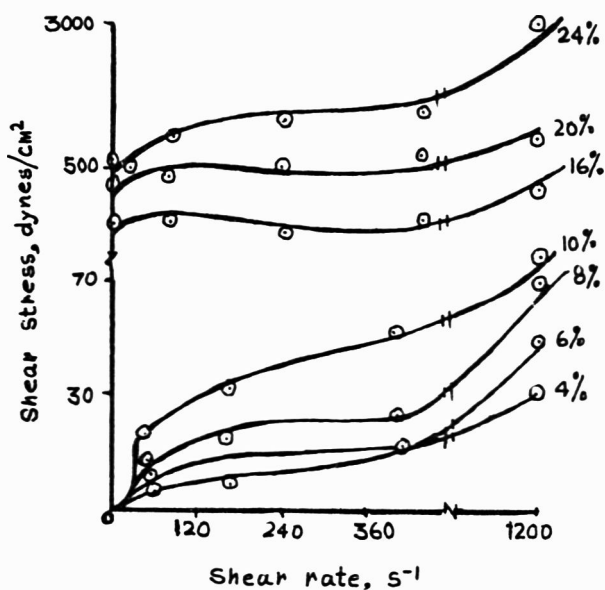


Fig.2. Flow curves of defatted rice bran protein concentrate in various protein concentrations at pH 9.0.

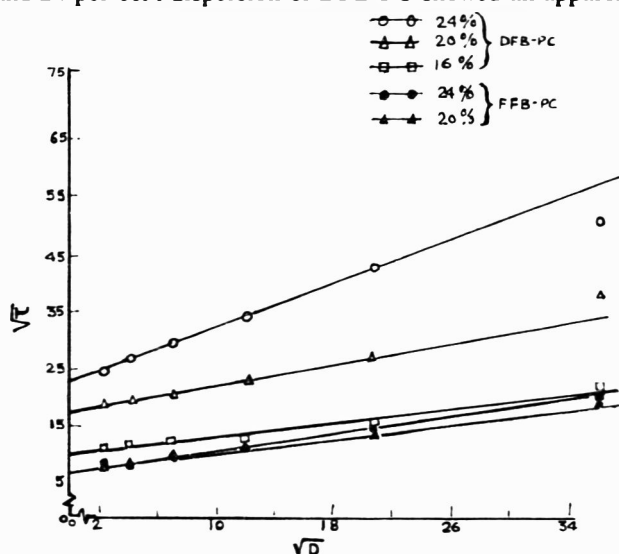


Fig.3. Casson's plot for various protein concentrations at pH 9.0.

TABLE 1. EFFECT OF RICE BRAN PROTEIN CONCENTRATE AT DIFFERENT CONCENTRATIONS ON POWER LAW CONSTANT AT pH 9.0

Conc. on w/v (%)	Full fat rice bran protein conc.			Defatted rice bran protein conc.		
	T_0	k	n	T_0	k	n
4	—	0.28	0.611	—	1.2	0.50
6	—	0.64	0.555	—	—	—
8	—	0.70	0.607	—	2.0	0.50
16	—	1.10	0.594	110.25	6.0	0.50
24	56.25	2.60	0.602	529.00	41.0	0.54

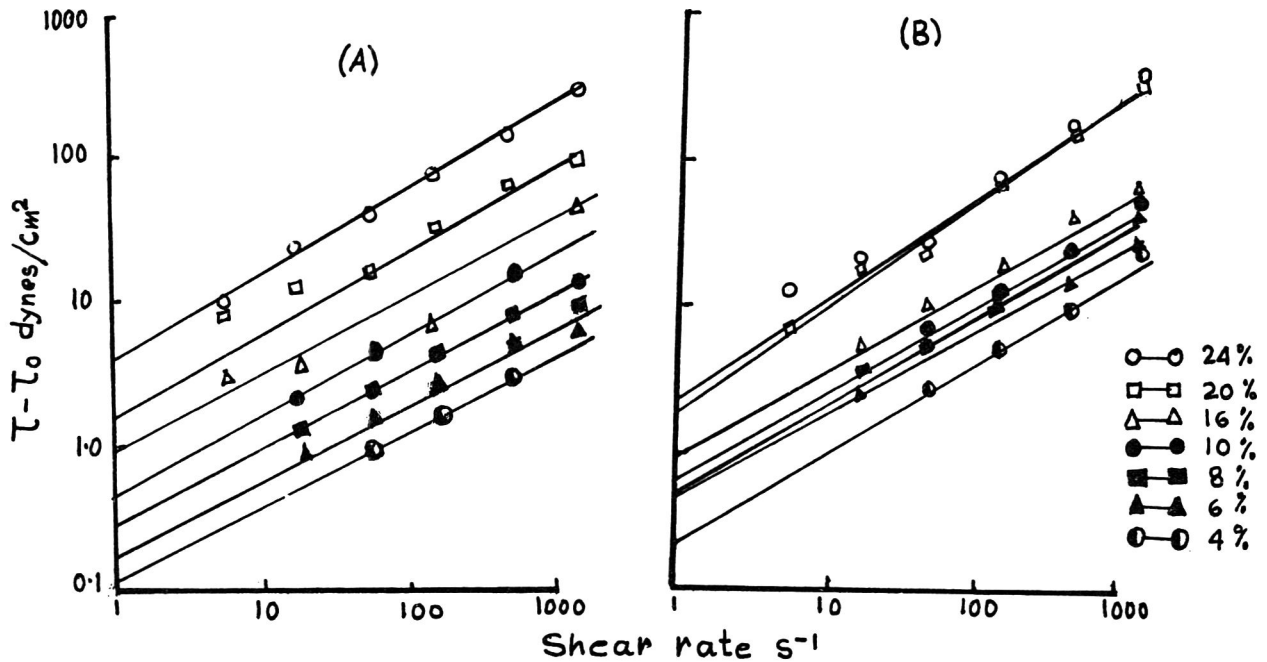


Fig.4. Double logarithmic plot of $T - T_0$ and Shear rate for dispersions of (A) defatted and (B) full-fat rice bran protein concentrate at various concentrations (pH 9.0).

TABLE 2. EFFECT OF pH ON POWER LAW CONSTANT OF DISPERSIONS OF RICE BRAN PROTEIN CONCENTRATE

Sample	Concn. (%)	pH 8.0			pH 9.0			pH 10.0		
		T_0	k	n	T_0	k	n	T_0	k	n
FFB-PC	10	—	0.33	0.660	—	0.76	0.611	3.24	1.35	0.511
	20	40.96	1.60	0.533	49.00	2.50	0.697	56.25	13.50	0.594
DFB-PC	10	—	2.40	0.520	—	3.10	0.500	16.0	3.70	0.500
	20	306.25	9.90	0.580	324.00	16.50	0.530	324.3	17.00	0.562

viscosity of 34 cp while the DFB-PC dispersion showed an apparent viscosity 250 cp (Fig 5). The apparent viscosity of 20 per cent FFB-PC and DFB-PC dispersions at pH levels of 8.0 to 10.5 were enormously high compared to 10.0 per cent dispersions. The high apparent viscosity at pH 9.0 may be due to aggregation and increased hydration of protein, while the increase at pH 10.5 may be caused by protein unfolding and dissociation into subunits and interaction.

The yield stress of food materials plays an important role in their flow characteristics. It is a desired property of hydrocolloids, and has a bearing on their sensory property (mouth

feel). Generally, with increasing solid contents, consistency index (k) increases and the flow behaviour index (n) which is a measure of the departure from Newtonian flow decreases. This type of behaviour was observed in case of both FFB-PC and DFB-PC samples.

The protein dispersion showed a shear thickening property during the initial shear rate; the viscosity increased rapidly and then decreased with the shearing time. The flow characteristics indicate that the increase in viscosity is likely to be due to protein-water interaction (hydration) rather than to a strong protein-protein interaction force. In this respect,

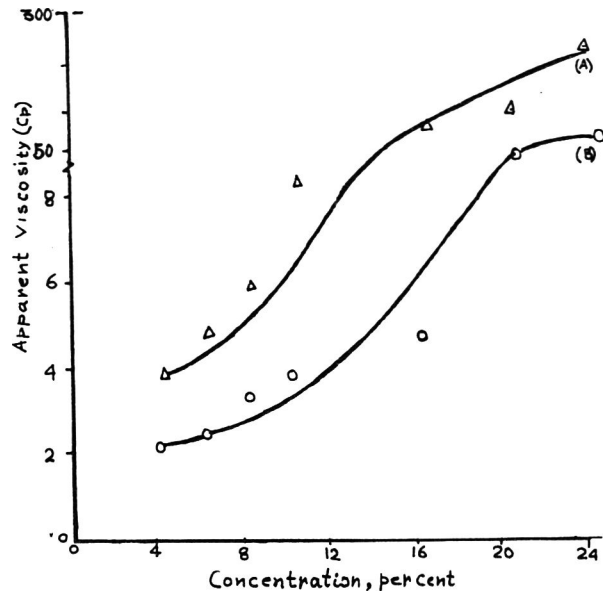


Fig.5. Effect of protein concentration on apparent viscosity of defatted (A) and full-fat (B) rice bran protein concentrate at pH 9.0 and shear rate 1312.0 S^{-1} .

rice bran protein differs from soybean protein and behave like groundnut protein. As swelling and viscosity are inter-related⁹, it is suggested that the amount of swollen, not fully solvated protein particles present are responsible for the observed increase in viscosity with concentration. The apparent viscosity of soy isolates has been reported to increase exponentially with protein concentration¹⁴, and this was affected by the swelling ability of the particular protein preparation. Swelling and unfolding increase the effective volume or hydro-dynamic volume and decreases the distance between the protein molecules, and thus viscosity or consistency is increased. Swelling, unfolding and flexibility of protein may also increase the axial ratio or axis of rotation and these can also increase the viscosity¹⁵. The rice bran protein concentrate dispersion of 4 to 10 per cent showed increased viscosity with increase in shear (shear thickening) upto a level (150 s^{-1}) and then gradually decreased (shear thinning) when shear rate increased further (Fig 1). This property indicates that the dispersions are of mixed type i.e; dialatent and pseudoplastic. This type of flow is only found in liquids that contain a high proportion of insoluble rigid particles in suspension¹⁶. However, in the case of 16 to 24 per cent dispersion, the viscosity decreased with shear rate which is typical of pseudoplastic materials.

Flaming *et al.*¹⁷ reported that alkaline treatment caused marked increase in viscosity of protein concentrate. thus pH

activation, as a means of increasing viscosity, may have practical significance in the formulation of gravies, beverages and comminuted food. Therefore, it appears now that rice bran protein concentrate could be used for making protein enriched gravies, and to all those food products where gelation is required since higher consistency index (K) and its relation to gelation property is considered important where prevention of flow during thermal processing and for imparting mouth feel are required.

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An Objective and Sensory Assessment of Cooking Quality of Some Rice Varieties Grown in Andhra Pradesh

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Cooking qualities of twenty varieties of milled paddy samples were evaluated by physical and chemical characteristics and palatability evaluation. Kernel dimension did not correlate significantly with the chemical characteristics. Palatability evaluation showed that short grain varieties had better palatability scores than long grain varieties. Significant correlations were found among some of the physico-chemical characteristics and the latter with palatability evaluations. Highly significant correlations were found among palatability evaluations. Grain length, elongation ratio, gel length, amylose content and protein content are some of the characteristics of rice used for predicting palatability.

Rice is an important cereal and staple diet in India. The information about nutritional quality and acceptability of several rice varieties limited.

The present study was undertaken to study the effect of physico-chemical characteristics on cooking quality of twenty varieties of rice by their palatability evaluation.

Materials and Methods

Paddy samples were milled by standard method (5-10 per cent degree of milling by weight). The rice samples were classified into five classes i.e; long slender, long bold, medium slender, short bold and short slender according to Mitra¹; long slender (LS) Length > 6 mm, L/W ratio > 3.0; long bold (LB) length > 6mm, L/W ratio < 3.0; medium slender (M.S) length 5-6 mm, L/W ratio 2.5-3.0; short bold (SB) length < 6 mm, L/W ratio < 2.5; short slender (SS) length < 6 mm, L/W ratio > 3.0. The percentage of moisture in the raw rice was estimated using "Moisture Determinator". The hulling percentage and head rice recovery were estimated following the method of Ghosh *et al.*²

Elongation ratio and volume expansion were determined by the method of Verghese³. For determination of water uptake ratio, method of Bhattacharya and Sowbhagya⁴ was followed. Cooking time was noted according to the method of Chakrabarthy *et al.*⁵ The alkali spreading and clearing values were determined by using the method of Little *et al.*⁶ Gel length of the varieties was determined by the method of Cagampang *et al.*⁷ Method by Juliano³ was followed for estimation of amylose. Nitrogen content in the milled grains was estimated by Kjeldahl⁹ method and the values were multiplied by factor 5.95 to get protein content.

A score card of five points was prepared for estimating colour, appearance, texture, cohesiveness, doneness, taste, flavour, aroma and overall quality of the cooked samples. A panel of ten judges from among the staff and students of Home Science were selected and trained. One hundred grams of each rice samples were weighed in a balance and washed thrice with 400 ml of water each time. 600 ml of water was boiled and rice was added and cooked till it was done. The doneness was determined by the absence of any white grainy appearance, when cooked rice grain was pressed between two glass slides. Excess water was strained. The cooked samples were cooled to room temperature and served along with drinking water to the panel members for evaluation between 10 and 11.30 A.M. Statistical analysis was carried by using correlation.

Results and Discussion

Eight of the varieties (RNR 74802, RNR 87877, RNR 29692, RNR 3070, RNR 99377, BPT 3291, BPT 1235, and IET 7575) were of long slender type. Only one i.e; 'RNR 74229' was of long bold type. Five varieties (RNR 31446, MTU 5182, MTU 5249, RNR 1446 and IR 13525) were of medium slender type whereas three (RNR 52149, RNR 36718 and MTU 7029) were of short bold type and three (RNR 32349, RNR 1535 and BPT 5204) were of short slender type.

There was not much difference in the moisture level of different classes of rice varieties (Table 1). There was not much difference between hulling percentage of different types as they ranged from 76 per cent for long slender type to 78.3 per cent for medium slender type. The head rice recovery ranged from 63.3 to 69 per cent.

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TABLE 1. GRAIN LENGTH, WIDTH, LENGTH/WIDTH (L/W) RATIO, AND MOISTURE CONTENT OF DIFFERENT CLASSES OF RICE VARIETIES

Class	No. of var	Length ^a (mm)	Width ^a (mm)	L/W ratio ^a	Moisture content ^b
LS	8	6.36 ± 0.055	1.89 ± 0.071	3.81 ± 0.143	10.94 ± 0.184
LB	1	6.1	2.11	2.89	11.6
MS	5	5.75 ± 0.075	2.09 ± 0.036	2.76 ± 0.081	11.4 ± 0.444
SB	3	5.17 ± 0.105	2.11 ± 0.068	2.39 ± 0.083	11.35 ± 0.267
SS	3	5.21 ± 0.328	1.93 ± 0.139	3.05 ± 0.012	10.56 ± 0.033

Values are mean ± SE

a — Values are mean of given number of varieties and each varietal value is mean of twenty replicates.

b — Values are mean of given number of varieties and each varietal value is mean of three replicates.

Short grain varieties elongated more than medium and long grain varieties, though the difference is less. Volume expansion was also less for short grain varieties than long grain varieties (Table 2). It was noticed that with the increase in temperature the apparent water uptake increased as the average apparent water uptake at 80°C was 0.34 whereas it was 1.56 at 96°C. Spreading values ranged from 2 to 5 whereas clearing values from 1 to 4. (Table 2). Short grain varieties showed lower spreading and clearing values than long grain varieties although some exceptions were there. The clearing value was found to be less than spreading value by one in all the cases. Amylose content ranged from 21.3 to 23.2 per cent. Protein content ranged from 6 to 8.7 per cent with an average of 7.1 per cent (Table 3).

Five point scale given by Ruth¹⁰ (i.e; 5-very good, 4-good, 3-fair, 2-average and 1-poor) was used for scoring the cooked rice samples. Only one variety 'RNR 52149' got a class of 'very good' with an average mean score of 4.13. Nine of the varieties (i.e; 'RNR 1535', 'RNR 74802', 'BPT 5204', 'BPT 3291', 'RNR 87877', 'MTU 7029', 'RNR 99377', 'MTU 5182' and 'LET 7575') got a class of 'good' and ten varieties (i.e. 'RNR 74299', 'MTU 5249', 'RNR 3070', 'BPT 1235', 'RNR 29692', 'RNR 31446', 'RNR 32349', 'RNR 36718', 'RNR 1446' and 'IR 13525') got class 'fair'. None of the varieties studied got mean score of 2 or less.

Most of the physico-chemical characteristics were not correlated significantly and it may be because of limited number of samples. Kernel dimensions were not useful indices of the chemical composition of rice grains. Grain length showed a significant negative correlation with elongation ratio. Cooking temperature seemed to be an influencing factor on water absorption as it increased with increase in temperature. Water uptake ratio showed a strong positive correlation with alkali spreading and this is in agreement with the report of Tomar and Nanda¹¹. Water uptake ratio showed a strong negative correlation with cooking time. Cooking time showed a strong negative correlation with alkali digestibility. Gel length showed a highly significant negative correlation with amylose content (Table 4).

The palatability scores are presented in Table 5. For overall quality, short slender type got maximum mean value of 3.5 whereas medium slender type got minimum mean value of 2.8. It was found that all the sensory evaluations were highly significantly positively correlated with each other and is in agreement with the report of Ghosh *et al.*¹² Flavour was found to be most strongly positively correlated with overall quality followed by cohesiveness and appearance. Thus flavour, cohesiveness, appearance and texture may be the important factors which affect the palatability of a particular rice variety.

TABLE 2. ELONGATION RATIO, VOLUME EXPANSION, WATER UPTAKE RATIO AND COOKING TIME OF DIFFERENT CLASSES OF RICE VARIETIES

Class	No. of var	No. of repl. of each var	Elongation ratio	Vol expansion	W' 80°	W' 96°	W = $\frac{W'80^\circ}{W'96^\circ} \times 100$	Cooking time (min)
LS	8	3	1.6 ± 0.041	4.2 ± 0.226	0.4 ± 0.035	1.5 ± 0.074	22.4 ± 1.686	23.3 ± 0.513
LB	1	3	1.6	3.8	0.3	1.6	20.8	24.0
MS	5	3	1.6 ± 0.040	3.8 ± 0.256	0.4 ± 0.059	1.6 ± 0.063	24.9 ± 2.612	24.0 ± 0.864
SB	3	3	1.8 ± 0.012	3.7 ± 0.067	0.3 ± 0.042	1.5 ± 0.135	22.4 ± 2.768	23.9 ± 0.867
SS	3	3	1.7 ± 0.143	3.8 ± 0.515	0.3 ± 0.025	1.6 ± 0.109	20.4 ± 3.072	23.1 ± 1.563

Values are mean ± SE

W'80 — Apparent water uptake at 80°C

W'96 — Apparent water uptake at 96°C

W — Water uptake ratio

TABLE 3. SPREADING/CLEARING VALUE, GEL LENGTH, AMYLOSE, AND PROTEIN CONTENT OF DIFFERENT CLASSES OF RICE VARIETIES

Class	No. of var	No. of repl. of each var	Spreading value	Clearing value	Gel length (mm)	Amylose (g %)	Protein content (g %)
LS	8	3	4.8 ± 0.861	3.8 ± 0.861	70.4 ± 7.975	22.2 ± 0.721	7.2 ± 0.655
LB	1	3	2	1	82.5	21.3	6.0
MS	5	3	4 ± 1.225	3 ± 1.225	61.4 ± 9.438	22.9 ± 0.869	6.5 ± 0.819
SB	3	3	5 ± 1.527	4 ± 1.528	60.3 ± 4.622	23.2 ± 0.549	7.1 ± 0.416
SS	3	3	2 ± 0	1 ± 0	77.5 ± 13.611	21.5 ± 1.362	8.7 ± 0.821

Values are mean ± SE

TABLE 4. CORRELATIONS (r) AMONG PHYSICO-CHEMICAL CHARACTERISTICS OF RICE

Characteristics	L/W ratio	Elongation ratio	Water uptake ratio	Cooking time	Alkali spreading	Gel length	Amylose	Protein
Grain length	0.692**	-0.600**	0.2511	0.005	0.239	-0.079	0.090	-0.158
L/W ratio	1.000	-0.035	0.183	-0.269	0.138	0.153	-0.183	0.304
Elongation ratio		1.000	-0.002	-0.168	-0.068	0.159	-0.163	0.206
Volume expansion			0.286	-0.401	0.154	4.270	-0.301	-0.031
Water uptake ratio			1.000	-0.640**	0.622**	0.099	-0.158	-0.222
Cooking time				1.000	-0.648**	-0.300	0.408	-0.041
Alkali spreading					1.000	0.024	-0.024	0.043
Gel length						1.000	-0.989**	0.308
Amylose							1.000	-0.289

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

TABLE 5. MEAN PALATABILITY SCORES OF COOKED RICE

Class	Colour	Texture	Appearance	Cohesiveness	Flavour/ Aroma	Doneness	Taste	Overall quality
LS	3.4 ± 0.145	3.3 ± 0.169	3.1 ± 0.188	3.1 ± 0.104	3.2 ± 0.083	3.5 ± 0.089	3.0 ± 0.084	3.2 ± 0.129
LB	3.7	3.2	3.0	3.13	3.3	3.7	2.9	3.0
MS	3.1 ± 0.246	2.8 ± 0.144	2.7 ± 0.134	2.6 ± 0.094	3.0 ± 0.054	3.3 ± 0.082	2.9 ± 0.082	2.8 ± 0.99
SB	3.6 ± 0.358	3.6 ± 0.522	3.4 ± 0.482	3.2 ± 0.407	3.3 ± 0.304	3.7 ± 0.289	3.2 ± 0.351	3.4 ± 0.439
SS	3.8 ± 0.253	3.6 ± 0.238	3.3 ± 0.396	3.4 ± 0.289	3.5 ± 0.258	3.8 ± 0.104	3.5 ± 0.169	3.5 ± 0.351

Values are mean ± SE

Number of varieties — LS — 8, LB — 1, MS — 5, SB — 3, SS — 3

Each varietal value is mean of three replicates.

It was found that only gel length, total amylose and protein content had significant correlations with some of the palatability evaluations. All the other physico-chemical characteristics studied did not have any significant effect on palatability evaluations. Grain length was significantly and positively correlated with all the palatability evaluations. Amylose was negatively and significantly correlated with palatability evaluations especially colour, texture, appearance, cohesiveness, flavour and taste and of course overall quality. Protein was positively correlated with palatability evaluations but not significantly except in the case of after taste. Grain length, elongation ratio, gel length, amylose and protein content are some of the characteristics of rice used for predicting palatability of rice.

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Secretion and Composition of Bile in Rats Fed Diets Containing Spices

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Feeding mustard and tamarind to experimental rats produced marked increase in bile flow rate. Onion and curry powder in the diet significantly increased the secretion of bile solids without affecting the bile flow rate. The bile acids output was noticeably higher in animals fed either mustard, tamarind, onion or curry powder, and this was accompanied by similar increases in biliary phospholipid and cholesterol. Dietary cinnamon and asafoetida increased biliary secretion of cholesterol and phospholipid without affecting bile acid content. Intragastric single dose administration of clove or cardamom did not influence the bile secretion and composition. The results suggest increased hepatic conversion of cholesterol to bile acids under the influence of dietary mustard, tamarind, onion and curry powder.

Spices are extensively used as food additives to enhance sensory quality of foods. This apart, several spices exhibit a wide range of beneficial physiological effects and are used as ingredients of many medicinal preparations^{1,2}. Spices have been generally believed to intensify salivary flow and gastric juice secretion, and hence aid in digestion³. They are consumed usually in the form of curry powder which is a mixture of several spices in varying proportions. Coriander, turmeric and red pepper form the main ingredients of curry powders while the other common ingredients are cumin, black pepper, fenugreek, dry ginger and cinnamon. Mustard and asafoetida are largely used in seasonings. Cloves and cardamom are widely used as flavouring agents in curry foods and in condiments. Tamarind is a principal souring agent in curries, sauces and chutneys especially in South India.

Earlier studies from our laboratory have revealed that the active principles of spices — turmeric and red pepper⁴, and the spices — fenugreek and ginger⁵ stimulate bile flow and also influence bile composition. On the other hand, coriander⁶, cumin⁶ and black pepper⁷ are shown to have no influence on bile secretion and composition. In the current study, we have investigated the influence of a few other commonly used spices and a spice mixture on secretion and composition of bile in experimental rats. The results obtained are discussed in the context of hypocholesterolemic effects of spices.

Materials and Methods

Male Wistar albino rats weighing 250 ± 10 g were used in the experiments. Spices studied here for their influence on bile are: clove (*Eugenia caryophyllus*), cardamom (*Elettaria cardamomum*), cinnamon (*Cinnamomum zeylanicum*), asafoetida (*Ferula foetida*), mustard (*Brassica nigra*), tamarind (*Tamarindus indica*), onion (*Allium cepa*) and curry powder, a spice mixture. Clove, cardamom, cinnamon,

asafoetida, mustard and tamarind powder were procured locally and powdered to pass through 50 mesh sieve. Onion obtained from local market was freeze dried and powdered (onion powder yield was approximately 12 g/100 g raw onion). Curry powder was prepared from the locally purchased dry powdered ingredients with the following composition (per cent): coriander, 40; turmeric, 20; red pepper, 20; black pepper, 5; cumin 5; mustard, 5; fenugreek, 2; dry ginger, 1; clove, 1 and cinnamon, 1. All the chemicals were of analytical grade and the solvents were distilled before use.

Animal treatment: In the feeding study, groups of six animals were maintained (*ad libitum*) on various spice containing diets for four weeks. The basal diet consisted of (per cent): casein, 21; cane sugar, 10; corn starch, 54; refined groundnut oil, 10; vitamin mixture, 1 and salt mixture, 4. Spices were incorporated into this basal diet substituting an equivalent amount of corn starch to give various experimental diets containing (per cent): cinnamon, 50 mg; asafoetida, 250 mg; mustard, 250 mg; tamarind, 2.5 g; onion powder, 0.6 and 3.0 g, and curry powder, 0.5 and 2.5 g. For single dose study, animals maintained for two weeks on a basal diet were used. After an overnight fast, animals (n=6) were force-fed by intubation two different doses (mg/kg body wt) of clove (3 and 15) and cardamom (3 and 15) as a suspension in two ml 10 percent (w/v) aqueous starch. Control rats were administered two ml 10 per cent aqueous starch. At the end of four weeks, spice feeding term, or 3 hr after the intragastric administration of spice, rats were anaesthetized by intraperitoneal injection of ethyl urethane (1.2 g/kg body wt). Laparotomy was performed and the common bile duct was cannulated with PE-10 tubing (Thomas Scientific Co., USA) and the bile was collected for 3 hr. Bile volumes were measured and kept frozen pending analyses.

Bile analysis: Bile solids were determined by gravimetry. Lipids were extracted with chloroform-methanol by the

method of Bligh and Dyer⁸. The chloroform layer was used for the determination of cholesterol and phospholipids according to Searcy and Bergquist⁹ and Marinetti¹⁰ respectively. Uronic acid in the methanolic layer was estimated according to Dische¹¹. Total bile acids in the methanolic layer were estimated by employing 3 α -hydroxy-steroid dehydrogenase according to the procedure of Turley and Dietschy¹². Individual bile acids in the methanolic extract were separated by thin layer chromatography using chloroform-methanol-acetic acid-water (65:24:15:9 v/v)¹³. Bile acid spots were visualised after reacting with phosphomolybdic acid spray reagent and quantitated by densitometry using a Camag TLC Scanner¹³. Statistical evaluation of the data was done by Students' t-test¹⁴.

Results and Discussion

In the present study, cinnamon, asafoetida, mustard and tamarind have been fed to animals at levels corresponding to about five times the average human dietary intake of these spices. The levels used here are based on calculated dietary intake of spices in the form of curry powder and on dietary survey conducted in India¹⁵. The two levels of curry powder and onion powder fed here will be comparable to normal and five times the normal human intake. Since clove and cardamom are not normally consumed in the daily diet, the present study with them is restricted to their single dose administration. The diet consumption and the body weights in all the experimental groups during the four weeks, spices treatment were comparable to controls (data not given).

Data on the influence of single dose administration of clove and cardamom on bile secretion and composition are given in Tables 1 and 2. Bile flow and solids were not changed by

clove and cardamom treatment at either doses. Phospholipid and cholesterol secretions in bile were significantly increased by clove and cardamom administration only at the higher dose, viz., 15 mg/kg body wt. Clove administration at the higher dose led to an increase in biliary uronic acid secretion suggesting that some of the metabolites of its constituents are probably excreted as glucuronides in bile. Bile acid output was not affected by single dose clove and cardamom administration (Table 2).

Table 3 shows the effect of dietary cinnamon, asafoetida, mustard and tamarind on bile flow and on biliary secretion of cholesterol, phospholipids and uronic acids. Feeding mustard and tamarind for four weeks significantly enhanced the bile secretion by as much as 30 per cent while the bile solid concentration remained normal. Biliary cholesterol and phospholipids were significantly higher in all these four spice fed groups. Increase in the cholesterol and phospholipid secretion was highest in mustard treatment (80 and 130 per cent). Uronic acid content in bile was also higher in mustard fed animals, implying that some of the metabolites of mustard constituents are probably excreted via bile as glucuronid conjugates.

The total bile acid output was significantly higher in rats fed mustard and tamarind (Table 4). Any change in the relative proportions of various individual bile acids was also examined. The increased bile acid output in bile produced by feeding mustard or tamarind was mainly due to increased taurocholic acid (Table 4).

Dietary feeding of onion and curry powder did not influence bile flow rate but produced significantly higher secretions of solids (Table 5). Biliary phospholipid secretion was increased by dietary onion and curry powder by over 100 and

TABLE 1. EFFECT OF INTRAGASTRIC ADMINISTRATION OF CLOVE AND CARDAMOM ON BILE SECRETION AND COMPOSITION

Spice	Quantity (mg/kg body wt)	Bile flow (ml/hr)	Bile solids (g%)	Phospholipids (μ mole/hr)	Cholesterol (μ mole/hr)	Uronic acids (μ mole/hr)
Control		0.48 \pm 0.04	3.65 \pm 0.21	2.42 \pm 0.28	0.282 \pm 0.029	0.817 \pm 0.081
Clove	3	0.48 \pm 0.04	3.29 \pm 0.12	2.71 \pm 0.28	0.277 \pm 0.038	0.853 \pm 0.097*
	15	0.53 \pm 0.03	3.43 \pm 0.22	3.64 \pm 0.21*	0.398 \pm 0.029*	1.116 \pm 0.079*
Cardamom	3	0.51 \pm 0.05	3.24 \pm 0.23	2.89 \pm 0.18	0.342 \pm 0.024	0.745 \pm 0.081
	15	0.55 \pm 0.05	3.20 \pm 0.10	3.70 \pm 0.41*	0.416 \pm 0.030*	1.028 \pm 0.083

Values are mean \pm SEM of five animals. *Significantly different from control ($P < 0.05$)

TABLE 2. EFFECT OF INTRAGASTRIC ADMINISTRATION OF CLOVE AND CARDAMOM ON BILE SECRETION

Spice	Quantity (mg/kg body wt)	Total bile acids	Taurocholic acid	Glycocholic acid	Taurodeoxycholic acid
Control		5.87 \pm 0.70	3.27 \pm 0.43	0.66 \pm 0.16	1.59 \pm 0.34
Clove	3	6.24 \pm 1.09	3.53 \pm 0.18	0.43 \pm 0.12	2.11 \pm 0.27
	15	7.13 \pm 0.89	4.13 \pm 0.20	0.47 \pm 0.06	2.74 \pm 0.34*
Cardamom	3	5.81 \pm 0.68	3.23 \pm 0.26	0.47 \pm 0.12	1.51 \pm 0.27
	15	6.38 \pm 0.48	3.90 \pm 0.43	0.49 \pm 0.16	2.13 \pm 0.40

Values (μ mole/hr) are mean \pm SEM of five animals. *Significantly different from control ($P < 0.05$)

50 per cent respectively. Cholesterol secretion was also slightly but significantly higher in onion and curry powder fed animals. Total bile acid output was markedly increased (40-70 per cent) by onion and curry powder feeding at both the dietary levels (Table 6). The increased bile acid output was mainly due to increased taurocholic acid, although increases in the secondary bile acid taurodeoxycholic acid were also seen.

Results of the current study indicate that unlike other spices such as fenugreek (*Trigonella foenumgraecum*), ginger (*Zingiber officinale*), black pepper (*Piper nigrum*)^{5,7}, and spice principles — curcumin (of turmeric: *Curcuma longa*), capsaicin (of red pepper: *Capsicum anrum*) or piperine (of black pepper: *Piper nigrum*)^{4,7,16}, the spices clove, cardamom, cinnamon, asafoetida, onion and curry powder do not have the hydrocholagogic effect. Further, unlike the other hydrocholagogic spices which stimulate bile flow with concomitant decrease in bile solid concentration, mustard and tamarind have stimulated the bile flow without affecting the bile solid concentration. Thus, the mechanism of stimulation of the bile forming function of the liver could be different

among these two groups of spices.

The secretion of phospholipid and cholesterol in bile depends on the secretion of bile salt in an approximately linear relationship at physiological rates of output¹⁷. Thus, higher rate of bile acid output produced by dietary mustard, tamarind, onion and curry powder was accompanied by similar increases in biliary phospholipid and cholesterol secretion. But an uncoupling between bile acid and lipid secretion appears to occur in cinnamon or asafoetida fed rats and in clove or cardamom administered animals, wherein increased secretion rates of phospholipid and cholesterol were accompanied by normal rate of bile acid secretion.

As the absorption of the bile salts is eliminated by diversion of the normal route through cannulation of the common bile duct, it can be inferred that the increased bile salts output in mustard, tamarind, onion and curry powder fed animals is due to the increased hepatic conversion of cholesterol into bile acids. A similar observation has been made in the case of curcumin⁴, capsaicin⁴, fenugreek⁵ and ginger⁵ all of which are known to have hypocholesterolemic activity. The hypocholesterolemic property of onion has been well

TABLE 3. EFFECT OF DIETARY CINNAMON, ASAFOETIDA, MUSTARD AND TAMARIND ON BILE SECRETION AND COMPOSITION

Dietary spice	Bile flow (ml/hr)	Bile solids (g%)	Phospholipids (μ mole/hr)	Cholesterol (μ mole/hr)	Uronic acids (μ mole/hr)
Control	0.57 \pm 0.04	2.99 \pm 0.11	1.68 \pm 0.08	0.259 \pm 0.013	0.964 \pm 0.077
Cinnamon (50 mg %)	0.58 \pm 0.05	3.25 \pm 0.14	3.22 \pm 0.06*	0.401 \pm 0.008*	0.979 \pm 0.067
Asafoetida (250 mg %)	0.62 \pm 0.05	3.31 \pm 0.15	2.63 \pm 0.32*	0.339 \pm 0.021*	0.892 \pm 0.056
Mustard (250 mg %)	0.74 \pm 0.04*	3.26 \pm 0.20	3.84 \pm 0.56*	0.463 \pm 0.049*	1.677 \pm 0.205*
Tamarind (2.5 %)	0.72 \pm 0.05*	3.33 \pm 0.20	2.81 \pm 0.26*	0.385 \pm 0.034*	1.046 \pm 0.056

Values are mean \pm SEM of five animals. *Significantly different from control (P<0.05)

TABLE 4. EFFECT OF DIETARY CINNAMON, ASAFOETIDA, MUSTARD AND TAMARIND ON BILE ACID SECRETION

Dietary spice	Total bile acids	Taurocholic acid	Glycocholic acid	Taurodeoxycholic acid
Control	6.30 \pm 0.70	4.29 \pm 0.20	0.94 \pm 0.12	1.21 \pm 0.15
Cinnamon (50 mg %)	6.46 \pm 0.21	4.74 \pm 0.33	0.94 \pm 0.10	1.72 \pm 0.23
Asafoetida (250 mg %)	6.93 \pm 0.70	4.89 \pm 0.39	1.11 \pm 0.12	1.74 \pm 0.18
Mustard (250 mg %)	9.45 \pm 0.91*	6.02 \pm 0.33*	1.21 \pm 0.12	1.99 \pm 0.21*
Tamarind (2.5 %)	9.96 \pm 1.33*	6.41 \pm 0.32*	1.11 \pm 0.18	2.34 \pm 0.27*

Values (μ mole/hr) are mean \pm SEM of five animals. *Significantly different from control (P<0.05)

TABLE 5. EFFECT OF ONION AND CURRY POWDER FEEDING ON BILE SECRETION AND COMPOSITION

Dietary spice		Bile flow (ml/hr)	Bile solids (g%)	Phospholipids (μ mole/hr)	Cholesterol (μ mole/hr)	Uronic acids (μ mole/hr)
Name	Quantity (%)					
Control		0.60 \pm 0.04	3.06 \pm 0.12	1.88 \pm 0.19	0.269 \pm 0.012	0.928 \pm 0.086
Onion powder	0.6	0.60 \pm 0.02	3.66 \pm 0.07*	4.25 \pm 0.37*	0.337 \pm 0.013*	0.728 \pm 0.127
	3.0	0.63 \pm 0.05	3.60 \pm 0.08*	4.33 \pm 0.47*	0.330 \pm 0.012*	0.760 \pm 0.081
Curry powder	0.5	0.51 \pm 0.05	3.62 \pm 0.21*	3.03 \pm 0.41*	0.301 \pm 0.011	0.788 \pm 0.101
	2.5	0.55 \pm 0.03	3.69 \pm 0.22*	3.22 \pm 0.50*	0.316 \pm 0.016*	0.897 \pm 0.082

Values are mean \pm SEM of five animals. *Significantly different from control (P<0.05)

TABLE 6. EFFECT OF ONION AND CURRY POWDER FEEDING ON BILE ACID SECRETION

Dietary spice		Total bile acids	Taurocholic acid	Glycocholic acid	Taurodeoxycholic acid
Name	Quantity (%)				
Control	—	8.14 ± 0.87	5.69 ± 0.46	0.51 ± 0.02	1.93 ± 0.32
Onion powder	0.6	11.51 ± 0.72*	7.47 ± 0.48*	0.82 ± 0.18	3.39 ± 0.27*
	3.0	11.98 ± 0.97*	7.84 ± 0.45	0.70 ± 0.24	3.31 ± 0.19*
Curry powder	3.5	11.18 ± 0.87*	6.50 ± 0.18	1.15 ± 0.24*	3.26 ± 0.44*
	2.5	13.70 ± 1.29*	8.14 ± 0.46*	1.19 ± 0.31	2.76 ± 0.56

Values (μ mole/hr) are mean \pm SEM of five animals. *Significantly different from control ($P < 0.05$)

recognized^{18,19}. Recent investigations have revealed absence of any effect of dietary curry powder²⁰, mustard²¹ and tamarind²¹ on serum and liver cholesterol in hypercholesterolemia induced rats.

Thus, the present study indicates that mustard, tamarind and the spice mixture (curry powder) though do not possess hypocholesterolemic property would still cause increased bile acid output presumably by enhancing hepatic cholesterol conversion. Onion and curry powder are shown here to stimulate bile acid formation without influencing the bile forming function of the liver. Lack of influence on secretion and composition of bile as observed here in clove and cardamom administered rats compares with similar observation made on a few other spices, viz., coriander, cumin and curry leaf⁶. The observation that mustard and tamarind stimulate the bile forming function of liver and also increase bile salt secretion justify their use in digestive disorders^{2,22}.

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INFLUENCE OF PACKAGING SYSTEMS ON THE MICROBIOLOGICAL QUALITY OF PROCESSED CHEESE DURING STORAGE

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Microbiological quality of processed cheese packaged in small lacquered tins (P1), polystyrene cups (P2), and low density polyethylene tubs (P3), when stored at 30°C/60% R.H. and 7-8°C/80% R.H., respectively, revealed that P1 was the best package, followed by P3 and P2 for storing processed cheese under the above two conditions, of temperature and R.H.

Packaging is a very essential step in the manufacturing line, as the product has to be saved from microbial spoilage which would make the food unsuitable for human consumption. Besides, pathogenic bacteria may occur in foods and result in food poisoning outbreaks. To meet the quality standards, certain package systems have been developed^{1,3}. Although tin containers are a perfect package system, it is quite expensive and involves the expenditure of valuable foreign exchange. In the present study, an attempt has been made to find out the microbiological quality of processed cheese packaged in indigenously manufactured, low cost, hygienic and attractive plastic containers, in comparison with tin containers.

Polystyrene (PS) cups with lids and low density polyethylene (LDPE) tubs with lids were procured from the leading manufacturers of the country. The capacity of each container was 150 g. The PS cups (P1) and their lids were opaque and white in colour, while the LDPE tubs (P2) and their lids were translucent. The height and top diameter of the PS cup were 7.1 and 6.2 cm respectively. The thickness of the cup sheet was 0.20 mm. The height and top diameter of the LDPE tub were 3.4 and 7.9 cm, respectively and the thickness of the tub sheet was 0.60 mm. Lacquered tins (P3) used in the investigation were obtained from well known Indian firms and had a capacity of 240 g. The height and diameter of the tins were 7.8 and 5.9 cm, respectively.

The plastic containers and their lids were cleaned by using 'teepol' and tap water. They were then chemically sterilized by keeping in chlorine solution (200 p.p.m.) for 3 min⁴. Thereafter, the packages were air dried. The sterilisation of the packages was done immediately before use as far as possible under aseptic conditions. The tin cans and their lids cleaned with hot detergent solution and tap water were

sterilized in a hot air oven at 165-170°C for 2 hr, immediately before use⁴.

The method of Kosikowski⁵ was followed. Medium ripened (upto 6 months old) Cheddar cheese (from cow's milk) collected from the Experimental Dairy of the Institute was used in the preparation of processed cheese. When the cheese mass became velvety, smooth and attained a temperature of 70°C, it was directly filled into the three types of pre-sterilized packages (P1, P2 and P3). Immediately after filling, the plastic containers were covered with their respective lids, which in turn, were further sealed by using cello tape of 2.5 cm width. The tins were sealed off. The sealed packages after 1 hr were stored under 2 different conditions, 30°C and 60 per cent R.H. (condition A), and 7-8°C and 80 per cent R.H. (Condition B).

Samples of processed cheese packaged in 3 different types of containers and stored under conditions A and B were examined for standard plate count, yeast and mould count, and anaerobic spore formers count. Sampling and the preparation of dilution blanks were carried out according to the Laboratory Manual⁶. The standard plate count (SPC) was determined using tryptone dextrose yeast agar⁶, and yeast and mould (Y&M) count using potato-dextrose agar medium⁷. The colonies were counted and expressed in log₁₀/g as per the recommended method⁸. The anaerobic spore formers count in the samples was found out by using Differential Reinforced Clostridial Medium (DRCM)⁹: Ten gram of processed cheese was macerated in sufficient 0.1 per cent peptone water to give a 0.2 or 0.1 (W/V) dilution of the sample. Decimal dilutions of the macerate were prepared in the same diluent and 1 ml of the original macerate and the next two decimal dilutions were transferred to the tubes containing DRCM. Before use, the DRCM broth tubes were steamed for 10 min to expel oxygen present initially in the tubes. Three tubes with each dilution¹⁰ were inoculated. The tubes after incubation at 37°C for 48-72 hr were observed for blackening of the medium, and the results were recorded as most probable number (MPN) counts.

During storage, the packaged cheese samples were also observed for any visual defect such as 'water weeping', bloating or mould growth. The methods and techniques of Snedecor and Cochran¹¹ were followed for analysing the data obtained during the present study.

The data for microbiological quality in terms of standard plate count (SPC), yeasts and moulds (Y&M) count and anaerobic spore formers (ASF) count of processed cheese samples stored in the 3 different types of packages (P1, P2 and P3) for different periods under conditions A and B are presented in log₁₀/g in Tables 1 and 2, respectively.

TABLE 1. MICROBIOLOGICAL COUNTS (\log_{10} /g) OF PROCESSED CHEESE STORED IN DIFFERENT PACKAGES AT 30°C AND 60 PER CENT R.H.

	0-day storage			10-day storage			20-day storage			30-day storage		
	Poly-styrene	LDPE	Tin	Poly-styrene	LDPE	Tin	Poly-styrene	LDPE	Tin	Poly-styrene	LDPE	Tin
Standard Plate Count												
Trial I	2.763	2.763	2.763	4.756	4.869	4.832	5.707	5.792	5.756	4.732	4.857	4.819
Trial II	2.813	2.813	2.813	4.793	4.845	4.813	5.770	5.903	5.785	4.778	4.813	4.785
Trial III	2.857	2.857	2.857	4.851	4.892	4.875	5.813	5.924	5.875	4.778	4.851	4.845
Mean	2.811	2.811	2.811	4.800	4.869	4.840	5.763	5.873	5.805	4.763	4.840	4.816
Yeasts and Moulds												
Trial I	0.778	0.778	0.778	1.380	1.518	1.602	2.602	2.748	2.699	1.342	1.477	1.544
Trial II	0.954	0.954	0.954	1.602	1.914	1.813	2.857	2.964	2.954	1.602	1.875	1.778
Trial III	1.114	1.114	1.114	1.903	2.204	2.146	2.813	3.097	3.130	1.880	2.079	2.041
Mean	0.949	0.949	0.949	1.628	1.879	1.854	2.757	2.936	2.928	1.608	1.810	1.788
Anaerobic Spore Formers (MPN/100g)												
Trial I	3.6	3.6	3.6	9.1	15	15	21	29	21	15	21	15
Trial II	9.1	9.1	9.1	15	21	15	21	43	23	15	29	20
Trial III	7.2	7.2	7.2	11	19	13	21	36	21	15	26	19
Mean	6.6	6.6	6.6	11.7	18.3	14.3	21	36	21.7	15	25.3	18

Storage at 30°C and 60 per cent R.H.: The processed cheese samples in all the three types of packages showed rapid microbial growth after 10 days of storage. Subsequent storage resulted in a gradual increase in SPC. However, the counts decreased slightly at the end of 30 days of storage (Table 1). The samples packed in P1 showed minimum growth of micro-organism (mean value in \log_{10} /g: 4.763) followed by the samples packed in P3 (4.816) and P2 (4.840) in an ascending order. Highly significant ($P < 0.01$) differences in the total counts were observed due to the 3 types of packages, and the intervals of storage, while the interaction between intervals and packages was not significant.

The samples packaged in P2 showed maximum increase in Y & M count (2.936), and minimum in P1 (2.757), with intermediary values for P3 (2.928), at the end of 20 days of storage. However, counts of Y & M decreased in all the packages after 30 days of storage, probably due to setting in of lag phase. The statistical analysis of the data revealed that the durations of storage and the type of package significantly ($P < 0.01$) affected the growth of yeasts and moulds. The interaction intervals and type of package were found to be not significant.

Increase from the initial ASF count (MPN/100g) of the processed cheese samples was minimum (21) in P1 followed by P3 (21.7) and P2 (36) in an ascending order after 20 days of storage. However, the counts decreased on prolonged storage of 30 days in all the samples packaged in the 3 types of containers, perhaps due to setting in of lag phase. The durations of storage, the 3 types of packages, and the interaction of type of package with interval, each individually, greatly ($P < 0.01$) contributed in the growth of anaerobic spore formers.

Storage at 7-8°C and 80 per cent R.H.: Samples of processed cheese packaged in P1 showed lower SPC (mean value : 3.878) followed by P3 (3.958) and P2 (3.994), respectively in an ascending order after 90 days of storage. A similar trend has been recorded by earlier workers^{12,13} who observed variation in total counts due to different types of packages used. The SPC of the processed cheese samples was significantly ($P < 0.01$) influenced by the type of package, intervals of storage and the interaction of intervals and packages.

The counts of yeasts and moulds increased to the maximum (3.234) in samples packed in P2, followed by P3 (3.217) and P1 (3.086) in descending order after 60 days of storage (Table 2). Similar to SPC, counts of yeasts and moulds also showed a decreasing trend at the end of 90 days of storage. Statistically, the durations of storage significantly ($P < 0.01$) influenced the growth of Y & M, but the type of package, and the interaction of intervals \times packages were not significant.

A perusal of Table 2 indicates that the minimum multiplication of ASF had been in the samples packaged in P1 (tins), followed by P3 (LDPE) and P2 (polystyrene), respectively, after 90 days of storage. The statistical analysis of the data also established that the duration of storage, the 3 types of packaging, and the interaction of packages \times intervals, each individually, significantly ($P < 0.01$) contributed in the growth of ASF.

The results obtained during the investigation are in agreement with the earlier findings^{12,14}, that in general, the microbial growth was a major factor in the selection of the most suitable packaging material for a particular product.

TABLE 2. MICROBIOLOGICAL COUNTS (\log_{10} /g) OF PROCESSED CHEESE STORED IN DIFFERENT PACKAGES AT 7-8°C AND 80 PER CENT R.H.

	0-day storage			30-day storage			60-day storage			90-day storage		
	Poly-styrene	LDPE	Tin	Poly-styrene	LDPE	Tin	Poly-styrene	LDPE	Tin	Poly-styrene	LDPE	Tin
Standard Plate Count												
Trial I	2.763	2.763	2.763	3.653	3.716	3.819	4.431	4.498	4.544	3.875	4.008	3.977
Trial II	2.813	2.813	2.813	3.681	3.832	3.724	4.414	4.476	4.446	3.857	3.991	3.954
Trial III	2.857	2.857	2.857	3.919	4.041	4.002	4.342	4.579	4.556	3.903	3.982	3.944
Mean	2.811	2.811	2.811	3.751	3.863	3.848	4.397	4.518	4.515	3.878	3.994	3.958
Yeasts and Moulds												
Trial I	0.778	0.778	0.778	2.380	2.415	2.477	2.819	2.903	2.963	1.301	1.544	1.477
Trial II	0.954	0.954	0.954	2.740	2.819	2.785	3.041	3.193	3.158	2.041	2.204	2.079
Trial III	1.114	1.114	1.114	2.845	2.914	2.875	3.398	3.602	3.531	2.301	2.556	2.447
Mean	0.949	0.949	0.949	2.655	2.716	2.712	3.086	3.234	3.217	1.881	2.100	2.001
Anaerobic Spore Formers (MPN/100g)												
Trial I	3.6	3.6	3.6	7.2	14	7.3	16	23	19	14	21	19
Trial II	9.1	9.1	9.1	12	19	14	19	39	23	15	28	20
Trial III	7.2	7.2	7.2	9.1	16	13	19	39	21	14	23	19
Mean	6.6	6.6	6.6	9.4	16.3	11.4	18	33.7	21	14.3	24	19.3

During the entire storage period (30 days at condition A and 90 days at condition B) of processed cheese, not even a single sample, packaged in plastic containers or tins, showed the defects of 'water weeping'¹⁵ or blowing of lid. However, some of the plastic containers (approximately 15 per cent) showed visible mould growth on the surface of processed cheese after 30 days of storage at condition A.

The study thus led to conclusion that from the consideration of least multiplication of microorganisms, tin cans proved best followed by LDPE tubs and polystyrene cups, respectively, for the packaging and storage of processed cheese at 30°C/60 per cent R.H. or at 7-8°C/80 per cent R.H.

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NICKEL IN VANASPATI

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Nickel in fine dispersed form is used as a catalyst in the manufacture of hydrogenated vegetable oils from which Vanaspati, Margarine and bakery shortenings are made. The nickel catalyst is removed by filtration along with filter aids, if required. However, nickel being in colloidal form in hydrogenated vegetable oils, permeates with the final product in traces. The presence of nickel even in traces is undesirable. A survey has been conducted to assess the intake of nickel through these hydrogenated food products. From the survey, it is recommended that a limit of 3 p.p.m. for nickel content may be laid down for these food products in the food standards.

Vanaspati, margarine and bakery shortenings are hydrogenated vegetable oil(s) products. In the process of hydrogenation of vegetable oils, nickel in the concentration of 0.02 to 0.1 per cent is employed as a catalyst¹. Nickel in the fine dispersed form is obtained from nickel formate or spongy form from nickel-aluminium alloy². The recent trend in hydrogenation industry is to use organo-nickel complexes, which leaves very low nickel residues in the product. In whatsoever form, it is removed by filtration with the use of filter aids. However, some of the nickel finds its way into the hydrogenated vegetable oil.

Nickel is found to be harmful and on ingestion may cause nausea, vomiting and diarrhoea^{3,4}. However toxicity of nickel salts is reported to be low, due to limited intestinal absorption of nickel⁵. It is also reported that nickel can produce subcutaneous cancer in rats and mice⁶. Schroeder *et al.*⁷ found a decreased concentration of copper in the lung and spleen of rats receiving 5 parts per million of nickel in drinking water.

From the above, it is essential to know the ingestion of nickel through these food products and a limit be laid down for the same. For this purpose, samples of Vanaspati in their sealed containers as well as from open tin were collected from the local market and analysed for nickel content.

Two methods have been adopted for the estimation of nickel content in vanaspati samples.

Spectrophotometric method: The estimation of nickel in the present study was carried out by adopting dimethyl-glyoxime method⁸. The absorbance maxima were recorded at 445 nm in the U.V. visible Shimadzu Spectrophotometer model 240.

Vanaspati (20 g) was taken in a platinum dish and was burnt on a low flame to avoid frothing. The burnt, sample was ashed in a furnace which generally requires 2 hr. The temperature of the furnace was kept below 500°C to avoid volatilization losses.

Ash was dissolved in 5 ml conc. HCL. Excess acid was removed by evaporation to dryness and dissolved the residue in water and volume was made upto 25 ml. Five ml of this solution was taken in 10 ml stoppered test-tube, 0.5 ml of saturated bromine water was added to it and allowed to stand for 1 min. then 1.0 ml of ammonia and 2 ml of 0.1 per cent dimethyl-glyoxime solution in alcohol (95 per cent) was added. Finally, volume was made to 10 ml with alcohol and mixed. Absorbance was recorded at 445 nm. The readings were taken within 10 min after adding dimethyl-glyoxime solution.

The standard solution of nickel was prepared by dissolving 2.2617 of nickel sulphate (99 per cent) in 30 ml conc. HCl and the volume was made upto 500 ml with distilled water. This solution contains 1000 μ g/ml of nickel. Calibration graph was prepared after diluting the stock solution ranging from 0.1 to 1.0 μ g per ml. It was observed that readings were linear upto 0.4 μ g/ml of nickel.

The method reported for determination of nickel by Atomic Absorption spectrophotometer in margarine⁹ was followed. The sample was ignited and the ash was dissolved in 5 ml acid mixture (HCl : HNO₃ : Water :: 7.5 : 7.5 : 10). Then it was boiled and evaporated till the volume was reduced to 2 ml. Finally, volume was made upto 10 ml with distilled water. The analysis of nickel was carried out on Varian Techtron Spectra AA 30 using acetylene-air flame at 232.0 nm, the most sensitive radiation of nickel.

To avoid the molecular absorption at 232.0 nm, the background correction has been applied by using hydrogen lamp. It was observed that calibration graph was nearly linear upto 10 μ g/ml and sensitivity of detection was 0.066 μ g/ml of nickel for 1 per cent absorption. The calibration graph was drawn after preparing standard solution of 1 to 10 μ g/ml of nickel.

It can be observed from the data in Table 1 that there is a wide variation in the nickel contents of different samples of vanaspati of various brands. Nickel content varied from a minimal of 0.4 p.p.m. (average of 0.43 and 0.36) to a maximum of 15.4 p.p.m. (average of 15.89 and 14.95). The higher nickel content in samples of some of the brands could be attributed to non adherence of quality standards during hydrogenation and subsequent post-refining of hydrogenated oils. It is also observed that nickel content was found to be around 3 p.p.m. for all the samples except one. Therefore, it is recommended that a maximum limit of 3 p.p.m. may be fixed.

TABLE I. NICKEL CONTENT (P.P.M) IN VANASPATI SAMPLES

Description of sample	Spectro- photometer	Atomic absorption spectrophotometer
Dalda (sealed polythene pack of 1 kg)	0.65	0.58
Dalda (from local market from open tin)	0.59	0.73
Rath (sealed plastic pack of 1 kg.)	0.43	0.36
Rath (from local market from open tin)	0.88	0.69
Panghat (from local market from open tin)	1.26	1.09
Gagan (sealed polythene pack of 1 kg.)	2.16	2.42
Gagan (from local market from open tin)	3.48	3.66
Uttam (from local market from open tin)	15.89	14.95
Vanaspatti without label (from local market from open tin)	3.07	3.31
Vanaspatti without label (from local market from open tin)	3.42	3.57

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ENHANCED α -AMYLASE PRODUCTION ON WHEAT BRAN MEDIUM BY *BACILLUS SUBTILIS*-159

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Effect of wheat bran on the production of amylase by *Bacillus subtilis*-159 was investigated. Wheat bran, at 1% concentration gave about 1.85 fold increase in amylase production than it is produced from Tendler's non-synthetic (TNS) medium (2.0-2.6 units/ml) within 48 hr; however, at higher concentrations of wheat bran the amylase production was retarded. Addition of CaCO_3 to the medium containing 4% wheat bran enhanced amylase production to 20 units/ml at 96 hr.

Thermostable alpha-amylases from *Bacillus* spp. are used extensively in textile and paper industry. A bacterial strain, *Bacillus subtilis*-159 produced an amylase active at high temperatures¹. It was shown further that the amylase is a better desizing agent when compared with other commercially available amylases². *B. subtilis*-159 amylase also finds its use in the preparation of low viscosity starch suitable for sizing purposes³. It was found that Tendler's non-synthetic (TNS) medium with slight modifications was best suited for the growth of *B. subtilis*-159 which produced 2.0-2.6 units/ml of amylase in 42-48 hr at 30°C⁴. Attempts were made to obtain nutritional conditions for higher yields of amylase by incorporating carbon and nitrogen sources in TNS medium. These studies showed only a 2.0-3.5 fold increase in amylase production⁴. Wheat bran is a cheap natural by-product available abundantly from industrial flour mills. It contains carbohydrates, proteins, fats, minerals, vitamins, phytic acid, etc⁵. The present studies were undertaken to assess the production of amylase on wheat bran

The wheat bran was obtained from Shaw Wallace Flour Mills, Bombay. It was analysed by standard methods and found containing (on per cent basis) starch, 12.5, reducing sugars, 4.8, protein, 14, and ash, 6.6.

Bacillus subtilis-159 used in the present investigation was obtained from Indian Institute of Science, Bangalore. It was maintained on nutrient agar slopes by subculturing at three month intervals.

Tendler's non-synthetic medium contained the following ingredients was modified slightly by replacing glucose with

0.2 per cent starch. The medium was used for preparation of inoculum as well as a control in nutritional studies. It had the following composition (A): (g/l): tryptone, 10; yeast extract, 4; NH_4NO_3 , 1; sodium citrate, 0.5; starch, 2 and (B): (g/l) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5; K_2HPO_4 , 0.3; and microsals (mg/l): $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 20; $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$, 2; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.5; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.8; $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1; H_3BO_3 , 0.1. For the growth and amylase production, the above medium was further modified whereby all the ingredients given under (A) were replaced with peptone (15 g/l) and supplemented with wheat bran; the pH was adjusted to 7.2. A 42 hr old actively growing culture having O.D. 0.1 at 540 nm was added at the rate of 2 ml per 50 ml medium and incubated at 30°C on a rotary shaker (200 strokes/min) for 48 hr.

After the growth, the cells were harvested by centrifuging at 12000 \times g in Sorvall RC2-B centrifuge. The amylase activity of the cell-free supernatant was estimated by the starch-iodine method of Street⁶ as modified by Khandeparkar and Dhala⁷. The optimum temperature of the enzyme is 50°C at pH 6.0. Thus, one unit of amylase is defined as that amount of enzyme which hydrolyses 1 mg starch in 1 min at its optimum temperature and pH. The reducing sugars formed at various stages of growth were detected on TLC plates coated with silica gel G employing the solvent system n-butanol-ethanol-water (50 : 30 : 20). The chromatogram was run at 20°C and developed by spraying an saldehyde-sulphuric acid reagent⁸.

Studies were conducted using wheat bran as a substrate in TNS medium. Table I shows the effect of various concentrations of wheat bran on amylase production. Initially, the amylase activity increased with the concentration of wheat bran upto 1 per cent, where it reached to about 1.85 times higher than in control medium. However, the trend altered at higher concentrations and the enzyme activity started declining with increase in the substrate concentration. It was found that at 4 per cent wheat bran level, less than half the activity was retained. A simultaneous check on pH showed that the broth turns more and more acidic with increase in the substrate concentrations. Thus, at 2 per cent wheat bran the pH of the medium decreased from 7.2 to 6.5, and at 4 per cent it lowered to pH.6.0.

To control the pH of the medium CaCO_3 (0.1-0.5 per cent) was employed where 0.3 per cent of it was found to be the most suitable concentration for the purpose. Thus, the organism was grown in growth medium containing wheat bran at 1 per cent to 4 per cent in the presence of 0.3 per cent CaCO_3 for 48 hr. The results are incorporated in Table 1. It is seen that the amylase production in the medium containing 1 per cent wheat bran remained almost the same, however, there was a substantial increase in amylase produc-

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TABLE 1. EFFECT OF WHEAT BRAN IN PRESENCE AND ABSENCE OF CaCO₃ ON AMYLASE PRODUCTION BY *BACILLUS SUBTILIS*-159

Wheat bran (%)	Amylase (units/ml)	
	No CaCO ₃	With CaCO ₃
0.1	1.33	—
0.2	2.80	—
0.5	4.13	—
1.0	4.93	5.03
2.0	4.13	5.33
3.0	2.00	6.00
4.0	1.01	7.63
Control	2.66	2.60

tion in presence of CaCO₃ at higher concentrations of wheat bran. At 4 per cent wheat bran concentration, 7.35 units/ml of amylase were produced. This was approximately 7 times higher than that obtained without CaCO₃ in the medium.

To understand various changes taking place in the course of time, the organism was cultivated in growth medium containing 4 per cent wheat bran and 0.3 per cent CaCO₃ and alteration of pH, amylase production, increase in biomass, change in sugar concentration, disappearance of starch from the broth were studied for 120 hr. Fig. 1 shows the change in these parameters. It was observed that the pH of the medium, initially decreased from 7.2 to 6.5 upto a period of 24 hr. Thereafter, it increased gradually and finally arrived to 8.6 at 96 hr and maintained at this level on further incubation. The amylase production started after a lag of 24 hr, increased with biomass until both reached to a maximum level of around 84 hr to 96 hr. After this time, there was a fall both in biomass and amylase produced, at 96 hr the amylase activity reached to 20 units/ml. Several authors⁹⁻¹² showed that the alpha-amylase production in various *Bacillus* spp was growth limiting and occurs in the logarithmic phase of growth. In the case of *B. subtilis*-159, the growth pattern and amylase production are in good agreement with the findings of the above authors. Upton and Fogarty¹³ observed maximum amylase activity of *Thermomonospora viridis* during the logarithmic phase of growth and noted that approximately 70 per cent of the total starch was utilized by the organism before a major part of the amylase was produced. Similarly, the present strain also utilized all the starch by 42 hr and the enzyme production continued upto 96 hr. During the course of the growth, the starch was hydrolysed forming reducing sugars. The concentration of sugars in the medium increased with time upto 48 hr, then rapidly declined to a level at 72 hr which remained constant thereafter. TLC of the cell-free filtrate at 48 hr showed the presence of glucose, maltose and isomaltose. But by 72 hr, glucose and maltose were utilised by the organism from the medium leaving behind only isomaltose. Although there are reports¹⁴ that some strains of fungi utilize isomaltose, or the sugar acts as an inducer for amylase synthesis, it is clear from

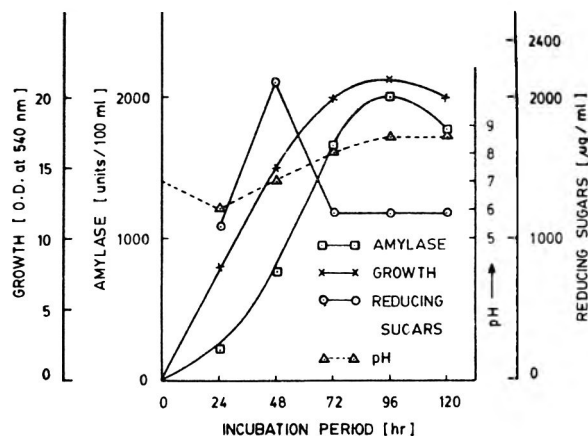


Fig.1 Effect of incubation period on amylase production, growth of organism, change in pH and reducing sugar.

the present results that *B. subtilis*-159 does not utilize isomaltose.

The foregoing results reveal that wheat bran is a good substrate for amylase production by *B. subtilis*-159. The effect of wheat bran on the enhancement of amylase production can not be attributed to starch alone but many other nutrients present in it particularly vitamins, phytic acid, etc.

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MINERAL REQUIREMENTS FOR GROWTH OF *VOLVARIELLA VOLVACEA* MYCELIUM BY SUBMERGED FERMENTATION

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A study was made on the mineral salt requirements of *Volvariella volvacea* for growth in shake-flask fermentation. It was observed that KH_2PO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ were required at concentrations of 0.1, 0.05 and 0.03% respectively. The elements Fe, Zn, B and Mo were required in concentrations of 3.6, 4.0, 15.0 and 0.5 $\mu\text{g/ml}$ respectively for maximum production of mycelium.

Mushrooms have been used as food and condiments from time immemorial. The submerged culture process has been employed by a number of investigators¹⁷ for production of mushroom mycelium as this technique has several advantages viz. smaller requirements of space, labour and time, easier to control contamination problems, higher efficiency of protein production, availability of the product throughout the year and better utilisation of low cost materials. The yield of the cell biomass by the submerged culture technique is related to the optimum cultural conditions, its requirements for nutrients, viz. carbon and nitrogen sources and minerals. Humfléd³ observed that phosphorus, potassium, sulphur, calcium, magnesium, iron, manganese, zinc, copper and cobalt were required for growth of *Agaricus campestris*. Litchfield⁶ reported that the rate of growth of *Morchella* species in a synthetic medium was enhanced by supplements such as corn steep liquor which is rich in trace nutrients, Srivastava and Bano⁸ studied the effect of several major elements like potassium, phosphorus, calcium and magnesium on the yield and crude protein content of *Pleurotus flabellatus* mycelium grown in a glucose-ammonium salt medium with a mixture of vitamins. The present investigation is aimed to determine the requirements of mineral salts for growth of *Volvariella volvacea* mycelia in a synthetic medium.

Volvariella volvacea culture was obtained from Tamilnadu Agricultural University, Coimbatore, India. The culture was grown on potato-dextrose agar slants for 4 days at 37°C and was subcultured weekly. The effect of minerals was studied in the selected medium consisting of the following (percentage) maltose—3 KH_2PO_4 —0.1, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ —0.05 Urea—0.24, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ —0.001 thiamine, riboflavin, calcium pantothenate, nicotinic acid each at 50 $\mu\text{g}/100\text{ ml}$,

biotin—0.5 $\mu\text{g}/100\text{ ml}$; folic acid—0.3 $\mu\text{g}/100\text{ ml}$, pyridoxine—20 $\mu\text{g}/100\text{ ml}$. For the preparation of the inoculum, the organism was first grown in 50 ml of the synthetic medium (pH 6.0) in a 250 ml Erlenmeyer flask at 32°C on a rotary shaker (120 r.p.m.) for 10 days and the mycelia were collected and washed twice with 30 ml of sterile water to remove all adhering contaminants. The mycelia were then transferred aseptically into a 250 ml Erlenmeyer flask containing 50 ml of demineralised water and 45 glass beads. It was shaken vigorously and 5 ml of this suspension was used as inoculum for each 250 ml flask containing 50 ml fermentation medium (pH 6.0). Inoculated flasks were incubated at 32°C on a rotary shaker (120 r.p.m.) for 10 days after which the cells were collected by filtering through Whatman No.1 filter paper. The mycelial mass was washed with distilled water to remove adhering medium constituents, dried overnight at 50–55°C and the dry cell weight in g/l was taken as the yield of the biomass. Demineralised water was used throughout the experiment. The solutions of sugar, urea and phosphate were purified by the chelation method as the use of adsorption method for removal of trace element contamination was not found suitable. In the chelation method⁹, 8-hydroxy quinoline was used for demineralisation of maltose, urea and phosphate. The solutions were mixed in required proportions and dispensed in the 250 ml Erlenmeyer flask along with other minerals and sterilized. Sterile maltose and KH_2PO_4 were added to the basal medium prior to inoculation. In a typical experiment, the mineral under observation was first omitted from the medium and then added in graded doses to the basal medium in separate flasks to determine optimal concentration. A triplicate set was taken for each experiment.

For studying the effects of salts KH_2PO_4 , MgSO_4 and CaCl_2 on the production of mushroom mycelia, rigorous

TABLE . EFFECT OF MINERALS ($\mu\text{g/ml}$) ON GROWTH OF *VOLVARIELLA VOLVACEA*

Fe	Mn	Zn	B	Mo	Cell biomass (g/l)
0.0	—	—	—	—	1.1
2.0	—	—	—	—	5.2
3.6	—	—	—	—	6.5
3.6	2.0	—	—	—	6.6
3.6	—	2.0	—	—	7.5
3.6	—	4.0	—	—	8.0
3.6	—	4.0	3.0	—	8.7
3.6	—	4.0	5.0	—	9.0
3.6	—	4.0	10.0	—	9.4
3.6	—	4.0	15.0	—	9.9
3.6	—	4.0	15.0	0.1	10.0
3.6	—	4.0	15.0	0.5	11.5

—, absence of mineral.

purification of the medium was not done as these were tested at higher concentration. The optimal concentrations of KH_2PO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ for the production of mycelia of *Volvariella volvacea* were 0.1, 0.05 and 0.03 per cent respectively (Results not shown). Table I shows that Fe, Zn, B and Mo were required in concentrations ($\mu\text{g/ml}$) of 3.6, 4.0 15.0 and 0.5 respectively. Manganese is not essential for growth of *Volvariella volvacea*. Copper and cobalt were inhibitory to the growth of the organism (Results not shown). It is interesting to note that the metals Zn, B and Mo were not absolutely essential for growth of the mushroom but these were necessary for maximum growth.

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EFFECT OF COMMON SALT IN PREVENTING SPOILAGE OF WET PADDY UNDER INCLEMENT WEATHER CONDITIONS

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Mixing of 1 or 2% common salt either with 20% paddy husk or powdered paddy husk were found effective in reducing the moisture content and incidence of fungi, increasing the total and head rice recovery of wet paddy and preventing the sprouting of grain completely. Among these, one per cent common salt with 20 per cent paddy husk which is easily adaptable and convenient was superior without any adverse effect on quality of cooked rice.

Spoilage of wet paddy grain due to sprouting, mould growth is a common phenomenon whenever the harvest coincides with rains¹. This is very common in Krishna-Godavari delta where the rice crop is adversely affected by cyclonic storms at the time of threshing of paddy. Sun-drying of the paddy is not possible during inclement weather. Also, the use of mechanical dryers are not encouraged because of its high cost and non-availability. Under these situations as an alternate remedy, the use of common salt (2.5 to 10 per cent) alone and in combination with powdered paddy husk or soil (as carrier material) for preservation of wet paddy has been studied by earlier workers and found effective in preventing the spoilage of wet paddy²⁻⁴. The usefulness of lower concentrations of common salt (1 and 2 per cent) along with paddy husk/powdered paddy husk in preventing the spoilage of wet paddy was studied in order to reduce the cost of

treatment and to make the method more convenient and easily adaptable. Consequent effects on milling and cooking quality were also studied.

A laboratory study was conducted using common salt at 1 and 2 per cent (w/w) concentrations along with 20 per cent (w/w) paddy husk/powdered paddy husk as carrier material. The paddy variety 'Masuri' used in the present study was soaked in water for about 14-16 hr in order to raise its moisture content to about 33.2 per cent (wet basis). The required quantities of common salt were weighed and thoroughly mixed with 5 kg of wet paddy along with 20 per cent paddy husk/powdered paddy husk as per the treatments (Table 1) and kept in the form of heaps on the floor in three replicates. Wet paddy without common salt and carrier material served as control. The samples from each treatment (comprising of 3 replicates) were collected at regular intervals to record moisture content, incidence of fungi and per cent sprouted grains. The moisture content was recorded by oven drying method⁵. Fungal isolation of the samples was made by standard blotter method⁶. At the end of experiment, the treated and untreated wet paddy were sun-dried separately so as to reach a safe level of 12 per cent moisture content. The dried paddy was separately milled using rubber roll sheller (Saitama Kawa goe, Japan), the total and head rice recovery were recorded on weight basis. The milled rice samples (400 g) were taken and separately cooked in domestic pressure cooker and cooking quality on the basis of texture, colour, smell and taste was analysed by rank sum method⁷ by a panel of 7 judges.

The reduction of moisture in all the treated samples was observed upto 5th day after treatment (DAT) and difference between treated and untreated samples at 10 day after treatment was not observed (Table 1). This indicated the effectiveness of treatments. The effectiveness of common salt with husk powder in reducing the moisture of wet paddy was also

TABLE 1. EFFECT OF COMMON SALT ON MOISTURE CONTENT AND SPROUTING OF WET PADDY*

Treatment		Initial moisture (% w.b)	Moisture content (% w.b) at indicated days after treatment				Sprouted grains** (%)
NaCl (%)	Paddy husk (%)		1	3	5	10	
1.0	20.0	33.2	28.2	27.9	27.0	19.5	0.0
2.0	20.0	33.2	27.8	27.5	27.3	18.9	0.0
1.0	20.0*	33.2	28.6	28.5	27.4	17.8	0.0
2.0	20.0*	33.2	28.5	28.7	27.4	19.0	0.0
Control		33.2	31.8	31.8	28.8	18.3	41.0
CD at 5%			1.30	0.98	0.91	NS	
CV %			2.44	1.90	1.62	—	

*Powdered paddy husk; * Mean of three replicates; **at 5th day after treatment.

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TABLE 2. EFFECT OF COMMON SALT TREATMENT ON THE MILLING QUALITY OF WET PADDY***

Treatment		Total rice** recovery (%)	Head rice** recovery (%)
NaCl (%)	Paddy husk* (%)		
1.0	20.0	74.5	72.4
2.0	20.0	74.4	71.7
1.0	20.0*	74.9	72.8
2.0	20.0*	74.5	71.9
Control		67.1	62.7
CD at 5%		1.33	1.46
CV %		0.97	1.10

*Powdered paddy husk;

***Moisture 33.2% on wet basis; **Mean of three replicates;

established earlier by other workers^{1,4,8-9}. All the treatments were equally effective in preventing the sprouting of wet paddy completely when compared to control which recorded 41 per cent on 5th day. Similar result was also reported by Anthoni Raj *et al.*¹⁰ Mixing of common salt at both the levels along with either paddy husk or powdered paddy husk could not inhibit the fungal growth completely. However, visually intensity of incidence of mould growth was more in control samples than in treated samples. The fungi *Aspergillus*, *Fusarium*, *Helminthosporium*, *Rhizopus* and *Trichoconiella* were isolated from treated as well as control samples. None of the treatments was free from any one of the above stated fungi. Prevention of fungal infection due to application of 5 per cent NaCl and 20-30 per cent husk powder to parboiled paddy was observed by Singaravadivel¹.

The data on milling quality of the paddy samples at 12 per cent moisture content are presented in Table 2. The total and head rice recovery were more than the control in all the treated samples. The total rice recovery in treated and control was about 74 and 67 per cent respectively; the head rice recovery in the corresponding samples was about 71 and 62 per cent respectively. Common salt either with paddy husk or powdered paddy husk has got its effect on head and total rice recovery. The report of Singaravadivel *et al.*¹ on parboiled paddy is a supporting evidence to the results of the present study. The percentage broken were the same in the treated and control. The low rice recovery in control might be due to sprouting. The data on cooking quality of the treated and untreated samples are given in Table 3. The samples of paddy treated with common salt at 1 per cent along with 20 per cent paddy husk or powdered paddy husk was within acceptable limits without showing any adverse effect on taste, colour, smell and texture.

In general, it may be concluded that the spoilage of wet paddy can be minimised using 1 per cent common salt with

TABLE 3. EFFECT OF COMMON SALT TREATMENT OF WET PADDY ON COOKING QUALITY CHARACTERISTICS — RANK SUM DATA

Treatment		Cooking quality characteristics			
NaCl (%)	Paddy husk (%)	Texture	Colour	Smell	Taste
1.0	20.0	10** Sup.	11** Sup.	10** Sup.	10** Sup.
2.0	20.0	24 n.s	23 n.s	24 n.s	23 n.s
1.0	20.0*	11** Sup.	10** Sup.	11** Sup.	11** Sup.
2.0	20.0*	25 n.s	26 n.s	25 n.s	26 n.s
Control		35** Inf.	35** Inf.	35** Inf.	35** Inf.

*Powdered paddy husk;

Sup : Superior; Inf : Inferior; **Significant at 1% level; n.s : Not significant.

20 per cent paddy husk (which is easily adaptable and convenient) and was equally effective as with 20 per cent powdered paddy husk without any adverse effect on cooking quality of rice.

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EFFECT OF DRYING ON MICROBIAL POPULATION OF WET PADDY USING BATCH TYPE DRYER

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Drying of wet paddy using batch type dryer at 45-55°C drying air temperatures significantly minimised the bacterial and fungal populations. Reductions of microbial population at different drying air temperatures were not consistent and did not show any definite trend.

In coastal areas of Andhra Pradesh, untimely rains and cyclonic storms soak paddy at the time of harvesting and threshing and cause serious losses. Such paddy gets spoiled due to sprouting, mould growth, etc., as sun-drying becomes impossible in the prevailing inclement weather. Under such circumstances, the rain soaked paddy has to be dried immediately within 24 hr to prevent spoilage¹. The improved technique of drying of wet paddy by mechanical drying system acts as an efficient tool in salvaging the damaged paddy which otherwise could not be milled as raw paddy². Therefore, to study the effect of drying on bacterial and fungal population (responsible for spoilage) of wet paddy, a batch type dryer of 1 ton capacity was used at different drying air temperatures.

The wet paddy variety 'IET 4786' caught under cyclonic rain was used for the present study. The wet paddy was

received from 7 different fields and the condition of the paddy was not uniform, since it was exposed to rain in different situations resulting in varying degrees of discolouration, mould growth and other foreign material. The paddy received from each fields was treated as a batch and the 7 batches of paddy were dried at air temperatures of 45, 47, 48, 50, 51, 52 and 55°C for 60 to 90 min so that the moisture level of the paddy reached a safe level of 12-14 per cent. The quantity of wet paddy used for drying of each batch was 0.5 ton. the moisture content of the dried and undried paddy was recorded using Aquatronic grain moisture meter. Four kg each of undried sample (kept aside at the start of drying) and dried sample at the end of drying were taken from each batch and stored in glass jars of 5 kg capacity under laboratory conditions for one month in 3 replicates. Standard dilution plate technique³ was adopted to grow the bacterial and fungal population by drawing 10g of sample at the end of storage period from each batch of stored dried and undried paddy. Nutrient agar and malt extract agar were used to isolate the bacteria and fungi respectively. The data were statistically analysed and the results are given in Table 1.

The results in Table 1 indicate that the bacterial and fungal populations were significantly minimised due to drying at all the drying air temperatures adopted in the study. The advantage of batch type dryer in reducing the spoilage due to mould growth has also been stated by Pillaiyar¹, the other advantage being less chance of over drying of the grain¹. Maximum reductions of bacterial and fungal population were observed at 52°C (45.44) per cent) and 47°C (89.01 per cent) respectively. Minimum reductions of bacterial and fungal population were observed at 47°C (8.40 per cent) and 51°C (32.44 per cent) respectively. The inconsistency of the

TABLE 1. EFFECT OF DIFFERENT DRYING AIR TEMPERATURES ON MICROBIAL POPULATION OF WET PADDY*

Batch No.	Drying air temp (°C)	Moisture after drying (%)	Decrease in no. of bacteria/g of paddy**	% reduction	Decrease in no. of fungi/g of paddy**	% reduction
1	45	13.0	0.24	42.49	0.43	63.09
2	47	14.0	0.04	8.40	0.96	89.01
3	48	14.5	0.08	15.90	0.78	83.41
4	50	12.0	0.20	36.53	0.42	62.41
5	51	12.5	0.08	18.58	0.17	32.44
6	52	13.0	0.27	45.44	0.54	70.89
7	55	14.0	0.22	39.32	0.62	75.73
S.E.			0.03		0.10	
't' value at 5%			4.70		5.71	

*Initial moisture content of paddy was 29%; ** transformed logarithm values

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variation in reduction of bacterial and fungal population with drying air temperatures is probably due to abnormal variation in the initial condition of the grain^{4,6} and duration of drying.

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DEHYDRATION OF BITTER GOURD (*MOMORDICA CHARANTIA* LINN) RINGS

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Catalase was the most heat resistant enzyme in bitter gourds. Blanching 1 cm thick rings for 2 min in boiling water inactivated all the enzymes present in rings. Blanching rings in 5% NaCl and drying them in a tray drier for 3 hr at 70°C followed by 4 hr drying at 60°C gave dark green, soft textured, slightly salty and less bitter product.

Bitter gourd is an important vegetable of summer and rainy seasons. It has typical bitter taste and many medicinal values have been attributed to it. Bitter gourd is said to tone-up liver and spleen, useful for diabetic people, diuretic and vermifugal and improve digestion. It is said to be a good vegetable for patients suffering from ascites, gout and pain in the joints. Because of these attributes and its special culinary taste, bitter gourd has good demand. However, it is available only during harvesting season due to its perishable nature. Drying and canning are two methods employed to preserve it¹. Dried product is preferred because of advantages like reduced mass and lower packaging requirements. It is noted that during drying bitter gourds develop rubbery texture and become brown in addition to losing some of its bitterness. Therefore, this study was undertaken to develop a suitable process of dehydration of bitter gourd slices.

Good quality raw bitter gourds of uniformly medium size and dark green colour are washed well in water, sorted and sliced transversely into 1 cm thick rings with sharp stainless steel knife. They are found to contain catalase and peroxidase. The index enzyme for blanching was established by heating rings for various periods of 0 to 3 min in boiling water and testing them for the presence of the above two enzymes. Complete inactivation of catalase took 2 min as against 1.5 min for peroxidase. Therefore, the blanching process was designed to destroy catalase. Slices are blanched in boiling water for 2 min and dipped in 0.2 per cent potassium metabisulphite (KMS) solution for 10 min at room temperature¹. But, according to Luh *et al.*² green vegetable like cabbage have higher pH and should be sprayed after blanching with 0.2-0.7 per cent solution of sulphites. Therefore, blanched samples are dipped in sulphite solution (1 per cent sodium

sulphite + 1 per cent sodium bisulphite) similarly. A common practice followed at home for reducing bitterness is to apply common salt lightly on the scrapped surface of bitter gourd, keep for sometime and to remove excess salt from the surface by washing in water. Therefore, bitter gourds are given salt treatment in two ways. They are blanched by boiling in 5 per cent sodium chloride solution or their surfaces are scrapped lightly with knife, smeared with common salt, kept at room temperature for 30 min, washed and blanched in boiling water for 2 min. Unblanched or water blanched and undipped slices are used as control. Treated samples are spread on perforated aluminium trays (tray loading: Ca. 6 kg/m²) and dried in a cabinet drier at 70°C for the first 3 hr followed by 4 hr drying at 60°C¹. Dehydrated slices are packed in polyethylene packets sealed and stored in desiccators.

Moisture content is determined by air-oven drying method³. Shrinkage ratio (SR), dehydration ratio (DR), rehydration ratio (RR) and extent of non-enzymatic browning (NEB) were determined according to the methods outlined by Ranganna³. Total chlorophyll and pheophytin contents were estimated by the method of Vernon⁴ using 80 per cent acetone as solvent. Presence of catalase and peroxidase was tested using 3 per cent H₂O₂ and 1 per cent guaiacol³, respectively. Reconstituted samples of bitter gourd are subjected to descriptive sensory analysis for colour, taste and texture by a panel of 5 trained panelists drawn from the laboratory^{3,5}.

Bitter gourd contained 2.8 per cent total soluble solids. Their moisture content (83 per cent) is higher than that reported by Kalra *et al.*¹ Activity of catalase and peroxidase is higher in the outer scales and seeds. Thermal resistance of catalase is greater and therefore, it is used as an index enzyme for the blanching process. Two minutes blanching of 1 cm thick rings in boiling water is found to be adequate for catalase inactivation. The rings are dried to about 5 per cent moisture during two stage dehydration. All the dehydrated samples except the unblanched ones are free from catalase and peroxidase. SR of the salt-smeared samples is the highest due to loss of material through scrapings. DR (7.5-10.0) are close to the reported values of 8.2-10.0¹ but RR values of 5.0-5.8 are lower than the reported values of 6.2-8.1¹ (Table 1). Ratio of RR to DR is used as an index of reconstitutability. Though this ratio does not differ significantly among the samples, its value is highest (0.66) for the sulphite-dipped and also brine-blanched samples (Table 1) which indicates their superior rehydration characteristics. Discolouration in dried product may be caused by changes in sugars during drying and/or due to formation of

TABLE 1. QUALITY CHARACTERISTICS OF DEHYDRATED BITTER GOURD RINGS

Pretreatment ¹	SR	DR	RR	Reconstitutability (RR/DR)	NEB (OD) at 440 nm	Total chlorophylls ⁴ (mg/100 g solids)	Total pheophytins ⁴ (mg/100 g solids)	Sensory qualities
No blanching	11.3	10.0	5.8	0.58	1.00	13.745	2.212	Pale green, bitter and leathery
Water blanched	9.4	8.0	5.2	0.65	0.82	19.105	11.708	Brownish, bitter and leathery
Water blanched, KMS dipped ²	9.4	8.0	5.2	0.65	0.80	19.467	4.955	Pale green, bitter and leathery
Water blanched, sulphite dipped ²	8.9	7.5	5.0	0.66	0.49	23.787	8.455	Dark green, bitter and slightly leathery
Salt treated, water blanched ³	11.4	8.7	5.2	0.59	0.79	11.527	3.796	Brownish, less bitter and soft
Brine blanched	8.7	7.7	5.1	0.66	0.60	24.579	10.498	Dark green, less bitter, salty and soft

¹ 2 min blanching in boiling media; ² Dipped in 0.2% KMS or 1% NaHSO₃ + 1% Na₂SO₃ solution for 10 min at room temperature; ³ Bitter gourd surface scrapped, smeared with salt for 30 min, washed and blanched; ⁴ Total chlorophyll and total pheophytin contents (mg/100 g solids) of raw samples 28.68 and 2.90; NEB: Extent of non-enzymatic browning; SR: Shrinkage ratio; DR: Dehydration ratio; RR: Rehydration ratio.

pheophytin from chlorophylls. Therefore, NEB, total chlorophylls and total pheophytins are determined to objectively express the visual colour of dehydrated bitter gourd samples. Though pheophytin content of control samples is minimum, its chlorophyll content is also low and NEB is high (Table 1). The visual rating for its colour is pale green and texture is leathery. The product is rated poor even though it has retained its bitterness. KMS dipping has been reported¹ to give a good dehydrated product. But in this study, it is found to yield a pale green and leathery product and its NEB is higher (0.80). Green vegetables like cabbage, etc., have higher pH and sulphite solution has been reported to be more effective than KMS in checking browning during dehydration^{2,6}. Rahman and Buckle⁷ reported greater retention of chlorophylls during 12 months storage in capsicums dipped in sulphite solution before freezing. In this study also, sulphite-dipping improved the colour, reduced NEB but the texture is still not liked. Salt-smearing is found to improve the texture but it reduced bitterness and induced browning. Among the five treatments tried in this study, brine-blanching is found to be the best at it gives a dark green

product of soft texture although it reduced bitterness and imparted saltish taste.

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PRESERVATION OF RAW MANGO SLICES (VAR. NEELUM) FOR USE IN PICKLE AND CHUTNEY

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Steeping preservation in a chemical solution containing 5% salt, 1.2% acetic acid and 0.1% KMS was found to be the best followed by 3% salt, 0.8% acetic acid and 0.2% KMS than by dry salting. Addition of 0.5% calcium chloride helped to retain texture of slices during subsequent storage. Dry salting showed more softening and browning of slices.

Mango occupies about 942.56 thousand hectares of land yielding 8214.51 thousand tonnes of fruits annually. Both young and unripe fruits, because of their acidic taste, are utilized for culinary purposes as well as for preparing pickles, chutneys and amchur^{1,2}. About 2000 tonnes of mango slices in brine are exported annually mainly to European countries and about 7000 to 8000 tonnes of pickles and chutneys mainly to U.K, Canada, U.S.A. and Germany.

Raw mango slices during the season are preserved with salt for processing during off-season and for export. Johar and Anand³ studied the microbiology of brined slices and recommended storage in 15 per cent brine. An improved method has been suggested by Anand and Johar⁴ employing 10 per cent brine containing 200 p.p.m. sulphur dioxide for primary salting for 20 hr followed by five per cent powdered salt with 200 p.p.m. sulphur dioxide for storage and finally, filling the empty space in the barrel with 5 per cent brine solution containing 200 p.p.m. SO₂. Effect of dry salting on chemical composition and texture of green mango slices has been studied by Das and Bose⁵. The present study was conducted to find out a suitable method for preserving raw mango slices for use in pickles and chutneys.

Fresh raw mangoes (var. 'Neelum') was obtained from the orchard of the Division of Fruit and Horticultural Technology, IARI, New Delhi. Fruits were washed with water thoroughly, peeled with stainless steel knife and cut into longitudinal pieces of 1.5 cm average thickness. Slices were preserved both in acidified brine solutions and by dry salting as such and together with potassium metabisulphite. Slices were packed in glass jars and stored at room temperature (30 ± 5°C).

Soluble solids (°Brix) content was determined with a hand refractometer and the values corrected to 20°C. Acidity, pH and sugars were determined by the standard methods of

A.O.A.C.⁶ Ascorbic acid was determined by the titrimetric method⁷. Analysis for sulphur dioxide and NEB were carried out by the methods of Ranganna⁸. Sensory evaluation was carried out by a panel of six judges using a 9 point Hedonic scale⁹. Total microbial counts were estimated by Seeley and van Demark¹⁰.

Data presented in Table 1 show that during 10 months storage of raw mango slices, there was decrease in total soluble solids (T.S.S.) and SO₂ retention, lowering of acidity, pH and microbial load in all the treatments. Stored slices retained 5.0 to 6.0 per cent T.S.S., while acidity and pH ranged from 1.19 to 1.55 per cent and 2.20 to 2.65, respectively. Sulphur dioxide retention was 61.5 to 73.6 per cent in different treatments. In the steeping solutions different concentration of potassium meta bisulphite (KMS) (0.1 to 0.2 per cent), salt (2.5 to 5.0 per cent) and acid (0.6 to 1.2 per cent) did not show marked effect on non-enzymatic browning (NEB) during storage which ranged between 0.1500 and 0.2300 as O.D. Microbial counts/g decreased tremendously during storage.

It was observed that a quality of raw mango slices was better when preserved with 10 per cent salt and 0.1 per cent KMS as compared to 15 per cent salt with 0.1 per cent KMS with regard to non-enzymatic browning, microbial load and organoleptic quality (Table 2), while the latter treatment showed better retention of vitamin C during 5 months storage. Although dry salting of slices showed better retention of nutrients, it showed more non-enzymatic browning and softening as well as higher microbial load as compared to slices when preserved in acidified brine solutions by steeping preservation technique (Tables 1 and 2).

Effect of calcium chloride on the texture of preserved slices was also studied during steeping preservation of raw mango slices (Table 3.) By organoleptic evaluations, it was observed that by addition of 0.5 per cent calcium chloride to the acidified brine, texture of slices could be improved to some extent. There was slight increase in T.S.S and acidity and lowering of pH during 3 months storage at room temperature. Apparently, addition of calcium chloride did not affect colour and flavour of the preserved material. It was observed that the slices could be preserved safely upto one year in an acidified chemical solution with good retention of colour, flavour and texture. A steeping solution containing 5 per cent salt, 1.2 per cent acetic acid 0.1 per cent KMS and 0.5 per cent CaCl₂ was found to be the best followed by 3 per cent salt, 0.8 per cent acetic acid, 0.2 per cent KMS and 0.5 per cent CaCl₂ (Table 1). Dry salting was not good with regard to colour and texture although retention of nutrients was slightly better as compared to steeping preservation. Overall quality of the preserved product was very good by steeping preservation. This technique can be used successfully

TABLE 1. PHYSICO-CHEMICAL CHANGES IN RAW MANGO SLICES PRESERVED BY VARIOUS CHEMICAL SOLUTIONS AT ROOM TEMPERATURE

Treatments			Storage period (months)	°Brix (20°C)		SO ₂ (ppm)		Acidity (% as CA, W/W)		Microbial load (millions/g)		Organoleptic quality (overall scores)	
Salt (%)	Acetic acid (%)	KMS (%)		Slices	Brine	Slices	Brine	Slices	Brine	Slices	Brine	Slices	Brine
2.5	0.6	0.2	0	6.81	—	—	1000	1.84	—	29.22	—	Colour, flavour texture good (10)	White, transparent (10)
			10	5.00	4.90	620.12	566.84	1.10	1.10	2.09	3.15	Colour off white, flavour slight acceptable, texture good (6)	Light yellow, slight cloudy (5)
3.0	0.8	0.2	0	7.32	—	—	1000	3.16	—	66.80	—	Colour, flavour, texture good (10)	White, transparent (10)
			10	5.40	5.38	615	598.20	1.39	1.40	1.98	3.30	Colour off white, flavour, texture good (7)	Light yellow, transparent (6)
5.0	1.2	0.1	0	6.81	—	—	500	1.84	—	29.22	—	Colour, flavour texture good (10)	White, transparent (10)
			10	6.02	6.00	368	302.20	1.55	0.94	1.28	3.08	Colour white, flavour texture very good (8)	Light yellow, transparent (6)

Values are the average of 2 determinations for both the replications

TABLE 2. EFFECT OF DRY SALTING ON PHYSIO-CHEMICAL CHANGES IN CURED RAW MANGO SLICES AT ROOM TEMPERATURE

Treatments		Storage period (months)	Vitamin C (mg/100 g)	Microbial load (million/g)	Non-enzymatic browning (O.D)	Organoleptic quality (overall scores)
Salt (%)	KMS (%)					
10	0.1	0	13.89	66.8	0.0862	Colour, flavour and taste good (10)
		5	4.00	4.12	0.3010	Colour white, flavour and texture good (7)
		0	10.89	10.4	0.0862	Colour, flavour and taste good (10)
15	0.1	5	3.26	5.62	0.2926	Colour off white, flavour acceptable texture soft. (6)

Values are the average of 2 determinations for both the replications.

for preservation of mango slices in processing industry for indigenous and export market. The preserved slices were used directly after taking out from the brine for the preparation of pickles and chutneys after adding rest of the materials like

salt, acid, various spices, oil, etc. Those cured slices can also be dried or dehydrated for further storage for the preparation of various products like powder, amchur, chutney, pickles, ketchup, sauces, curries and spiced beverage preparations.

TABLE 3. EFFECT OF CALCIUM CHLORIDE IN PRESERVATION OF RAW MANGO PEELED SLICES DURING 3 MONTHS STORAGE AT ROOM TEMPERATURE

Salt (%)	Treatments			Storage period (months)	Organoleptic quality (overall scores)	
	Acetic acid (%)	KMS (%)	CaCl ₂ (%)		Slice	Brine
3	0.8	0.2	0.5	0	Colour, flavour and taste good (10)	White, transparent (10)
				3	Colour off white, texture very good, flavour good (9)	V. light yellow, transparent (7)
3	0.8	0.2	—	0	Colour, flavour and taste good (10)	White, transparent (10)
				3	Colour off white, texture and flavour good (8)	V. light yellow, transparent (7)

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**CHEMICAL QUALITY OF SOME MARKETED
INDIGENOUS MILK PRODUCTS.
I. MAJOR CONSTITUENTS AND MINERAL
COMPOSITION OF PANEER**

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Analysis of samples of paneer collected from shops in Karnal City market showed wide variations in moisture and fat. The ash content also varied. The average moisture content ranged between 56.2 and 61.4%. The fat content ranged from 18.30 to 22.7%. Overall value of 21.4% fat in samples on dry basis was just above Prevention of Food Adulteration Act's limit of 50% on dry matter basis, while samples from some shops did not correspond to the legal minimum limit. Shop to shop variation for pH values was also observed.

According to prevention of Food Adulteration Rules¹ 1983, paneer means a product obtained from cow's or buffalo milk or a combination thereof by precipitation with sour milk, lactic acid or citric acid. The fat content shall not be less than 50 per cent of the dry matter. The published information on mineral composition of market or laboratory paneer samples is not available. Therefore, chemical composition of paneer was studied to assess the quality of paneer sold commercially.

Market samples of paneer were collected in pre-sterilized polyethylene bags from five different shops of Karnal market. The samples were transported in a ice-box and stored at 5°C

until analysed. Moisture and total solid contents were determined as per the procedure described² with minor modification which involved addition of 5 ml of hot distilled water to break the curd particles and disperse it uniformly. Fat was estimated by the Gerber test³. The protein content of samples was determined by micro-kjeldahl method⁴. Lactose was calculated by difference. Ash content was estimated according to the method described for chhana⁵. Calcium content was estimated from the ash solution⁴. Phosphorus content was estimated colorimetrically by the method of Fiske and Subba Row⁶. Citrate was determined by modified colorimetric method of White and Davies⁷. Chloride was estimated by the semi-micro mercurimetric titration method of Roy and Yadav⁸ with minor modification. Sodium and potassium contents were determined from ash solution in a systronic digital flame photometer⁹ (with sodium and potassium filter) Model-121 at a constant air pressure of 0.5 kg/cm² using regulated indane gas as a source of fuel. Magnesium, copper, iron and zinc in ash solution were estimated with the help of Pye-Unichem SP-191 atomic absorption spectrophotometer¹⁰.

pH of paneer samples (immediately after sampling) was measured by the method as devised by Kosikowski¹¹ for soft cheese.

The results pertaining to the major milk constituents and pH of paneer from different shops are shown in Table 1. Moisture content of paneer ranged from 56.2 to 61.4 per cent with an overall value being 57.9 per cent for different shops. These values were considerably higher than those (51.43 per cent) for market samples of paneer from Karnal and Delhi cities as reported by Rajorhia *et al*¹².

TABLE 1. MAJOR MILK CONSTITUENTS OF MARKET PANEER

Shops	Moisture (%)	Total solids (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	pH
1	57.3 ± 0.8	42.7 ± 0.8	21.8 ± 0.7 (51.1)	17.0 ± 0.5 (39.5)	2.2 ± 0.1 (5.2)	1.6 ± 0.0 (3.8)	5.5 ± 0.1
2	56.2 ± 0.8	43.8 ± 0.8	22.4 ± 0.9 (50.9)	17.5 ± 0.2 (40.1)	2.3 ± 0.1 (5.2)	1.6 ± 0.0 (3.7)	5.6 ± 0.1
3	56.8 ± 1.2	43.2 ± 1.2	22.7 ± 0.9 (52.6)	16.2 ± 0.4 (37.6)	2.4 ± 0.1 (5.6)	1.8 ± 0.1 (4.2)	5.5 ± 0.1
4	61.4 ± 1.5	38.6 ± 1.5	18.3 ± 1.3 (47.0)	16.0 ± 0.6 (41.8)	2.2 ± 0.2 (5.6)	1.8 ± 0.1 (4.7)	5.9 ± 0.1
5	57.7 ± 1.0	42.3 ± 1.0	21.6 ± 1.0 (51.1)	17.1 ± 0.4 (40.5)	2.1 ± 0.2 (5.1)	1.8 ± 0.1 (4.2)	5.8 ± 0.1
Overall	57.9 ± 1.1	42.1 ± 1.1	21.4 ± 1.0 (50.5)	16.8 ± 0.4 (39.9)	2.3 ± 0.1 (5.4)	1.7 ± 0.1 (4.1)	5.7 ± 0.1

Figures in parenthesis indicate value on dry matter basis. Mean of eight samples. Mean ± S.E.

TABLE 2. MINERAL COMPOSITION (MG PER 100 G) OF MARKET PANEER

Shops	Cal	Mag	Phos	Citrate	Sod.	Pot.	Chlo.	Cu	Iron	Zinc
1	467.5 ± 19.6 (1092.1)	25.1 ± 2.5 (52.2)	344.6 ± 12.1 (809.3)	122.7 ± 6.2 (287.9)	31.1 ± 5.0 (73.1)	57.8 ± 4.6 (134.7)	51.4 ± 1.5 (120.7)	0.4 ± 0.1 (0.8)	4.4 ± 0.6 (10.4)	3.0 ± 0.1 (7.1)
2	422.5 ± 17.4 (964.6)	27.1 ± 2.1 (61.8)	349.4 ± 15.8 (800.7)	149.8 ± 11.1 (330.4)	35.7 ± 4.2 (82.6)	61.3 ± 4.3 (145.4)	53.8 ± 2.7 (122.9)	0.9 ± 0.1 (2.2)	2.7 ± 0.6 (6.1)	3.1 ± 0.1 (6.9)
3	450.00 ± 17.2 (1042.4)	27.2 ± 1.4 (63.6)	349.4 ± 16.7 (811.1)	122.7 ± 11.0 (269.7)	28.9 ± 5.6 (65.9)	51.6 ± 7.7 (118.8)	47.2 ± 5.4 (97.7)	0.6 ± 0.1 (1.4)	3.4 ± 0.6 (7.9)	2.9 ± 0.1 (6.8)
4	412.5 ± 16.1 (1068.6)	26.3 ± 1.9 (68.7)	330.6 ± 17.0 (856.1)	100.7 ± 11.1 (259.9)	32.7 ± 5.1 (77.7)	38.8 ± 3.5 (100.5)	44.3 ± 1.8 (121.8)	0.35 ± 0.1 (0.9)	5.1 ± 0.8 (13.3)	3.1 ± 0.1 (8.3)
5	475.0 ± 11.2 (1123.9)	29.1 ± 1.7 (69.7)	347.5 ± 24.1 (820.9)	122.3 ± 9.1 (288.4)	34.3 ± 3.6 (80.7)	57.2 ± 5.4 (135.3)	55.6 ± 2.62 (131.4)	0.3 ± 0.0 (0.7)	2.9 ± 0.3 (6.9)	3.1 ± 0.1 (7.4)
Overall	445.0 ± 9.7 (1058.8)	27.0 ± 1.3 (65.2)	344.3 ± 18.7 (819.6)	123.7 ± 10.0 (287.3)	32.5 ± 5.1 (76.0)	53.7 ± 3.5 (126.7)	40.5 ± 3.2 (118.9)	0.5 ± 0.1 (1.2)	3.7 ± 0.4 (8.8)	3.0 ± 0.1 (7.3)

Figures in parentheses indicate values on dry matter basis.
Values are mean of 8 determinations, Mean ± S.E.

The PFA standards for paneer describe a minimum of 50 per cent fat on dry matter basis. Overall value of 21.4 per cent fat in paneer samples of present investigation on dry matter basis was just above PFA limit of 50 per cent. A wide variation in fat content (18.3 to 22.7 per cent) among shops was noticed, while samples from some shops did not correspond to the legal minimum limit of fat for whole milk paneer. This could be because of use of low fat or partially skimmed milk for the manufacture of paneer.

There were not much variations in protein and lactose contents of market paneer either among shops or samples. Similar values for protein (17.67 per cent) and lactose (2.07 per cent) were also reported by Rajorhia *et al*¹².

The ash content of market samples of paneer ranged from 1.6 to 1.8 per cent among shops, overall values being 1.7 per cent. However, in market situation, milk from variable sources and of different qualities, status of salts in milk, losses in whey, extent of lowering of pH of coagulation, pressing and chilled water treatment may have resulted the variations in ash contents.

The pH of paneer samples from different shops ranged considerably from 5.5 to 5.8; pH values show that the method of coagulation vary from shop to shop. The data on calcium, magnesium, phosphorus, citrate, sodium, potassium, chloride, copper, iron and zinc of market samples of paneer are given in Table 2.

Calcium content of paneer varied from 412.5 to 475.0 mg per 100 g among shops. Similarly, citrate content also showed wide variation from 100.7 to 149.8 mg per 100 g of paneer. Highly noticeable differences in copper and iron contents (0.3 to 0.9 mg and 2.9 to 5.1 mg per 100 g respectively) were observed among shops. The overall values for calcium, magnesium, phosphorus, citrate, sodium, potassium, chloride, copper, iron and zinc were 445.00, 27.00, 344.30,

123.7, 32.5, 53.7, 40.5, 0.5, 3.7 and 3.0 mg per 100 g paneer respectively.

The variations in mineral composition of various samples of paneer from different shops might explain some important influencing like type and quality of milk, concentration of various minerals present in natural or contaminated forms, method of preparation, nature, type and strength of a coagulant used, temperature and pH of coagulation, types of container used during manufacture and storage.

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CHEMICAL QUALITY OF SOME MARKETED INDIGENOUS MILK PRODUCTS. II. MINERAL COMPOSITION OF KHOA

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Forty samples of khoa from five different shops of Karnal city were analysed for minerals. A noticeable difference for magnesium, phosphorus and zinc between samples and a significant variation for calcium among shops were observed. The market samples of Khoa contained (mg/100g) calcium 654.00, magnesium 66.67, phosphorus, 376.55, citrate, 517.36, sodium 182.88, potassium 368.00, chloride 331.65, copper 0.16, iron 2.43, and zinc 2.43.

The chemical make up of khoa is very complex. The processing steps of heating and stirring introduce innumerable changes in the multi-component milk system. The investigations carried out in the past were mostly to estimate major milk constituents and to set legal requirements for them. The gross chemical composition, packaging, preservation and nutritional aspects of khoa have been reviewed in detail¹. However, the information on mineral composition in khoa is incomplete and very limited. To supplement the available information on some minerals in khoa, the market study was conducted.

Forty market samples of khoa were collected in pre-sterilised polyethylene bags from five different shops of Karnal city. The samples were transported in an ice-box and stored at 5°C until analysed. A representative sample from whole cross section of the product was taken. The portion was then passed through a fine mesh stainless steel grater and ground in a mortar. The ground product was placed in a sample container with lid and was used for chemical analysis.

The ash content in khoa samples was determined according to the method used for chhana². The ash as obtained was dissolved in 10 ml acid (1:1 mixture of HNO₃ and HCl) and transferred into 50 ml volumetric flask. The volume was made upto the mark with glass distilled water. The contents were mixed well and stored in polyethylene bottles until further analysis.

Calcium content was estimated from ash solution³. Phosphorus content was estimated colorimetrically as per the method of Fiske and Subba Row⁴. Sodium and potassium contents were determined from ash solution in a systonic digital flame photometer (with sodium and potassium filters), Model 121 at air pressure of 0.5 kg/cm² with Indane gas as

a source of fuel. Magnesium, copper, iron and zinc were estimated with the help of Pye-Unichem Sp-191 atomic absorption spectrophotometer. For magnesium estimation 1 ml of ash solution was diluted to 50 ml and estimated by atomic absorption spectrophotometer.

Two way analysis of variance without interaction was carried out for the data obtained from market survey of khoa using the following statistical equation:

$$V_{ij} = \mu + S_i + t_j + e_{ij}$$

Where,

V_{ij} = various milk minerals of khoa for j the sample belonging to its shop ($i=1,2,\dots,5\dots j+1,2,\dots,8$),

μ = general mean;

S_i = Effect of i th shop., t_j =Effect of j th sample and

e_{ij} = Random error component assumed to follow normal distribution with zero mean and constant variance $6e^2$.

Major constituents: The overall values for moisture, total solids, fat, protein, lactose were 41.14, 58.86, 19.35, 14.29 and 25.54 per cent respectively. The ash contents of market khoa varied from 2.59 to 3.04 per cent between samples and from 2.75 to 2.89 per cent between shops. Several workers⁵⁻⁶ have reported variable results for ash contents ranging from 2.98 to 3.7 per cent in khoa. The type and quality of milk used, extent of dehydration and presence of adulterant would cause such disparity.

The mean and overall concentration of various minerals of market samples of khoa from different shops are given in Table 1. The average values for calcium, magnesium phosphorus, citrate, sodium, potassium and chloride were found to range from 612.50 to 717.50, 64.99 to 68.09, 362.50 395.00, 504.06 to 551.56, 171.54 to 190.01, 373.89 to 384.01, 324.47 to 344.40 mg per 100 g respectively whereas the concentration of trace elements, viz. copper, iron and zinc varied from 0.13 to 0.19, 1.96 to 3.02 and 3.36 to 4.00 mg per 100 g respectively.

Report of Narain and Singh⁷ has shown enormous variations (429.50 to 1000 mg/100 g) in the calcium content of market khoa. Similarly, wide variations have also been reported for phosphorus content (335.66 to 490.0 mg/100 g) in samples from market⁸.

The iron content of market khoa was in close agreement with those (3.19 mg/100 g⁷) but the same was lower as compared with contents (9.42 mg/100 g⁹). However, all these values in market khoa were significantly higher than khoa samples prepared in stainless steel containers. This might probably be due to longer contact of milk with surface of iron Karahi usually used by the khoa manufacturers and metallic contaminated milk used.

The data for other minerals are not available in published literature. The variations observed in the values of different

TABLE 1. MINERAL COMPOSITION (MG/100 G) OF MARKET KHOA*

Minerals	Shops					Overall
	1	2	3	4	5	
Ash	2.76 ± 0.06 (4.58)	2.87 ± 0.05 (5.00)	2.75 ± 0.06 (4.55)	2.76 ± 0.05 (4.84)	2.89 ± 0.08 (4.96)	2.81 ± 0.05 (4.78)
Calcium	630.00 ± 25.07 (1031.77)	642.50 ± 24.62 (1138.36)	667.50 ± 13.59 (1103.93)	717.50 ± 19.06 (1259.66)	612.50 ± 18.87 (1049.88)	654.00 ± 20.24 (1112.72)
Magnesium	68.69 ± 2.24 (112.44)	64.99 ± 2.14 (113.26)	66.36 ± 2.17 (109.62)	66.11 ± 2.58 (116.03)	67.23 ± 2.29 (117.38)	66.67 ± 1.64 (113.75)
Phosphorus	384.38 ± 18.64 (629.76)	362.50 ± 15.64 (631.76)	395.00 ± 21.44 (651.36)	377.13 ± 18.99 (661.42)	363.75 ± 15.55 (602.92)	376.55 ± 14.93 (635.45)
Citrate	551.56 ± 26.77 (905.97)	517.13 ± 15.98 (898.97)	504.69 ± 22.02 (834.93)	509.38 ± 19.00 (894.90)	504.06 ± 15.62 (894.49)	517.36 ± 20.21 (879.85)
Sodium	171.54 ± 13.13 (281.78)	190.01 ± 7.86 (326.61)	181.07 ± 11.12 (299.53)	184.29 ± 7.41 (324.39)	187.50 ± 10.12 (321.38)	182.88 ± 10.25 (310.74)
Potassium	343.89 ± 12.55 (563.25)	381.92 ± 12.98 (664.23)	370.87 ± 19.99 (613.38)	359.97 ± 10.89 (632.68)	384.01 ± 12.10 (658.06)	368.00 ± 13.32 (626.32)
Chloride	332.26 ± 13.57 (564.87)	344.40 ± 12.09 (599.50)	330.98 ± 11.36 (546.93)	326.14 ± 9.25 (549.93)	324.47 ± 10.00 (555.63)	331.65 ± 11.45 (563.37)
Copper	0.19 ± 0.02 (0.31)	0.17 ± 0.02 (0.29)	0.15 ± 0.02 (0.20)	0.13 ± 0.01 (0.23)	0.16 ± 0.02 (0.27)	0.16 ± 0.02 (0.27)
Iron	3.02 ± 0.66 (5.00)	1.96 ± 0.15 (3.41)	2.14 ± 0.29 (3.60)	2.26 ± 0.33 (3.93)	2.75 ± 0.31 (4.70)	2.43 ± 0.34 (4.14)
Zinc	3.97 ± 0.30 (6.53)	4.01 ± 0.15 (7.17)	3.45 ± 0.27 (5.73)	3.36 ± 0.28 (5.90)	3.61 ± 0.26 (6.17)	3.68 ± 0.21 (6.30)

Figures in parentheses indicate values on dry matter basis.

*Mean of eight samples; values are Mean ± S.E.

minerals may be ascribed to the type and quality of milk, extent of desiccation, method of preparation and storage of khoa samples.

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FREE FATTY ACID CONTENTS AND OFF-FLAVOUR IN COMMERCIAL CANNED MUTTON CURRY

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Nine commercial samples of canned mutton curry exhibiting commercial sterility but possessing putrid smell showed very high levels of total free fatty acids (FFA) comprising mainly of palmitic, stearic and oleic acids. Laboratory studies have established that levels more than 2000 mg/100 g lipid are generally seen when substandard meats are used for processing. Analysis of the above samples showed a high degree of correlation between the FFA content and off-flavour.

Previous studies from this laboratory have shown that free fatty acids (FFA) as reliable indicators for detecting spoiling or spoiled meat used for canning^{1,5}. To validate the above observations certain commercial samples possessing such defects obtained through quality control agencies were analysed for their FFA contents.

Nine samples of commercially canned mutton curry (a-i) manufactured in 1986 were obtained. Of these, five were canned curried chunks (a-e) and four were canned curried keema (f-i). The cans had been left exposed to all vagaries of tropical climate. Ten g of uniformly mixed sample was taken for determining the standard plate count (SPC) and coliforms as per standard methods for canned meat analysis⁶. A panel of five judges examined the product for its appearance, colour and odour.

Lipids were extracted from 90 g curry using chloroform and methanol mixture⁷ and quantified. From the chloroform solutions of the lipids, free fatty acids were separated on TLC. For qualitative detection of FFA, 25 μ l of the chloroform solution containing 4-5 mg lipid was used. For quantification, preparative chromatography was employed. The distinctly resolved FFA band was eluted from the plate and quantified by gravimetry. For comparison, lipids were also extracted from canned curried meat samples prepared under laboratory conditions using fresh meat with a SPC less than 10^6 organisms/g (sample j); from spoiling meat with a SPC around 10^8 organisms/g (sample k) and from spoiled meat with SPC more than 10^9 organisms/g (sample l). About 5 mg of FFA obtained from each sample was esterified with boron trifluoride-methanol⁸ and the resultant methyl esters were resolved on a $10' \times \frac{1''}{8}$ O.D S.S. column packed with 'Famex' and operated at 200°C on a CIC (Chromatography Instruments Company, Baroda, India) gas chromatograph

equipped with flame ionisation detector. The compounds were identified and quantified with the help of authenticals also separated under the same conditions (nitrogen flow 45 ml/min, hydrogen flow 30 ml/min).

Table I gives data on the sensory qualities of the product, microbiological quality and FFA content (as per centage lipid) in commercial samples (a-i) and in laboratory prepared sample (j,k and l). All the commercial samples were judged as giving putrid smell to varying degrees, though in six samples the colour and appearance of the contents were not altered significantly. From our laboratory studies, it has become evident that the putrid smell becomes detectable when the initial counts on the meat were more than 10^8 organisms/g as seen in sample k and i. In one of the commercial samples (sample b) total disintegration of the muscle had taken place. Microbiological examination of the contents showed no evidence of any living organisms and the cans were found to be commercially sterile which indicated the adequacy of thermal processing. Lipid content in the commercial samples of chunks was about 2-4 per cent, in minced meat preparation it was 4-7 per cent. The pattern of FFA bands (R_f 0.41 \pm 0.1) was similar to those obtained in laboratory samples. A distinct increase in the width of FFA band (R_f 0.41 \pm 0.1) was noticeable in all the commercial samples compared to the control. The FFA content in the commercial sample (a-i) showed wide variation ranging from 1908 to 15830 mg/100g lipid. In the laboratory samples prepared from fresh meat, the FFA level has been always less than 1700 mg whereas canned samples prepared from spoiling and spoiled meat, it was more than 2500 mg going up to 8000 mg. Analysis of a few commercial samples of canned meat curry which were of acceptable quality possessing no off-odours revealed that the FFA content was around 1600 mg/100 g lipid. Based on the analysis of a large number of samples prepared under laboratory conditions, a tentative level of 2000 \pm 200 mg has been recommended. The above results show that in all the commercial samples possessing such flavour defects FFA content was higher than the lower limit (1800 mg) suggested. Seven out of nine samples had FFA content ranging from 2300 to 15830 mg and two samples were found to have 1900 - 2120 mg which were also reported to possess slight off-odours. This shows that in majority of samples, a high FFA content of more than 2200 mg is a positive indication of the use of spoiled meat for processing. However, some samples which lie in the border line (1800 - 2200 mg) can also be considered to be of doubtful quality only if they possess putrid smell. Since meat is a complicated tissue to arrive at a more reliable limit for acceptance, a large number of samples will have to be analysed and till such time the tentative limit suggested can be used as a guideline to relate the off-smell with a chemical index.

TABLE 1. APPEARANCE OF THE CONTENTS, MICROBIAL AND CHEMICAL QUALITY OF COMMERCIAL AND LABORATORY PREPARED SAMPLES OF CANNED MUTTCN CURRY

Sample description	Appearance/off-odour of the contents	Microbiological spoilage	FFA* (mg/100 lipid)
Commercial samples			
a — Chunks	Off odour, no discolouration	—ve	3562
b — „	Total discolouration, putrid foul smell	—ve	15830
c — „	Off odour, no discolouration	—ve	3541
d — „	Off odour, no discolouration	—ve	2350
e — „	„ „	—ve	3361
f — Keema (Mince)	Slight off odour	—ve	2118
g — Mince	—do—	—ve	3172
h — Mince	Slight off odour, no discolouration	—ve	1908
i — Mince	Contents frothy, slight off odour	—ve	4458
Laboratory prepared sample			
j — prepared from fresh meat mince with SPC 10^6 organisms/g	Contents normal, acceptable	—ve	1000—1700
k — prepared from spoiling meat mince with SPC 10^7 — 10^8 organisms/g	Slight discolouration, off odours	—ve	2500—4000
l — prepared from totally spoiled meat mince with SPC $> 10^9$ organisms/g	Total discolouration, foul, putrid smell	—ve	4000—8000

*Mean of two samples (90 g) drawn from each can**

**The values given for FFA refer to those obtained in several samples (more than 49 samples) prepared and analysed under laboratory conditions.

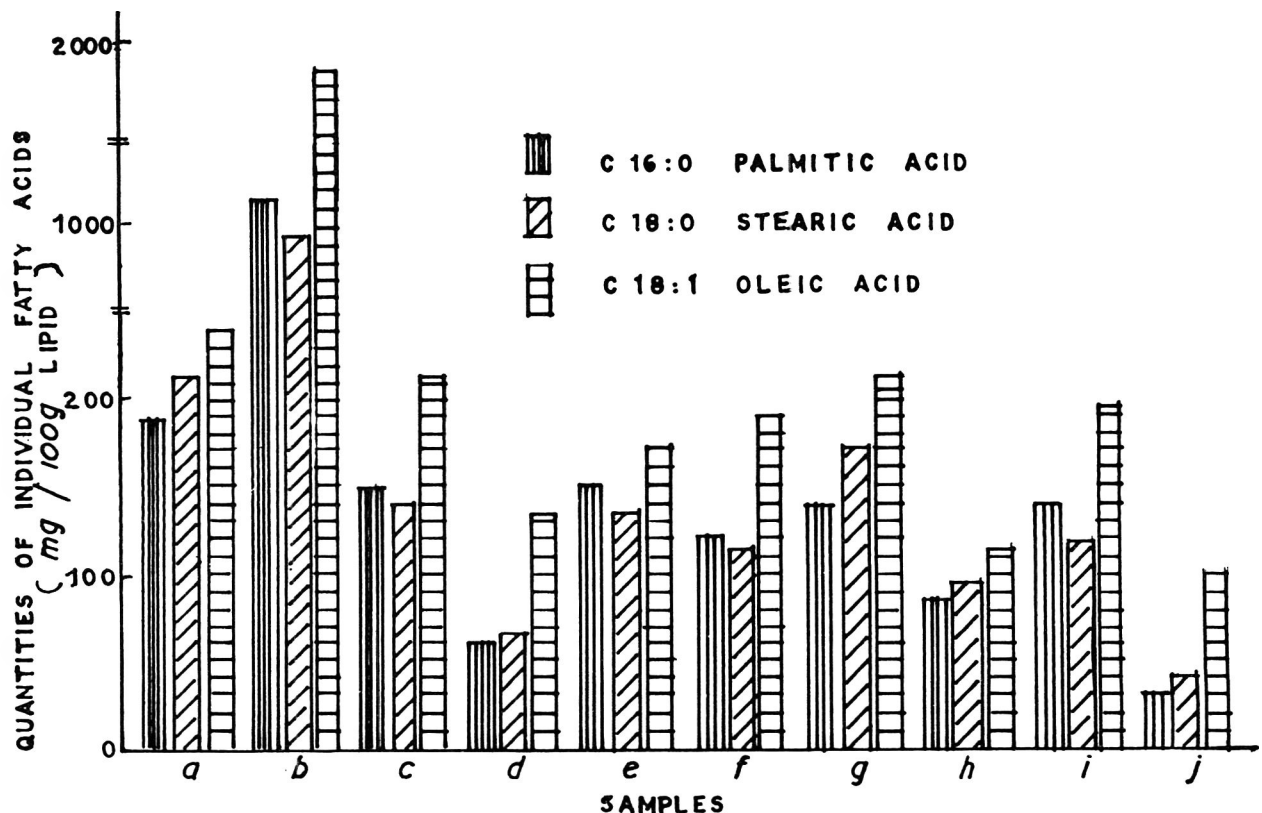


Fig. 1. Quantities of some individual fatty acids present in FFA mixture as determined by GLC analysis, a-i commercial canned curry samples. Sample j-control (Prepared in the laboratory using fresh meat).

Fig.1 represents the quantities of palmitic, stearic and oleic acids calculated from GLC analysis compared to control. In all the commercial samples possessing flavour defects, the quantities of the above three fatty acids in FFA fraction were very much higher. This shows that when FFA contents are about 1800-2200 mg, GLC technique is further useful for confirmation. From our earlier studies, it has been shown that when the initial microbial count increased from 10^6 — 10^7 to 10^8 — 10^9 the quantities of palmitic, stearic and oleic acids in FFA fraction showed 4-5 times increase in quantities and the flavour changes were distinct and reported to give putrid smell.

Therefore, it can be concluded that if the product conforms to all specifications but found to give putrid smell on opening, it can be ascribed to the use of spoiled meat for processing which can now be detected by FFA marker using the TLC and GLC techniques.

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EXTRACTION OF JUICES FROM PEACHES, PLUMS AND APRICOTS BY PECTINOLYTIC TREATMENT

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Addition of pectinolytic enzyme (pectinol) to the pulps of plum, peach and apricot increased the juice yield, causing a slight change of total soluble solids, pH and acidity, and a drastic decrease in the apparent viscosity. However, it improved the colour and clarity without affecting the flavour. Optimum enzyme concentration for addition was found to be 0.5%.

Stone fruits like Plum (*Prunus salicidna*), Peach (*Prunus persica*) and Apricot (*Prunus armeniaca*) constitute about 20 per cent of the total fruit production of the state of Himachal Pradesh¹. Out of these fruits, only free stone peaches are canned commercially. But apricot and plums are not being commercially preserved at present. These fruits can be used for beverage but extraction of juice is difficult. Pectic enzymes have been used for separating serum from pulpy fruits like banana², grapes^{3,4}, mango⁵, etc. But the information on the use of this enzyme for juice extraction from plum, peach and apricot is scanty. Experiments were, therefore, conducted to

evaluate quality characteristics of juices prepared by treating their pulps with pectinolytic enzyme. The results obtained are reported in this communication.

Fully ripe fruits of plum ('Santa-Rosa'), peach ('July-alberta') and apricot ('Newcastle' variety) were obtained from the orchard of this University. They were pulped by adding water (about 100 ml per kg of fruit), cooked for 10-15 min under 5 p.s.i. steam pressure, and passed through a pulper. Pectinol enzyme used was manufactured by M/S Triton Industries Ltd., Mysore, India. Enzyme at the rate of 0.5, 1.0, 1.5 per cent was added to the respective pulps and incubated at 40°C for 4 hr. The respective controls without any added enzymes were also kept along with samples. Treated pulps were passed through a cheese cloth and the filtrates were heated to 95°C to inactivate the added pectinases. All the juices were analysed for T.S.S., pH and titrable acidity by the methods prescribed⁶. The colour was compared in the Tintometer and relative viscosity was determined with Ostwald viscometer⁶. The sensory qualities of juices were compared on the Hedonic scale⁶ by a panel of five trained judges. Samples were presented in the coded form to the judges in the separate chambers. These were evaluated in the day light. The data from sensory analysis were analysed by F-test to determine the significant differences between treatments as per prescribed methods⁷.

Addition of enzyme even at the level of 0.5 per cent was found to increase the juice recovery in all the fruits (Table 1). The increase in juice yield was minimum for peach

TABLE 1. EFFECT OF ENZYMATIC TREATMENT ON PHYSICO-CHEMICAL CHARACTERISTICS OF JUICES FROM PLUM, PEACH AND APRICOT

Enzyme level (%)	Juice yield (%)	T.S.S. (°Brix)	Titrable acidity (% MA)	pH	Relative viscosity (flow in min)	Tintometer (colour units)		
						Yellow	Red	Blue
Plum Fruit								
No Enzyme	60.0	9.6	2.0	3.36	1.80	0.9	8.0	0.8
0.5	79.0	9.0	2.1	3.30	0.29	0.8	19.7	0.1
1.0	82.0	9.0	2.1	3.30	0.33	0.8	18.2	0.1
1.5	78.6	9.2	2.1	3.30	0.29	0.8	18.2	0.1
Peach Fruit								
No Enzyme	22.5	9.8	1.0	3.58	1.28	3.0	0.8	0.3
0.5	56.0	10.0	1.1	3.50	0.36	4.8	1.4	0.1
1.0	59.0	10.4	1.2	3.45	0.40	4.7	1.5	0.1
1.5	61.0	10.0	1.2	3.45	0.39	4.9	1.7	0.1
Apricot Fruit								
No Enzyme	52.0	13.8	1.8	3.12	1.50	14.2	6.6	0.1
0.5	87.1	14.0	1.8	3.14	0.38	13.2	6.0	0.0
1.0	88.5	14.0	1.8	3.15	0.36	13.2	5.1	0.0
1.5	85.7	14.0	1.8	3.14	0.36	13.1	6.0	0.0

TABLE 2. SENSORY SCORE ON HEDONIC SCALE OF JUICES OF DIFFERENT TREATMENTS

Treatment	Taste	Clarity & colour	Flavour
<i>Plum Juice</i>			
Without enzyme	7.0	6.0	6.2
With enzyme	6.9	7.2	6.1
<i>Peach Juice</i>			
Without enzyme	7.1	5.0	6.3
With enzyme	7.2	6.8	6.5
<i>Apricot Juice</i>			
Without enzyme	6.8	5.7	6.1
With enzyme	6.7	6.9	6.2
C.D. (P<0.05)	0.34	0.23	0.21

but even in this case increase was 50 per cent. Thus, addition of enzyme is advantageous in increasing the yield of juice considerably. However, increasing the enzyme concentration beyond 0.5 per cent did not increase the yield appreciably (Table 1). Therefore, 0.5 per cent is the optimum concentration of enzyme. The addition of enzyme did not alter the T.S.S., titrable acidity or pH (Table 1). But it decreased the relative viscosity of the juices sharply due to the enzymatic hydrolysis of pectin as has also been reported⁵ without altering their colour. Due to the improved clarity, the colour appeal of juices increased considerably. During sensory

evaluation (Table 2) the enzyme treated juices were preferred to those from the untreated pulps with respect to the colour and clarity. But no differences were observed between tastes and flavours of the treated and untreated juices, probably due to the presence of mild fruity rather than caramelised flavour in the enzyme treated juice as has been reported in the similar study on prune juice⁸.

The study showed that treatment of the pulps of plum, apricot and peach with 0.5 per cent enzyme increased juice yield without affecting their quality characteristics.

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EFFECT OF DIETARY TALLOW ON CARCASS FAT AND SERUM CHOLESTEROL PROFILE IN WHITE LEGHORN COCKRELS

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Studies carried out to assess the effect of cockrel growth rations based on deoiled rice bran (DRB) and its enrichment with graded levels of tallow (2.4 and 6%) revealed significant elevations of serum cholesterol and carcass (body) fat content at 6% level compared to other groups.

The ever increasing demand for poultry meat is one of the key factors for the rapid development of the broiler industry. These broilers grow at a faster rate hence requires diets which are rich in energy and proteins, viz. cereals, whose availability is very scanty and thus competes with the human beings. This has necessitated the usage, as well as the nutritional evaluation of diets based on agro-industrial by-products such as deoiled rice bran, supplemented with non-edible fats like tallow.

Majority of the documentary evidence suggests that saturated fat has a dominant role on the level of serum cholesterol. It also appears that there are distinct differences in the ability of the saturated fatty acids in bringing about an elevation of serum cholesterol level. Therefore, in the present work attempts have been made to study the effect of dietary tallow on carcass fat and serum cholesterol level in white leghorn cockrels.

Experiments have been carried out with 200 one day old white leghorn (WLH) male chicks obtained from the same hatch and were divided into five equal groups of 40 birds each. They were allotted randomly to five dietary regimen and reared upto the age of 12 weeks under the identical menageriel practices. The control group (T₁) was fed with a normal starter ration consisting of rice polish (R.P.) as one of its feed ingredients. The second group (T₂) was offered the same ration as that of the control, but rice polish was totally replaced (W/W) by the deoiled rice bran (DRB). The groups III (T₃), IV (T₄) and V (T₅) were given T₂ group ration supplemented with 2.4, and 6 per cent of tallow, respectively. The tallow was incorporated with a view to increase the energy density of the diet.

At the completion of the growth studies, six birds from each group were randomly selected to study the carcass fat and serum cholesterol levels. They were kept on 12 hour fast before being slaughtered. A representative sample of the thigh

muscle piece was collected to determine the carcass fat content by following the standard AOAC procedures¹. The clear serum separated from the blood was collected individually from each bird at the time of slaughter and stored at low temperatures for determining the total serum cholesterol as per the procedure described by Varley *et al.*² Statistical analysis of the data was carried out as per Snedecor and Cochran³.

The data generated from the present study have been presented in Table.1. The carcass of the birds fed with DRB (T₂) diet showed a significant (P<0.01) decrease in the amount of body fat as compared to R.P. diet (T₁), which might be due to the difference in the intake of dietary fat (1.16 against 2.61 g/bird/day). Incorporation of tallow at graded levels has resulted in a gradual increase in the body fat of the birds. However, the increase in the amount of body fat obtained with the dietary treatments T₃ and T₄ was found to be non-significant statistically when compared with either control (T₁) or (T₂) (DRB) diets, Addition of tallow at 6 per cent (T₅) level has resulted in highest deposition of body fat (12.6 per cent) which is significantly (P<0.01) different from remaining treatments. The results obtained in the present study are in accordance with those of Ramdel and Latshow⁴ and Laurin *et al.*⁵.

The total serum cholesterol (mg/100ml) was observed to be significantly higher (130.15) in the group of birds fed with rice polish (T₁) diet, when compared with those fed with DRB based (T₂) diet (114.74). Addition of tallow at 2 per cent (T₃) level has resulted in an increase of serum cholesterol level, as compared to DRB (T₂) diet, but was significantly lower (P 0.01) than that of R.P. (T₁) diet. Similarly, the incorporation of tallow at 4 (T₄) and 6 (T₅) per cent levels has resulted in the increase of serum cholesterol levels, the highest (150.00) being in T₅ group which was highly significant (P<0.01) statistically as compared to the rest of the treatment groups. Miller *et al.*⁶ reported only small differences in the serum cholesterol level when fat was added to the diet. On the contrary, March and

TABLE 1. EFFECT OF DIFFERENT DIETARY TREATMENTS ON CARCASS (BODY) FAT AND SERUM CHOLESTEROL PROFILE

Treatments	Carcass fat (%)	Total serum cholesterol (mg/100ml)
T ₁	10.12 ^b	130.15 ^c
T ₂	8.69 ^c	114.74 ^d
T ₃	9.42 ^{bc}	121.80 ^d
T ₄	10.00 ^{bc}	139.49 ^b
T ₅	12.56 ^a	150.00 ^a

Figures with the same superscript in each column do not differ significantly.

Biely⁷ and Panda *et al.*⁸ reported an increase in the level of serum cholesterol due to the addition of dietary fat. The results obtained in the present study are in agreement with the above workers.

Epidemiological studies carried out worldwide revealed a correlation between the type and amount of the dietary fat and the prevalence of coronary heart diseases (CHD). Documentary evidence suggests that the serum cholesterol level is influenced by the type of fat in the diet. Saturated fatty acids appear to be about twice as effective in elevating the serum cholesterol level as polyunsaturated fatty acids in decreasing it. Even though tallow is rich in stearic acid which is highly saturated, does not increase the level of serum cholesterol⁹. However, the results obtained in the present study are not in agreement with the above observation, since there is an increasing trend in the serum cholesterol level with the addition of dietary tallow of graded levels, which was found to be highly ($P < 0.01$) significant at 4 and 6 per cent levels. The magnitude of this influence of dietary tallow on the serum cholesterol profile and its possible role on the occurrence of CHD is yet to be determined.

The authors express their gratitude to Dr. V.M. Jhala, the then Principal, presently Vice-Chancellor, Gujarat

Agricultural University, Dr. C.H. Joshi, Director of Animal Husbandry and Dr. N.I. Ghasura, Deputy Director, Regional Poultry Breeding Farm, Makarba for providing the facilities and necessary help required for the completion of this study.

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BOOK REVIEWS

Yeast Biotechnology and Catalysis: by Hubert Verachtert and Rene De Mot (Ed.) Marcel Dekker, Inc, 270, Madison Avenue, New York, N.Y.10016, 1990; pp: XV+522; Price: \$150 (US and Canada), \$180 (all other countries).

This book, the 5th volume in a series of books published under the broad category of Bio-process Technology consists of 15 chapters dealing with recent developments and advances made in processes and products in Yeast Bio-technology. The following paragraphs give briefly the review on groups of closely related topics.

The first chapter discusses various methods of classifying yeasts based on their morphological, physiological and biochemical properties and the second is a critical review on the latest genetic engineering techniques used in the improvement of industrial yeasts.

In chapter 3, recent advances made to improve the ethanol productivity by increasing the cell density (cell recycle and cell immobilization avoiding ethanol inhibition (vacuum and membrane fermentations) improving ethanol tolerance (supply of oxygen or sterols and modification of cell membrane) and preventing the contamination (acid wash of cells and use of antibiotics) have been discussed. Chapter 4 covers the progress made on the shortening of total brewing period by immobilized yeast fermentation. Though encouraging, it has been concluded that the associated problems such as reactor design for the evolution of CO₂, prevention of contamination and maintenance of yeast performance must be solved before it can qualify for industrial use. The topic on "Yeast proteases and brewing" (chapter 6) deals with the classification of proteases with an emphasis on yeast extra-cellular proteases and their application (including immobilized forms) in the chill-proofing of beer. In this, an exciting prospect would be to genetically engineer a brewer's yeast that can secrete chill-proofing enzyme. Yeast in mixed culture fermentations such as lambic beer forms the subject matter of chapter 14. It reviews the fermented foods and beverages involving mixed cultures of yeasts and bacteria, their interactions and metabolic complexities. The biochemical features and characteristic flavours of such foods and beverages have been presented and discussed at length.

More attention is paid in chapter 5 to the most recent development in baker's yeast production as well as its application in bread making. While focussing on relevant near discoveries, it specifies some of the unsolved problems in yeast physiology. Special emphasis has been laid on metabolism of carbon, energy and nitrogen during growth of baker's yeast.

In chapter 8, attempts have been made to review the potential of yeast with regard to producing single cell oils

(SCO) as substitutes for more expensive plant oils. Aspects of using yeast lipases in improved hydrolysis of fats and inter-esterification of cheap fats into more expensive ones as well as using other micro-organisms as a means of utilizing and upgrading low value fats have been discussed. In chapter 15, harnessing the potential of yeast r-DNA technology for the production of macromolecules of human therapeutic interest has been reviewed. The standing example is the Hepatitis-B vaccine produced in yeast and is currently in market place. The chapter gives the details regarding: (a) the tools that are available for r-DNA technology work in yeast, (b) factors affecting expression levels, (c) authentic recombinant protein, (d) co-translational and post-translational modification of heterologous protein in yeast and (e) safety and regulatory issues.

A review on the biotechnological potential of yeasts for the production of single cell protein (SCP) or ethanol from starch biomass has been presented in chapter 7. It includes sections on methods of amylase estimation, extra-cellular yeast amylases, production of SCP and ethanol as well as genetic improvement of industrial yeast strains for amylolysis. Chapter 9 deals with Inulin conversion by yeasts wherein, the aspect of: (a) sources and properties of inulin (b) inulinases from yeasts and their relation to other fructan hydrolases and (c) application of these enzymes or yeast strains in the production of fructose, ethanol and biomass are considered in detail.

The tenth chapter on "Lactose metabolism by yeasts" embodies a review on current literature on various aspects of transforming lactose in whey to more valuable products such as SCP, enzymes (lactases), ethanol and oils, and to solve the problem of pollution. Chapter 12 reviews the fermentation of D-xylose and cellobiose by yeasts. Though many yeasts are known to assimilate these substrates, the number of yeasts that can ferment them is less. General metabolism and utilization of these substrates by yeast to useful products is discussed at length. A review on methylotrophic yeasts is presented in chapter 13. Utilization of methanol for production of SCP and other fine chemicals is a topic of recent origin. This has been backed up with the available information on isolation of yeasts, their cellular organization and metabolism of methanol along with different pathways.

Bio-degradation of naturally occurring aromatic compounds by yeasts have been reviewed in chapter 11. The involvement of oxygenases, lactonases, thiolases etc. in the metabolism of phenol and resorcinol by yeasts has been discussed with an emphasis on the utilization of such yeasts for industrial and environmental purposes. Bio-degradation of keratin, plasticizers etc. by yeast has also been documented in the chapter.

All the chapters in the book are well written, comprehensive, supported with several tables, figures and photographs and with extensive references (2000 citations). The book is elegantly brought out in spite of some errors: Table 2 (P.152) *Mucor* and *Cephalosporium* are not yeasts moisture content (for dry matter content, p 104, 4th para) interesting (for intestine, p 445, 1st para) etc.

The book, as claimed by the publishers, is an excellent reference for microbiologists, molecular biologists, biotechnologists, food scientists and technologists, fermentation scientists, geneticists, environmental and chemical engineers, food and agricultural chemists and biochemists.

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Biotechnology and Food Industry: Edited by J. Hollo and D. Torley, Akademiai Kiado, Pubing. House of the Hungarian Academy of Sciences H-1363, Budapest, P.O.B.24, Hungary, 1988, pp.707; price: \$ 69.

This volume is 'The proceedings of an international symposium' held in Hungary, in October 1987. There are 64 technical papers under four sections: General Topics; Genetic Engineering, Physiology; Enzymes; Microorganisms; Technological Applications. Major representation of papers are apparently from Europe. There are however, a few papers from countries like India, USA Canada, China and Zimbabwe. Under the section of 'Genetic Engineering Physiology' papers relating to induced mutations, recombinational mechanisms by protoplast fusion and gene cloning have been included. It is noteworthy that these techniques have been employed in specific instances of the genetic improvement of industrial microorganisms, such as the improvement of winery, brewery and baker's yeasts, isolation of glucamylase over-producing strains of *Aspergillus niger* and the cloning of α -amylase gene of *Bacillus subtilis*. In the section on 'Enzymes, Microorganisms' the papers deal with the basis technology of production and application of enzymes that are of particular importance to the food industry. These include lipases, amylases, proteinases, glucose isomerase, cellulases, macerases milk clotting enzyme and β -galactosidase. Many papers in this section deal with enzyme and cell immobilization. The technology application section has very interesting presentation of novel and innovative biotechnological approaches to application of enzymes and microbial processes. Some of the papers in this section contain convincing economic parameters to indicate commercial feasibilities. For ethanol production, several non-conventional substrates have been suggested. A paper describes the application of aspartame-hydrochloride (usal) which has better solubility profile than aspartame. A good number of papers deal with utilization of food industry wastes.

A unique feature of the volume under review is that most

of the papers included in it deal with very practical and down-to-earth application in biotechnology. It should therefore be of value both to the students and research scientists in the area of biotechnology. The biotechnological industries are also likely to find in these papers very useful hints and processes of potential commercial importance.

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

Chitin and Chitosan: Edited by Gudmund Skjak-Braek Elsevier Science Publishers Ltd, Crown House, Linton Road, Barking, Essex IG 11 8JU, England pp; 835; Price not mentioned.

The book is based on the proceedings of the fourth International Conference on Chitin and Chitosan held on August 22-24, 1988 at the University of Trondheim, Trondheim, Norway. The previous three International Conferences on Chitin and Chitosan were held at MIT Cambridge, USA (1977), Sapporo, Japan (1982) and Ancona, Italy (1985). It deals with the advances made in the studies on Sources, Chemistry, Biochemistry, Physical properties and Applications of Chitin and Chitosan conducted in different countries. With 6 sections and 87 original works presented by specialists, the book covers the recent understandings on chitin and chitosan in biological, technological, biotechnological and medical applications and related fields.

The first section contains 11 plenary lectures on source of chitin biomass, chitin synthesis in fungi and nematodes, biochemical cytology, structure and chemical modifications, molecular biology, derivatives of chitosan, production, commercial uses and potential applications of chitin and chitosan. The deliberations on control and inhibition on chitin synthesis in fungi and nematodes give thrust on the development of potential fungicides and nematocides which may find wide application in agriculture. The discussion on various derivatives of chitin and their possible applications open a new field for further research and development.

The section on Sources and Biochemical aspects of chitin consists of 23 papers. The opening paper gives an account of the chitin stocks in marine sediments of various origin. The second paper outlines the basic and applied investigations carried out in Poland for the production of chitin and chitosan from krill and the applications in sciences, cosmetic and pharmaceutical industries and medicine. Screening of microorganisms suitable for chitosan production by fermentation, classification of enzymes hydrolysing chitin, chitinolytic activities in plants, characterisation of chitosanase, purification and assay of chitinase are dealt with in subsequent papers. Microbial production, hormonal regulation of chitin synthesis, degradation of chitin and chitosan by enzymes, lysozyme susceptibility and substitution site by chemical modification

are also discussed.

The third section contains 12 papers on the structure and chemical modification of chitin and chitosan. The advances in the preparation of different derivatives such as iodo chitins, chitosan oligomers, D-glucosamine oligomer series, hydroxy propylated chitosan, Endo- α -1, 4-polygalactosaminidase, N, O-carboxymethyl chitosan are described in eight papers. The remaining papers deal with the studies on the structure of chitin and chitosan using NMR, X-ray and CP/MAS ^{13}C NMR and the reaction kinetics of depolymerisation of chitosan. Physical chemistry and functional properties of chitosan and derivatives of chitosan are presented in the fourth section in 10 papers. Each one describes different aspects such as chitosan gel, copper ion binding, viscosity, foam enhancing, interaction of D-glucosamine and D-glucos-ammonium ion.

The recent innovations in the application of chitosan in medicine and biotechnology are discussed in 21 papers. The aspects covered include selective flocculation of *E. coli*, recovery of single cell protein, preparation and use of chitin beads in chromatography, encapsulation of mammalian cell culture, immobilization of enzymes, chitin collagen complex, pharmaceutical standards and dosage of chitosan, therapeutic applications, controlled release of drugs, antimetastatic effect on lung carcinoma and contact lense.

The ten papers presented in the last section give an account of various applications of chitosan in agriculture and biotechnology. They describe the use of chitosan to reduce plant virus infection, its effectivity in pesticides and fertilizers, increase of seed germination rate by chitosan coating, new solvent system for chitin, production of chitosan fibre and chitosan coated fibres, micro-crystalline chitosan and chitosan membrane for separation of alcohol water mixture.

The book is up-to-date and scholarly and the reader will find the text elaborate covering a wide range of topics in chitin and chitosan. People working in medicine nutrition, biotechnology, pollution, cosmetics, pharmaceuticals, agriculture, pesticides, fertilizers and many other areas can get useful and up-to-date informations on the application of chitin and its derivatives in their fields. Each paper is well written and references used in most papers are latest and pertinent. There are over 1000 such references, 178 tables and 313 illustrations. This makes the book an invaluable source of knowledge for those involved in chitin and chitosan research in developed and developing countries. The new ideas projected in the text will definitely stimulate much research particularly in the development of new derivatives of chitin and in their numerous applications. There is a comprehensive and useful subject index along with an index of contributors. Altogether the book is a good reference document for those engaged in chitin research.

P. MADHAVAN

CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY,
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Diet for Heartcare: Edited by D.P. Sen and Souvik Sen, Published by Dr. D.P. Sen, E-13/3, Karunamayee Salt Lake City, Calcutta-700 091; 1990, pp:44; Price: Rs.18.

This book is very informative and gives extensive details about the role of diet in development of coronary heart disease. The epidemiological details about the incidence of CHD in various parts of the world and its relation to the local food habits have been nicely brought out. The appendices are quite useful and give details about the contents of saturated fatty acids and PUFA in various foods. On the whole, contents of the book clearly explain in detail the relationship between the diet and CHD and how to prevent it. This book can be recommended for heart patients for ready reference. I suggest, the book should contain a few more illustrations and printed with a better get up and bolder types in its next edition.

C. D. SREENIVASA MURTHY
PHYSICIAN AND CARDIOLOGIST
MYSORE

Process Engineering in the Food Industry — Developments and Opportunities; R.W. Field and J.A. Howell, (Ed) England. PP:317; 1989; Price: £46.

This book is the culmination of a project run by the Food and Drink Subject Group of the Institution of Chemical Engineers.

The first section on 'New Process Opportunities and Novel Foods' contains six articles. Ohmic heating described in one of the articles is a new process which permits rapid, uniform sterilisation of liquid and particulate foods. Other than this, there are two articles on extrusion cooking, one on ready meal production, one on simulation of processing systems for cooling or cooking and an over-view of continuous sterilisation operation for aseptic packaging.

The second section on 'Plant Fouling and Cleaning' starts with a review article on fouling of heat exchangers, followed by an article on a modelling of fouling. The third article compares two new fouling monitors. The other two articles are on cleaning. While the first one describes how to design food processing equipment with particular regard to hygiene, the second article deals with the development from the concept to installation of a commercial micro-processor to control CIP systems.

The third section on 'Process Measurement and Control' covers two articles on modelling and optimisation and two articles on some novel sensors. The last article describes a portable equipment for measuring the efficiency of a Refrigeration Plant.

The last section on 'Membrane Processes' contains six articles. After reviewing the principles of membrane processing in food and dairy in the first article, their applications in cheese manufacture, brewing industry and concentration

of fruit juices are discussed in the subsequent articles. There is also one article on sterilisation of process air and one on removal of toxic compounds using ultrafiltration.

In short, this book contains some reviews and articles on new and important topics useful for food process engineers working in Research & Development and Industry.

P. RAMAKRISHNA
TATA TEA LIMITED
MUNNAR, P.O. KERALA-685 612.

Status of CFCs — Refrigeration Systems and Refrigerant Properties: International Institute of Refrigeration 177, Boulevard Maiesherbes, F-75017, Paris, 1988; pp:437; Price : Not indicated.

This is the proceedings of meetings of commissions B₁, B₂, E₁, E₂ in 1988 at Purdue, USA sponsored by the US National Committee for IIR. This is a compilation of 51 papers presented by specialists originating from various countries. The communications are presented in three sections: (1) Thermodynamic properties of refrigerants and refrigerant mixtures (2) Refrigeration systems (3) CFC forum and CFC issue.

Section I contains 9 papers and covers aspects on performances of refrigerant mixtures in heat pump, properties and refrigerant mixture, design of intercoolers/subcoolers, condensation of refrigerant mixture in a horizontal structure of CFCs and Ozone depletion potential.

This section enlightens clearly the increase in efficiency and coefficient of performance (COP) of heat pump using non-azeotropic mixture R₂₂/R₁₁₄ and R₁₃/R₁₂; improvement in heating capacity of heat pump using R₁₃ B₁/R₂₂ with rectifying circuit when the outside temperature is low. The thermodynamic properties are worked out well for pure refrigerants/mixed refrigerants and they are useful especially when they are calculated considering the effect of an alkyl benzene based oil. The molecular approach for search of alternative refrigerants presented in this section is very interesting and it advises to avoid the radical proposal such as elimination of R₂₂. The design technique of intercoolers illustrated in this section is a helpful tool for engineers.

Section II containing 19 valuable papers deals with refrigerating machine suitable for microbiology industry, the spray water room of air-conditioning system, LiBr double effect absorption refrigeration, optimization of refrigeration machinery, dynamic behaviour and failures of domestic refrigerator, gas fuelled engine driven refrigeration system, a new type regenerative gas refrigerator, condensation of steam/air mixture, peripheral pump, thermal comfort control for residential heat pump, computer assisted design of plate-tin-type evaporator, heat exchangers with fins/in-tube enhancement, ejector-injector, rotary regenerators with fresh air purging and Hartmann - Sprenger tube.

The significant outcomes of this section are: Improved economy benefits estimated by utilization of refrigeration and heating capacity of heat pump for production of protein-vitamin concentrate from paraffins, a new cycle of LiBr series-parallel compound cycle which has 10% higher COP in comparison with parallel cycle; refrigeration with gas fuelled engine drive, regenerative gas refrigerator, ejector-injector and Harmann-Sprenger tube are alternatives for conventional vapour compression system and energy saving measures; a new type regenerative gas refrigerator with a practical temperature of refrigeration at - 40°C and 16.5% coefficient of refrigeration; reduction in power input by 4% and increase in cooling capacity by prevention of refrigerant migration during on-off cycle of an air conditioner; the systematic approach by thermo economics for optimization of heat pump which indicates the importance of efficiency of compressor motor; study on dynamic behaviour and failures of small refrigeration system provides valuable informations; reduction of the size of condenser by 25% and evaporator by 12% through use of selected configuration and micro-fins.

The twenty two papers presented in last section deal with Montreal Protocol signed by USA and 23 other countries, in September 1987, conveying the depletion of stratospheric ozone layer due to harmful emission of chlorinated and brominated compounds. This section stresses the need for CFC restrictions and criticises the impact of the restrictions on refrigeration and air conditioning industries. It also describes the effect of various CFCs emitted by various sources of production, consumption, applications etc., and suggest alternative refrigerants/systems.

On the whole, this book would be a valuable addition to libraries and very much useful to scientists and engineers who are working in the area of refrigeration and air conditioning.

R. THIAGU
C.F.T.R.I., MYSORE.

Evaluation of Certain Veterinary Drug Residues in Food: World Health Organisation of the UN, Geneva, 1989; pp.66; Price:Sw fr.9.

Consumer awareness is increasing in recent times for better quality of meat free from drug residues. Large number of veterinary drugs are in use to control diseases among food producing animals. With the result, there are chances of these drugs entering into the animal products. Information on the drug residue content in animal products is limited. It is noteworthy that a publication on 'Evaluation of Certain Veterinary Drug Residues in Food' has been brought out in right time by FAO/WHO.

This publication is the outcome of a meeting of Joint FAO/WHO Expert Committee on Food Additives. The Expert Committee reviewed the presence of residues of veterinary

drugs in food. The report contained principles governing safety evaluation of residues of veterinary drugs in food, maximum residue levels, bound residues, bioavailability and food factors and intake data. The report mentioned the comments on residues of specific veterinary drugs. Daily intake values for individual edible tissues and animal products have been revised and incorporated in the report in order to protect all segments of the population.

The report included for the safety and residue of specific veterinary drugs:

- 1) Albendazole (anti-helminthic drug),
- 2) Dimetridazole, I pronidazole, metronidazole and ronidazole (anti-protozoal drugs)
- 3) Sulfadimidine and sulfathiazole (anti-microbial sulfonamides)
- 4) Trenbolone acetate (growth promotor)
- 5) Diminazone and Iso-metamidium (Trypananocides)

The report dealt with the toxicological and residue data of the drugs in-depth. It contained the recommendations on the acceptable daily intake for human beings and maximum residue levels of the drugs except for certain drugs due to insufficient toxicological information. The information presented in the report is highly useful in regulatory control of veterinary drug residues in food producing animals. The report also indicates the need for further toxicological studies of the drugs.

The findings of the evaluation of certain veterinary drugs in food are well presented in the report. The book is a valuable addition to any library catering to the information needs of research workers and also to the personnel involved in the regulatory control laboratories.

D. NARASIMHA RAO
C.F.T.R.I., MYSORE.

Environmental Health Criteria: World Health Organisation of the UN, Geneva, 1989, Price : Swiss Francs 10. Allethrins (allethrin, d-allethrin bioallethrin and s-bio-allethrin) 87- (pp 75) 92 - Resmethrins (resmethrins, bioremethrin and cismethrin) (pp 79).

Both these books form a part of the efforts of WHO Task Group on the synthetic pyrethroid insecticides used for control of household and stored grain pests, under the International Programme on Chemical Safety (IPCS). The books are published jointly by the UNEP, ILO, and WHO.

In both the books, efforts have been made to compile and summarise all the available information on various aspects of both groups of pyrethroids, such as history of development, properties, use patterns, metabolism, persistence and environmental behaviour, degradation, residues and toxicology. The Introduction gives a concise information on the history of

development of synthetic pyrethroids in general, followed by the Summary of the contents of the book.

Chapter 2 deals with the physicochemical properties, structures, isomers and list of analytical methods of the allethrins or resmethrins. The third chapter consists of information on environmental behaviour of the said pyrethroids, particularly on industrial production, use patterns, environmental transport, distribution and transformations of each compound, followed by levels of human exposure and residues in food. While chapter 5 furnishes the details of metabolism in mammals, toxicity to different aquatic animals and birds form the subject matter of chapter 6.

All the toxicological studies on mammals including acute, subacute, irritation and chronic toxicities have been reviewed and the results summarised in next chapter. Effects on the isomer composition on the toxicity values have also been discussed and information on potentiation and mode of action have been indicated in this chapter.

The most aspects on human health risks due to the use of these insecticides based on the available data and the experience of the WHO Task Group have been included in chapters 7 and 8. The overall view of the Task Group on the data have been included in the chapters on conclusions and recommendations. The mode of action of different groups of pyrethroid insecticides have been summarised under appendix.

It may be noted that allethrins are mostly used in mosquito mats, coils, aerosols and space sprays, in the sprays either alone or in combination with other insecticides. On the other hand, aerosols, space sprays and grain storage are the avenues through which resmethrins are used. Most of these household insecticides are extensively used in the urban areas and cities to avoid the nuisance by mosquitoes, flies etc. As such, there is a fear among the population regarding the safety of those formulations to humans in the dwellings where these are used, particularly to children. The two books reviewed here, furnish enough evidence on this line and one can get a clear picture of the safety and precautions needed in the use of formulations containing the said active ingredients.

However, certain information like the effects of synergists on toxicities, metabolism/persistence in insects, residual effects on sprayed surfaces etc are missing and these would have added to the value of the books.

Both these books are highly valuable as source of information and reference materials for Research Workers, Teachers, Administrators, Public health personnel, Medical professionals and Chemical Industries.

M.S. MITHYANTHA
RALLIS AGROCHEMICAL RESEARCH STATION, PEENYA,
BANGALORE-560 058.

JUST PUBLISHED

Monograph on PAPAYA

The Central Food Technological Research Institute Mysore, has just brought out an updated version of the monograph entitled "PAPAYA IN INDIA — Production, Preservation, and Processing". This publication aims to stimulate entrepreneurs on the varied uses of Papaya and its future potentialities. Latest information on such varied topics and cultural features, varietal characteristics, fruit chemistry, manufacture of papain, recovery of pectin and a host of new product possibilities and manufacture of Tutty-Fruity and fruit bars are some of the outstanding features. Trade information pertaining to suppliers of equipment and also the enzyme papain have been included. Price is nominally fixed at Rs.20/- per copy + V.P.P. charges.

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

- (a) *Research Paper:* Jadhav S S and Kulkarni P R, Presser amine; in foods, J Fd Sci Technol, 1981, 18, 156.
 - (b) *Book:* Venkataraman K, The Chemistry of Synthetic Dyes, Academic Press, Inc, New York, 1952, Vol, II, 966.
 - (c) *References to article in a book:* Joshi S V, in The Chemistry of Synthetic Dyes, by Venkataraman K, Academic Press Inc, New York, 1952, Vol. II, 966.
 - (d) *Proceedings, Conferences and Symposia Papers:* Nambudiri E S and Lewis Y S, Cocoa in confectionery, Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India, Mysore, May 1979, 27.
 - (e) *Thesis:* Sathyanarayar. Y, Phytosociological Studies on the Calicicolous Plants of Bombay. 1953. Ph.D. Thesis Bombay University.
 - (f) *Unpublished Work:* Rao G, unpublished, Central Food Technological Research Institute, Mysore, India.
8. Consult the latest issue of the *Journal* for guidance. For "Additional Instructions for Reporting Results of Sensory Analysis" see issue No. 1 of the Journal.

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Vol.28, No.2

Contents of forthcoming issue

March/April 1991

Research Papers

- DEHYDRATED COCONUT CHUTNEY by *T. S. Satyanarayana Rao, N. M. Kaverappa and H. P. Reddy*
- EFFECT OF PRESOAKING ON COOKING TIME AND TEXTURE OF RAW AND PARBOILED RICE by *C. M. Sowbhagya and S. Zakiuddin Ali*
- STUDIES ON BEER PRODUCTION FROM NIGERIAN MILLET by *Reginald C. Agu and Zak A. Obanu*
- A STUDY ON INSTANTISATION OF TUR-DHAL by *B. W. Bhuibhar, V. K. Andhare and A. B. Kadu*
- APPLICATION OF OSMOSIS-OSMO-CANNING OF APPLE RINGS by *R. C. Sharma, V. K. Joshi, S. K. Chauhan, S. K. Chopra and B. B. Lal*
- SOY-WHEAT FLOUR BLENDS: CHEMICAL., RHEOLOGICAL AND BAKING CHARACTERISTICS by *Pratima Misra, M. S. Usha and Surjan Singh*
- EFFECT OF INCORPORATING WHEAT BRAN ON THE RHEOLOGICAL CHARACTERISTICS AND BREAD MAKING QUALITY OF FLOUR by *P. Haridas Rao and Hema Malini Rao*
- INFLUENCE OF CASEIN/FAT RATIO OF MILK ON BAKING, RHEOLOGICAL AND SENSORY CHARACTERISTICS OF BUFFALO MILK MOZZARELLA CHEESE by *M. Ravi Sundar and K. G. Upadhyay*
- EFFECT OF ICE STORAGE ON PROTEIN AND RELATED CHANGES IN PINK PERCH (*NEMIPTERUS JAPONICUS*) by *G. Vidya Sagar Reddy and L. N. Srikar*
- NATURAL PLANT ENZYME INHIBITORS: PROTEASE INHIBITORS IN CANAVALLIA SEEDS by *N. Nagaraja Kumari and T. N. Pattabiraman*

Research Notes

- QUALITY OF EXTRACTED OIL FROM SOME COMMERCIALY SOLD DEEP-FAT-FRIED SNACKS AND USED OIL by *A. Neelima and G. Sarojini*
- AMYLOLYTIC ACTIVITIES OF CULTURE FILTRATES OF *RHIZOPUS ORYZAE* AND *BOTRYODIPLODIA THEOBROMAE* by *Ekundayo O. Afe and B. A. Oso*
- PROTEIN, LYSINE, MINERAL AND PHENOL CONTENTS OF SOME INDIAN WHEAT (*TRITICUM AESTIVUM* L.) VARIETIES by *Charanjeet K. Hira, Anita Kochhar, Ealwinder K. Sadana and Kanta K. Sharma*
- STUDIES ON COOKING RATE EQUATION OF DHAL by *M. B. Berc, S. Mukherjee, R. K. Singh and M. Gurung*
- RECOVERY OF DHAL FROM REDGRAM STORED UNDER DIFFERENT CONDITIONS by *Usha Kumari and N. S. Reddy*
- NUTRIENT CONTENT IN *FLACOURTIA* FRUITS FROM WESTERN MAHARASHTRA by *D. K. Kulkarni, M. S. Kumbhojkar, V. V. Agte, N. S. Joshi and V. N. Joshi*
- CHANGES IN PHOSPHOLIPID BROWNING OF HEN'S WHOLE EGG POWDERS PACKED IN DIFFERENT PACKING MATERIALS by *T. S. Satyanarayana Rao*
- DETECTION OF MUTTON, BEEF AND BUFFALO BEEF WITH ANTISERA TO SPECIES LIVER BY DOUBLE GEL IMMUNO-DIFFUSION, IMMUNO-ELECTROPHORESIS AND COUNTER IMMUNO-ELECTROPHORESIS by *S. Srinivas, P. M. Reddy and K. S. Reddy*
- ESSENTIAL AMINO ACIDS PROFILE IN EIGHT TRADITIONAL CEREAL CULTIVARS OF TRANS-HIMALAYAN REGION by *S. K. Katiyar and A. K. Bhatia*
- FUNGITOXICITY OF FOUR OXIDIAZOL THIONE DERIVATIVES TOWARDS FUNGI DETERIORATING MOONG (*PHASEOLUS AUREUS* ROXB.) SEEDS by *Archana Rathore and N. Misra*