ISSN-0022-1155

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS, INDIA

VOL. 24. NO. 2. MARCH / APRIL 1987

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(INDIA)

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Effect of Conditioning Methods on Physico-chemical, Milling and Functional (Baking) Properties of Soaked Wheat

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Received 30 *November* 1985; *revised* 8 *July* 1986

The physico-chemical, milling and baking characteristics of soaked wheat WL-1562 and S-308 as affected by methods of conditioning were investigated. Kernel weight, density, pearling index, water uptake, protein and ash contents of grains decreased with soaking treatment. Soaking and conditioning did not significantly increase the flour yield. Soaking of grains yielded coarser flour, the proportion of which increased with hot and steam conditioning. Diastatic activity and total sugars increased significantly with soaking whereas damaged starch and gluten considerably decreased. Steam conditioning of soaked wheat seriously injured the gluten properties and produced flour of low damaged starch seviously. Bake absorptions of flour and whole meal were considerably reduced upon soaking; but loaf volume improved with the treatment. Warm or hot conditioning improved the loaf volume and internal characteristics, but steam conditioned wheat exhibited poor bread making properties. Cookie's spread increased with soaking, but conditioning methods were of no use in improving its quality. Cake volume and internal characters were better when soaked wheat was hot conditioned. The doughs from soaked wheat meal were sticky and baked to inferior *chapatis* as compared to the *chapatis* from sound wheat.

Wheat grains get soaked and germinated before and after harvest which is a common problem all over the world. The detrimental effects of germination include cumulative losses of grain yield, grain quality, flour yield and baking quality[1-5].

Punjab is the leading wheat producing State of India and there is an increasing use of Punjab wheats in the milling and baking industry all over the country. In the recent past, there had been instances of untimely light to moderate showers during harvesting season which led to the wetting of grains in the ear or in the markets. Reseaıch about such damage has been concerned primarily with the development of alpha-amylase activity; however, information on effects in the early stages of germination, is scanty[6,7]. Besides variety and environmental factors which affect the baking quality of flour, proper conditioning method and milling are of high importance in realizing full potential of any wheat. Our first objective in the present investigation was to examine the effects of soaking on physico-chemical, milling and baking properties of two commercial varieties of wheat. The second objective was to see if there is any effect of conditioning method on these soaked wheats in improving their utilization.

Materials and Methods

Two cultivars of bıead wheat, 'WL-1562' and 'S-308' were procured from the Department of Plant Breeding, Punjab Agricultural University, Ludhiana from 1983-84 crop.

Soaking treatment: The cleaned wheat samples (10 kg) from each variety were soaked to 35 per cent moisture at 30°C (approximately for 6 hr). The excess water was drained off and the wheat dried in a forced air drier at 30°C to a moisture content of about 13 per cent.

Conditioning of wheat: The soaked and dried samples were conditioned using cold, warm, hot and steam conditioning methods as indicated in Table 1.

Table 1. Methods of conditioning the soaked wheat samples

Conditioning method	Tempering moisture (%)	Temp. (°C)	Time (min)	Resting period (at 30 °C) (hr)
Cold*	16.0	30	5	30
Cold	14.5	30	5	24
Warm	14.5	45	90	20
Hot	14.5	88	12	16
Steam	14.5	103	1/3	8

*Control, not soaked;

Milling: The tempered samples were milled in the Buhler pneumatic Laboratory Mill (MLU-202) under conditions of appropriate roll setting and feed rate. The yields of straight grade flour, bran and shorts were calculated on the basis of recovered products.

Physico-chemical properties of grain and flour: 1000-kernel weight, pearling index, protein (N $\times$5.7) and ash contents of grains were determined according to AACC methods[8]. Granulation of flour and colour grade values were estimated using Henry Simon's sedimentation apparatus and Kent-Jones and Martin Colour Grader[9].

Flour ash, protein, gluten, total sugars, damaged starch and diastatic activity were determined according to AACC methods Free-amino acids were determined using the methods of Lie[10].

Baking studies: For bread making the straight dough method[8] with remixing procedure of Irvine and McMullan[11] was followed with the difference that doughs were mixed optimally using required quantity of water. Cookies and cakes were made as per the AACC methods[8] and scored accordingly. *Chapati* (unleavened pan cake) was made by the method of Austin and Ram[12] using optimum water for dough kneading.

Statistical analysis: All analyses were performed in duplicates. Analysis of variance was conducted according to Pence and Sukhatme[13]. Least significant ranges (LSR) were computed at 5 per cent level of significance and tested with the Duncan's multiple range test.

Results and Discussion

Grain characteristics: Kernels of 'S-308' variety were bolder (45.6g) than those of 'WL-1562' (41.3g). Kernel weight, and density of kernels decreased with soaking treatment. 'WL-1562' variety was distinctively harder than 'S-308' as inferred from pearling index values. The decrease in pearling index with soaking treatment from 70.5 to 65.5 and from 65.1 to 53.7 per cent for 'WL-1562' and 'S-308' wheats respectively, indicated that grains got softened by soaking. Imbibition of water during soaking may be the reason for change in physical structure of the kernels. The protein and ash contents of grains decreased slightly by soaking in both the cultivars.

The soaked wheats showed lower uptake of water when immersed in water at different temperatures and times. A typical line diagram of 'WL-1562' showing the effect of soaking on water uptake is given in Fig. 1. The moisture content of sound grains of 'S-308' variety increased to about 20 per cent at 40°C as compared to 17.4 per cent of 'WL-1562'. The soaked grains at the same temperature reached the moisture content of 17.0 and 16.9 per cent respectively. These results are in agreement with those reported by Nagi and Bains[14]. The greater uptake of water by 'S-308' wheat may be due to the softness of the grains than 'WL-1562' wheat.

Milling characteristics: The data on the flour yield, shorts, bran, ash and flour colour as affected by soaking treatment and method of conditioning the soaked wheat are given in Table 2. Soaking treatment of grains did not significantly increase the flour yield in both the varieties. The flour yield of the soaked samples was 74.5 and 76.6 per cent for 'WL-1562' and 'S-308' varieties respectively as against 71.9 and 74.2 per cent for sound samples. Consequently, the percentage of bran and shorts decreased in the soaked samples. The higher yield of flour may be because a

TABLE 2. EFFECT OF METHOD OF CONDITIONING OF SOAKED WHEAT ON MILLING PROPERTIES

Parameters	Variety	Sound wheat	Soaked wheat			
		Cold	Cold	Warm	Hot	Steam
Flour yield (%)	WL-1562	71.9	74.5	76.4	75.9	76.0
	S-308	74.2	76.6	76.8	76.7	73.9
Bran (%)	WL-1562	13.9	13.0	9.6	13.1	14.4
	S-308	14.7	14.2	12.0	13.0	14.0
Short (%)	WL-1562	14.2	12.5	14.0	11.0	9.6
	S-308	11.1	9.2	11.2	10.2	12.1
Ash (%)	WL-1562	0.47	0.45	0.46	0.46	0.46
	S-308	0.46	0.41	0.46	0.43	0.42
Colour grade	WL-1562	1.7	1.8	1.9	1.4	1.3
	S-308	1.9	1.7	1.7	1.6	1.0

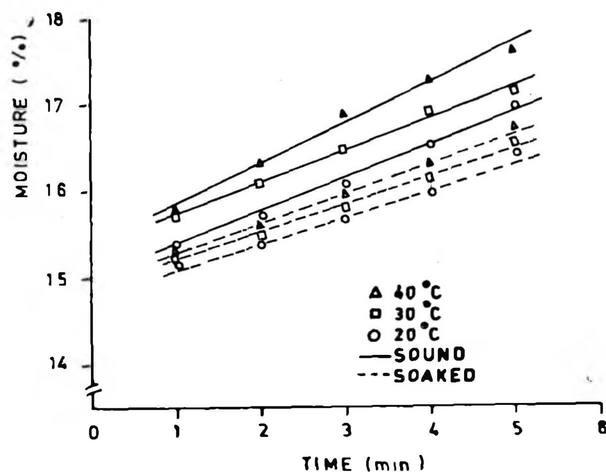

Fig 1. Effect of soaking on uptake of water during immersion in water by variety WL-1562

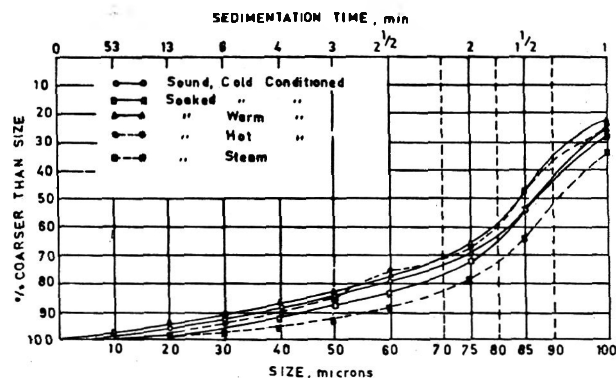

Fig 2. Effect of method of conditioning of soaked wheat on particle size distribution curves from variety WL-1562

part of endosperm is shifted to break and reduction flours which under normal milling would be conveyed to the shorts stream.

The method of conditioning the soaked wheat favourably influenced the flour yield in both the varieties. The highest flour yield was found by warm conditioning of soaked wheat samples in the two varieties. Hot as well as steam conditioned samples also increased the recovery of flour; however, the increase was non-significant. The varietal mean for 'S-308' was higher (75.6 per cent) than that of 'WL-1562' (74.9 per cent) but this difference was not significant.

Flour characteristics: The granulation curves revealed that soaking treatment increased the coarser size particles in flour in both the cultivars. The particle size distribution in a sample of flour had a significant bearing on the water absorption capacity. Warm conditioning of soaked wheat resulted in flour of finer granularity. The particles of less than 50 μ size increased from 13.3 to 18.3 per cent in 'WL-1562' and from 16.7 to 25.0 per cent in 'S-308' flour. This shifting of coarser size particles to smaller ones was lower with hot conditioning of soaked wheat. Steam conditioning of soaked wheat produced flour of comparatively coarser than warm or hot conditioned samples and in case of 'WL-1562' about one-third of the flour had the particle size more than 100μ. So, warm conditioning was more conducive to soaked wheats for getting flour of finer granulation. The typical granulation curves for 'WL-1562' wheat as affected by soaking and use of various conditioning methods on soaked wheat are given in Fig. 2.

Flour colour and ash values of flours (Table 2) revealed that the ash content reduced with soaking treatment in both the varieties though the flour extraction was more in those samples. Similarly, Kent-Jones and Martin Colour grade values also reflected the decreaee

TABLE 3. EFFECT OF METHOD OF CONDITIONING OF SOAKED WHEAT ON PHYSICO-CHEMICAL PROPERTIES OF FLOUR

Parameter	Variety	Sound wheat	Soaked wheat			
		Cold	Cold	Warm	Hot	Steam
Protein (%)	WL-1562	9.90	9.60	9.60	9.60	10.10
	S-308	8.80	8.90	8.90	8.80	9.40
Gluten, dry (%)	WL-1562	10.00	8.80	8.70	8.90	1.10
	S-308	7.90	7.40	7.80	7.70	5.20
Free amino acids (glycine mg/g)	WL-1562	1.17	1.29	1.26	1.19	1.29
	S-308	0.62	0.64	0.58	0.55	0.65
Diastatic activity (mg maltose/10 g)	WL-1562	204	310	208	188	120
	S-308	153	269	134	127	83
Damaged starch (%)	WL-1562	6.57	5.66	5.52	4.72	3.68
	S-308	5.72	3.69	3.94	3.61	3.21
Total sugars (%)	WL-1562	0.97	1.37	1.13	0.96	0.79
	S-308	1.10	1.15	1.06	0.91	0.73

indicating whiteness of flour with soaking. Decrease in the ash content of soft wheat flour was also observed by Hwang and Bushuk[3] and Lukow and Bushuk[6]. The later stated that this decrease in the ash content resulted from leaching of ash yielding components during soaking and the utilization of inorganic ions in metabolism/respiration. Method of conditioning did not have any noticeable impact on ash content of flours.

The perusal of the data (Table 3) showed that the varieties registered non-significant decrease in flour protein with different methods of conditioning. However, the varieties differed significantly in the protein content. Translocation of amino acids to the developing embryo may be the reason for decrease in protein content of flour during soaking. Nagi *et al.*[7] reported a significant decrease in the protein of flour upon soaking of wheat while Hwang and Bushuk[3] and Lukow and Bushuk[6] reported only a slight decrease with soaking and germination of grains.

Gluten content decreased with soaking treatment and 'WL–1562' variety showed a greater decrease than 'S–308' essentially due to varietal characters. The decrease in the gluten was attributed to its breakdown by proteolytic enzymes developed during soaking. Warm or hot conditioning of soaked wheat had no influence on the gluten content but steam conditioning resulted in a drastic decrease in the gluten especially in 'WL–1562' wheat where it decreased to merely 1.1 per cent.

Free-amino acids of the flour increased with soaking in both the cultivars. Tkachuk[15] stated that the free amino acids production began few hours after sound wheat was wetted and their level represented a sensitive measure of germination. Warm or hot conditioning of soaked wheat resulted in the slight decrease of free amino acids but steam conditioning had no influence on the free amino acids.

Diastatic activity and free sugars increased significantly in both the varieties with soaking treatment. The reduction in the damaged starch content was also significant. The diastatic activity in 'WL–1562' increased from 204 to 310 mg maltose/10 g and in 'S–308' from 153 to 269 mg maltose/10 g upon soaking. A number of authors[16–19] had also shown increased activity with soaking and sprouting.

A significant decrease in the damaged starch content of soaked wheat flour (6.57 to 5.66 per cent in 'WL–1562' and 5.72 to 3.69 per cent in 'S–308') was observed. Warm, hot or steam conditioning decreased the diastatic activity and damaged starch contents in both the varieties. As expected, the results showed that increased temperatures applied during conditioning destroyed the enzyme system and particularly steam conditioning caused a heavy reduction in diastatic activity and damaged starch contents. Total sugars increased significantly with soaking in the two varieties; however, no significant differences were found in the non-reducing sugar component. Conditioning methods significantly reduced the amount of sugars in the flours.

Baking properties: The data on bread making quality of flour milled after conditioning are given in Table 4 and typical loaf characteristics are shown in Fig. 3. Soaking of wheat had a favourable influence on the bread making properties of flour in both the varieties. Bake absorption of the varieties differed significantly. However, the conditioning methods had no impact on the bake absorption in the two cultivars. Loaf volume increased significantly with soaking in both the

TABLE 4. EFFECT OF METHOD OF CONDITIONING OF SOAKED WHEAT ON BAKING CHARACTERISTICS

Parameters	Variety	Sound wheat	Soaked wheat			
		Cold	Cold	Warm	Hot	Steam
Bread volume (ml)	WL–1562	430	450	520	520	230
	S–308	420	450	460	490	330
Bake absorption (%)	WL–1562	64	62	61	62	60
	S–308	58	55	57	58	60
Cake volume (ml)	WL–1562	920	920	940	980	835
	S–308	1000	1010	955	1020	1000
Cookie spread (W/T)	WL–1562	6.2	6.4	6.1	6.1	6.1
	S–308	6.5	7.5	6.5	6.3	5.7
Whole meal water absorption (%)	WL–1562	64.0	60.0	—	—	—
	S–308	55.7	57.4	—	—	—

— Not determined.

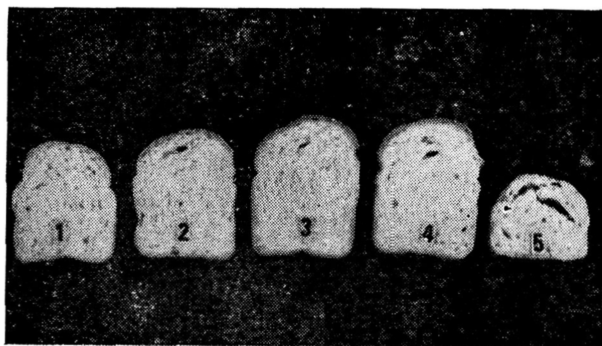

Fig. 3. Effect of soaking and method of conditioning on bread making properties of WL-1562 wheat (1) control, cold conditioned; (2) soaked, cold conditioned; (3) soaked warm conditioned; (4) soaked, hot conditioned and (5) soaked, steam conditioned.

cultivars which further increased with warm and hot conditioning of samples. Steam conditioned samples produced hard and smaller loaves with poor bread characteristics. The higher temperatures applied during steam conditioning may have destroyed the gluten properties with poor gas retaining power. Soaking of wheat had a negligible effect on crust and crumb characteristics but hot conditioning improved the crust colour, crumb texture and grain of loaves. Hot conditioning also improved handling properties of soaked wheats.

The method of conditioning the soaked wheat and soaking did not significantly influence cookie making properties. Cookie spread factor decreased with different conditioning methods. Warm or hot conditioning of soaked wheat increased the thickness of cookies but width decreased whereas steam conditioned samples produced a noticeable decrease in spread factor.

The varieties differed significantly in cake volume; however, soaking treatment had no effect on cake volume. Variety 'S-308' showed a slight increase in cake volume, whereas no difference was found in 'WL-1562' with soaking treatment. The varietal mean for cake volume in 'WL-1562' was 835 ml and in 'S-308', was 1000 ml. Hot conditioning of soaked wheat improved the cake volume in both the varieties. Thickness of walls and grain score reduced with soaking in both the varieties. Poor cake baking quality was observed by Lorenz and Valvano[5] in flour of 1-day sprouted wheat. They found that cakes had a smaller volume and had a dip in the centre, a coarse grain and firm texture. Cakes from variety 'S-308' had a dip in the centre in the present studies also. The cake characteristics ('WL-1562') as affected by soaking treatment and use of conditioning methods on soaked wheat are shown in Fig. 4.

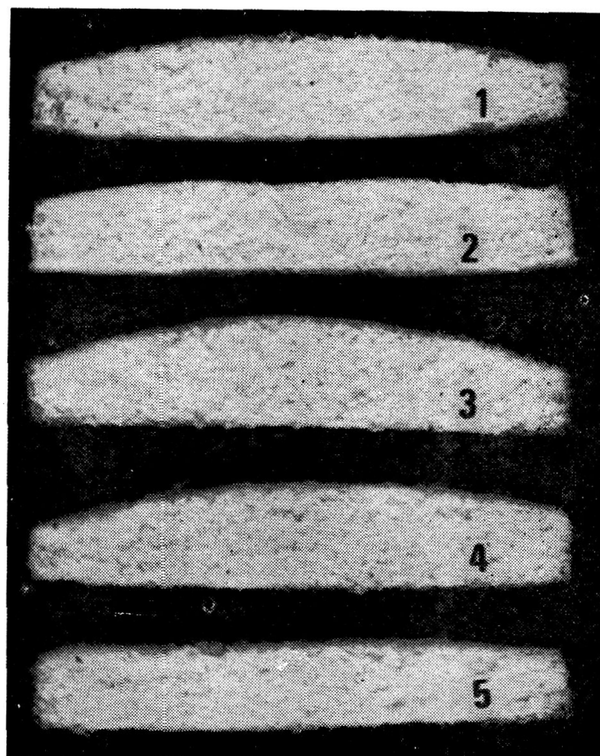

Fig. 4. Effect of soaking and method of conditioning on cake making properties of WL-1562 wheat (1) control, cold conditioned; (2) soaked, cold conditioned; (3) soaked, warm conditioned; (4) soaked, hot conditioned and (5) soaked, steam conditioned.

Chapati (unleavened pan cake) made from soaked wheat meal revealed a deterioration of the doughs, being sticky and having reduced water absorption. Sound wheat meal *chapati* was creamish to light brownish while a dull white *chapati* resulted from soaked wheats. The texture of *chapati* made from soaked wheats was semi-hard as compared to the soft, smooth and pliable texture from sound wheats.

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Physical Properties of Unsweetened and Sweetened Soymilk

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Receieved 18 *December* 1985; *revised* 30 *June* 1986

Effect of temperature on density, viscosity, specific heat and thermal conductivity for unsweetened and sweetened soymilk was determined. The density of unsweetened soymilk varied from 1.03459 at 10°C to 1.00365 g/ml at 90°C whereas that of sweetened soymilk varied from 1.0369 at 10°C to 1.0061 g/ml at 90°C. The viscosity decreased from 7.52 cp at 10°C to 0.86 cp at 90°C and from 8.62 cp at 10°C to 1.04 cp at 90°C for unsweetened and sweetened soymilk respectively. Specific heat for unsweetened soymilk increased from 0.9525 to 0.9623 cal/(g.°c) when the temperature was increased from 20 to 92.9°C. In case of sweetened soymilk, it increased from 0.9145 to 0.9234 cal/(g.°c) when the temperature was increassed from 20.1 to 92.7°C. Thermal conductivity increased from 0.33271 to 0.41341 Kcal/(hr-m-°C) when average temperature was increased from 37.68 to 92.49°C for unsweetened soymilk, in case of sweetened soymilk, it increased from 0.31152 to 0.40385 for the raise of temperature from 38.30 to 89.79°C. Different models were developed correlating the temperature and physical properties.

Heating and cooling of the products are probably the most frequently occurring processes in a liquid food processing plant. Therefore, information on product properties that govern flow and heat transfer behaviour is of importance. How the density, viscosity, specific heat and thermal conductivity change with temperature has considerable bearing on design and operation of related process equipments. Soymilk is a milk-like beverage. It is a suspension of fine soy solids in water. It has considerable potential as a milk extender and beverage. Very little information is available on the physical properties of soymilk although such studies have been made on skim milk[1], whole milk[2-4] and other milk products[5-9]. Hence, the effect of temperature on density, viscosity, specific heat and thermal conductivity of soymilk was studied.

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Materials and Methods

Soymilk was obtained from the Food Research Laboratory, G. B. Pant University of Agriculture and Technology, Pantnagar[10]. Unsweetened soymilk had 3.2 per cent protein, 1.7 per cent fat, 3.2 per cent carbohydrate, 1.5 per cent sucrose and 0.5 per cent minerals. In the sweetened soymilk, all the other solids were kept at the same level except sucrose which was 6 per cent . Samples were chilled to 5°C before actual tests were conducted.

Specific gravity bottle and Hoppler viscometer were used to determine density and viscosity, respectively. Method of mixtures was used to determine specific heat. The guarded hot plate method, described by Kaye and Higgins[11] was used to determine thermal conductivity. For density and viscosity, five replications were made to get the average value at each temperature. In case of specific heat and thermal conductivity, three replications were done.

Results and Discussion

Density, ρ, (g/ml): The densities of unsweetened and sweetened soymilk decreased with temperature and the two curves were almost parallel to each other (Fig. 1). The density of unsweetened soymilk varied from 1.03459 at 10°C to 1.00365 g/ml at 90°C whereas that of sweetened soymilk varied from 1.0369 at 10°C to 1.0061 g/ml at 90°C. The decrease in its density with increasing temperature seems to be influenced by the change in the density of water alone, since the density of soy solids is likely to remain constant as the coefficient of thermal expansion of biological solids is negligibly small.

An empirical equation has been derived to correlate the density of soymilk (ρ) at any temperature (t) to its density at 20°C (ρ_{20}) $\rho=\rho_{20}\ (1-\alpha\theta-\beta\theta^2)$ where $\theta=(t-20)$°C and α and β are Empirical constants.

For unsweetened soymilk, $\alpha=2.8594\times10^{-4}$, $\beta=1.6058\times10^{-6}$

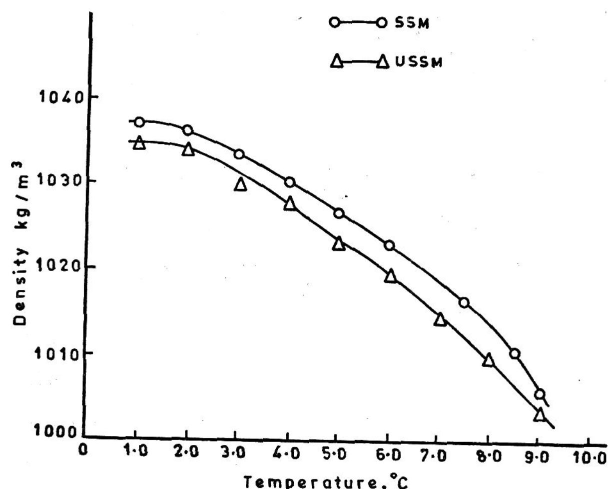

Fig 1. Effect of temperature on density

For sweetened soymilk, $\alpha=2.1924\times10^{-4}$, $\beta=2.5086\times10^{-6}$

The constants α and β have been determined using least squares method. The density of soymilk at any temperature in the experimental range can be predicted with an accuracy of $\pm$ 7.0 per cent.

Lal *et al*[12] reported that the density of cow's milk and buffalo milk decreased with the increase in temperature. In that case also, the variation of density with temperature is not linear.

Viscosity, μ(cp): The viscosity decreased from 8.62 cp at 10°C to 1.03 cp at 90°C and from 7.52 cp at 10°C to 0.86 cp at 90°C for sweetened and unsweetened soymilk, respectively. Lal *et al.*[12] reported that the viscosity of cow's milk varied from 3.43 at 10°C to 0.38 cp at 90°C whereas that of buffalo milk varied from 2.45 at 15°C to 0.76 cp at 90°C. Soymilk shows a much higher viscosity than normal milk at ower temperatures though this difference is much less at higher temperatures.

The higher viscosity of sweetened soymilk is due to higher sucrose content. The difference between the viscosities of 1.5 and 6 per cent aqueous sucrose solution is 0.1136 cp while that between unsweetened and sweetened soymilk is 0.5834 cp. This indicates that the effect of sucrose concentration on viscosity is greater when added to soymilk.

The relationship between viscosity of soymilk and temperature is depicted in Fig. 2 and can be represented by the equation

$$\mu=A\ \exp\ (E/RT)$$

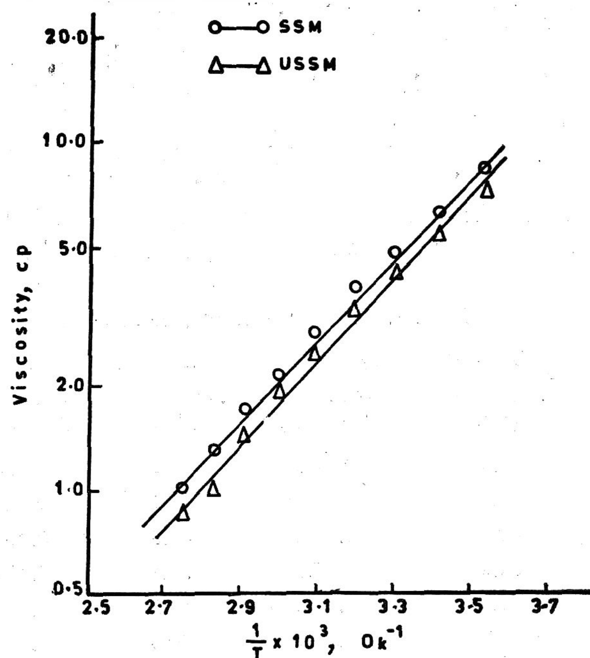

Fig 2. Relationship between log (viscosity) and temperature

Where μ = Viscosity, cp
R = Universal gas content, K cal/(g–mole.°K)
T = Absolute temperature, °K
A and E = Empirical constants
For unsweetened soymilk: $A = 3.5605 \times 10^{-4}$, and $E = 5.6339$
For sweetened soymilk: $A = 5.6024 \times 10^{-4}$, and $E = 5.4348$.

The equation has the same mathematical form as that of Arrhenius equation for reaction velocity. Therefore the quantity E can be interpreted as some kind of energy of activation for the molecular momentum transfer of viscous flow which is quite consistent with the concept of fluidized vacancies which provides a simple mechanism for the flow of a liquid in which the molecules are packed nearly as closely as they are in a crystal[13]. The activation energies for unsweetened and sweetened soymilk are close to each other.

Specific heat, C_p (cal/g–°C)*:* Specific heat of soymilk increases with increase in temperature (Table 1). Lal *et al.*[12] observed similar trend for cow's milk and buffalo milk. This smooth variation with temperature agrees with Fernandez–Martin[6] findings. The relationship can be expressed by

$$C_p = C_o + C_1 t$$

Where,

C_p = Specific heat, $\frac{\text{K cal}}{(\text{kg}°\text{C})}$
t = Temperature, °C
C_o and C_1 = Empirical constants
For unsweetened soymilk $C_o = 0.94903$ and $C_1 = 1.349 \times 10^{-3}$
For sweetened soymilk $C_o = 0.91044$ and $C_1 = 1.259 \times 10^{-3}$

Constants C_o and C_1 were calculated by the least squares method. The equation predicts the specific heat value of soymilk at any temperature in the range of 20 to 90°C with a maximum error of ± 0.4 per cent. The specific heat of unsweetened soymilk is higher than that of cow's milk, sweetened soymilk and buffalo milk, in the order given. This may be attributed to the water content of the product which plays the most dominant in role the specific heat of different milks. Higher the water content, higher is the specific heat of the product. Many workers [6, 9, 14–16] have proposed equations for specific heat on the basis of composition. However, only the equation of Fernandez–Martin[6] includes the effect of temperature and total solids. Specific heat values calculated through his equation compared well with the data obtained in the present study.

Thermal conductivity, K (kcal/m-hr-°C): Thermal conductivity of soymilk increased linearly with temperature. Data can be represented through the equation.

$$K = K_0 + K_1 t$$

Where,

K = Thermal conductivity Kcal/(m-hr-°C)
t = Temperature, °C
K_o and K_1 = Empirical constants.
For unsweetened soymilk, $K_o = 0.26752$ and $K_1 = 0.001592$
For sweetened soymilk, $K_o = 0.23372$ and $K_1 = 0.001901$

The constants K_o and K_1 were calculated by the least squares method.

Thermal conductivity of soymilk is lower than normal fluid milk[12]. Unsweetened soymilk is less dependent on temperature than sweetened soymilk. Addition of sucrose to unsweetened soymilk resulted in decrease in thermal conductivity. The degree of decrease in thermal conductivity is more at lower temperatures than at higher temperatures (Table 2).

Acknowledgement

The study reported was conducted at G. B. Pant University of Agriculture and Technology, Pantnagar as a part of an ICAR sponsored project "Heat transfer in processing of milk and milk products."

TABLE 1. SPECIFIC HEAT (Cp) OF SOYMILK

Unsweetened soymilk		Sweetened soymilk	
Av. temp (°C)	Sp. heat Cal/(g-°C)	Av. temp. (°C)	Sp. heat Cal/(g-°C)
20.0	0.9525	20.1	0.9145
29.5	0.9532	28.7	0.9150
40.2	0.9526	40.8	0.9140
53.0	0.9572	51.1	0.9161
67.1	0.9581	67.3	0.9164
80.2	0.9590	80.4	0.9220
92.9	0.9623	92.7	0.9234

TABLE 2. THERMAL CONDUCTIVITY (K) OF SOYMILK

Unsweetened soymilk		Sweetened soymilk	
Av temp. (°C)	K K. cal/(hr.m-°c)	Av. temp. (°C)	K K. cal/(hr.m-°C)
37.68	0.33271	38.30	0.31152
50.37	0.34072	49.90	0.32487
63.00	0.36431	62.85	0.34735
75.47	0.39437	79.83	0.39084
92.49	0.41341	89.79	0.40385

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Studies on Chemical Characteristics and Nitrate Nitrogen Content of a Few Cultivars of Tomato in Relation to Tin Pick up by Canned Juice*

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Received 15 *April* 1985; *revised* 20 *June* 1986

Nine cultivars—'Pusa Ruby', 'Sioux', 'Ogasta', 'Gemed', 'Punjab Chhuhara', 'NMR', 'Punjab Kesari', 'Sanmerzano' and 'Roma' were grown during different seasons at Kukkerhalli Farm, Mysore. Ripe fruits of first harvest were analysed for pH, acidity, ascorbic acid, total sugars, viscosity, alcohol insoluble solids, lycopene and nitrate nitrogen. Juices of first five cultivars were canned in 1 lb jam plain cans having a coating of 1 lb/base box (E 100) and tin pick-up studied. Cultivars differed widely in acidity, ascorbic acid, total sugars, viscosity and lycopene content. Seasonal variation in chemical composition was also observed. Slight increase in acidity, ascorbic acid and decrease in sugars were observed in September harvests compared to January harvests. In none of the cultivars, nitrate nitrogen content exceeded 1 p.p.m. in any season. Tin pick-up by canned juice of different cultivars at the end of 90 days storage at 37°C was in the range of 43 to 60 p.p.m.

Tomato juice and whole fruits are generally canned in plain cans as the aroma and taste are better retained[1]; in addition, stability of ascorbic acid is better.[2] Some times, during storage, corrosion of plain cans results in

*Part of Ph.D. thesis submitted by the Senior author (L. V. K.) to the University of Agricultural Sciences, Bangalore.

dissolution of tin in the product giving it a metallic taste. Occasionally, the tin pick-up exceeds the limit laid down under Fruit Product Order. Corrosion of tin plate may be due to constituents of raw material used, process variables, tin plate quality and can size.[3] Nitrate is one such constituent of tomato which accelerates the corrosion at a very fast rate. This is especially so if the pH is below 5.5[4,5]. To prevent the heavy tin dissolution in canned tomato products, use of tomato fruits having less than 3 p.p.m. nitrate nitrogen concentration is suggested[2]. Nitrate accumulation in plants is influenced by genetic make-up of the plant, locality and nutrition[6]. In United States of America, a number of cases of severe and erratic detinning in canned tomato and other vegetables led to organization of a collaborative research programme on the problem.[7]

Present studies were undertaken to ascertain the variability in chemical characteristics and nitrate content of some leading cultivars of tomato. Tin pick-up by canned juice of a few cultivars was also studied.

Materials and Methods

Nine cultivars of tomato 'NMR', 'Pusa Ruby', 'Sioux', 'Punjab Kesri', 'Ogasta', 'Gemed', 'San-merzano', 'Roma' and 'Punjab Chhuhara' were grown during different seasons at Kukkerhalli Farm, Mysore. Fertilizers were applied at the rate of N, 100 kg+P_2O_5 100 kg and K_2O 60 kg/ha. Phosphorus and potash were applied through single super phosphate and muriate of potash respectively, six days before transplanting while nitrogen was applied through urea in three split doses as follows: (i) 20 per cent within ten days of transplanting, (ii) 40 per cent after 20 days of transplanting, and (iii) 40 per cent after 40 days of transplanting.

Estimation of certain quality attributes: Fruits of first harvest were analysed for quality attributes such as pH, total solids, acidity, ascorbic acid, lycopene and nitrate nitrogen content. Red fruits of uniform size and shape formed the sample. Each sample contained not less than ten fruits. Each observation is the mean of a minimum of two samples.

Canned studies: Juices of cultivars 'Pusa Ruby', 'Punjab Chhuhara', 'Sioux', 'Ogasta' and 'Gemed' were extracted by hot break method and canned in 1 lb jam size plain cans having a tin costing of 1 lb/base box (E-100).

Titrable acidity and alcohol insoluble solids were estimated using A.O.A.C. methods[8]. pH was determined using digital pH meter. 2-6-dichlorophenol indophenol visual titration method was employed for determination of ascorbic acid[9]. Sugars were estimated by Lane and Eynon's method[8]. Lycopene content was determined by the method of Beerh and Siddappa[10]. Viscosity was measured by using Brooke-field Synchrolectric Viscometer. Nitrate nitrogen was determined using cadmium column method[11]. Tin was estimated by the method of McKenzie[12] with modifications suggested by Gowramma *et al.*[13].

TABLE 1. CHEMICAL COMPOSITION OF SOME TOMATO CULTIVARS

Cultivar	Season	pH	Titratable acidity (%)	Ascorbic acid (mg/100g)	Sugars reducing (%)	Sugars total (%)	Sugar/acid ratio	Viscosity (centipoise)	Alcohol insoluble solids (%)	Lycopene (mg/100g)
'NMR'	Sept. 1978	3.95	0.63	19.50	1.96	2.07	3.29	90	0.63	4.95
'Pusa Ruby'	Sept. 1978	4.00	0.59	23.40	2.65	2.86	4.85	160	0.85	5.71
,,	Jan. 1980	4.00	0.58	20.20	2.88	3.02	5.21	185	*	5.84
'Sioux'	Sept. 1978	4.10	0.48	17.50	2.42	2.75	5.73	75	0.60	5.56
,,	Jan. 1980	4.05	0.43	19.00	2.55	2.78	6.47	195	*	5.68
'Punjab Kesri'	Sept. 1978	4.13	0.51	15.53	2.98	3.08	6.04	415	1.16	5.84
'Ogasta'	Sept. 1978	4.18	0.43	22.40	2.78	2.91	6.77	380	1.02	5.80
,,	Jan. 1980	4.20	0.39	18.55	2.85	2.98	7.64	400	*	5.62
'Gemed'	Sept. 1978	4.20	0.41	15.77	2.65	2.74	6.68	310	1.10	6.10
,,	Jan. 1980	4.20	0.38	16.80	2.77	2.88	7.58	285	*	5.85
'San marzano'	Sept. 1978	4.22	0.46	15.98	1.95	2.21	4.80	310	*	5.75
'Roma'	Sept. 1978	4.25	0.40	16.00	2.98	3.17	5.92	*	*	6.15
'Punjab Chhuhara'	Sept. 1978	4.26	0.40	16.10	2.67	2.81	7.02	395	1.19	5.69
,,	Jan. 1980	4.30	0.37	14.50	2.74	2.85	7.70	430	*	5.76

*=Not analysed.

Results and Discussion

Table 1 shows the chemical composition of the different cultivars. Excepting 'Pusa Ruby' (0.58 per cent) and 'NMR' (0.63 per cent), all other cultivars had acidity in the range of 0.4–0.5 per cent. pH of juices among different cultivars ranged from 3.95 in cv. 'NMR' to 4.25 in cv. 'Roma'. The consistency of juice of cv. 'Pusa Ruby', 'Sioux' and 'NMR' was thinner and these were also observed to be low in alcohol insoluble solids content. Excepting these, fruits of all other cultivars were either pear or oval shaped. Similar observations have been made in a few other cultivars by Roy and Choudhuri.[14] Except 'NMR' and 'Sanmerzano', fruits of all other cv. contained total sugars of 2.90±0.17 per cent. Reducing sugars constituted the major portion of total sugars; non-reducing sugars formed about 10 per cent in most of the cultivars. Lycopene content was higher in cv. 'Roma' and 'Gemed'.

Seasonal variation in some quality attributes was also evident in all the cultivars. In general, slight increase in acidity, ascorbic acid and decrease in sugars was observed in the fruits of the September harvest. In almost every cv, sugar/acid ratio of juice was higher for January harvest. Long photoperiod is known to favour increase in sugar content of the fruit September was marked by cloudy weather and low sunlight. High increase in soluble solids and fall in acidity in respect of spring tomatoes have been reported by Saimbi[15] in a few cv. under Punjab conditions.

None of the cv. under study in different seasons accumulated nitrate in excess of 1 p.p.m. (Table 2). Only 'Pusa Ruby' and 'Sioux' were found to contain nitrate in all the seasons. In other cv. the pattern was not consistent. From these studies in different seasons under prevalent conditions, it can be concluded that none of the cultivars has a tendency to accumulate high nitrate in fruits.

TABLE 2. NITRATE NITROGEN (P.P.M.) CONTENT IN RED-RIPE FRUITS OF DIFFERENT CULTIVARS OF TOMATOES GROWN DURING DIFFERENT SEASONS

Cultivar	May 1978	Sept. 1978	Jan 1980
'NMR'	0	*	*
'Pusa Ruby'	+	0.5	0.7
'Sioux'	0.7	0.5	1.0
'Punjab Kesri'	+	0.5	*
'Ogasta'	+	+	0.7
'Gemed'	0	0.7	0
'San merzano'	0	+	*
'Roma'	0.4	+	*
'Punjab Chhuhara'	+	+	+

+ = <0.4 ppm * Not analysed.

TABLE 3. ANALYSIS OF CANNED TOMATO JUICE OF DIFFERENT CULTIVARS

Cultivar	Storage period (days)	Can content	Vacuum (inch/Hg)	pH	Viscosity (cp)	NO_3N (ppm)	Tin (ppm)	Can interior
'Pusa Ruby'	0	338	12.5	4.00	160	0.7	XX	Normal
	30	338	13.0			—	35	VLF
	60	342	12.0			—	48	VLF
	90	340	11.0	4.00	110	—	55	LF
'Punjab Chhuhara'	0	340	13.0	4.30	430	+	XX	Normal
	30	340	12.0			—	24	VLF
	60	341	11.5			—	35	VLF
	90	345	11.0	4.25	340	—	46	VLF
'Sioux'	0	340	13.0	4.15	95	1.0	XX	Normal
	30	338	12.5			+	36	VLF
	60	338	11.0			—	52	LF
	90	340	10.5	4.10	65	—	60	VLF
'Ogasta'	0	340	12.5	4.15	410	0.7	XX	Normal
	30	345	12.0			—	28	VLF
	60	345	12.0			—	44	VLF
	90	342	9.5	4.15	330	—	56	LF
'Gemed'	0	345	12.5	4.20	310	—	XX	Normal
	30	345	13.0			—	20	VLF
	60	342	12.0			—	29	VLF
	90	340	11.0	4.15	195	—	43	VLF

1 lb jam (E 100) cans were used and stored at 37°C.

XX — Not analysed. VLF — Very light feathering. + — <0.4 ppm.
* — Pooled samples of two cans analysed for tin. LF — Light feathering. — — Nil.

No substantial loss of vacuum during storage of canned juice was observed (Table 3). Though the cv. differed in their various chemical composition, the differences in respect of tin pick-up by canned juice was very small. In none of the cv., tin pick-up by canned juice was more than 60 p.p.m. at the end of 90 days storage. Tin pick-up of this order after 90 days storage at 37°C is minimal. Initial nitrate nitrogen contents in juices of all the cv. were very low (0–1.00 p.p.m.) to accelerate corrosion. Rapid lowering of consistency could be attributed to degradation of pectin because of higher storage temperature.

Acknowledgement

The authors are grateful to the Director, Central Food Technological Research Institute, Mysore for laboratory facilities and Director of Horticulture, Karnataka State for providing field facilities to undertake the work. The kind help of Dr. Mahadeviah, Dr. Eipeson, Mr. N. S. Singh and Mrs. Gowramma of C.F.T.R.I., Mysore during this work is gratefully acknowledged.

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Statement about ownership and other particulars about the periodical entitled JOURNAL OF FOOD SCIENCE AND TECHNOLOGY as required to be published under Rule 8 of the Registration of Newspapers (Central) Rules 1956.

FORM IV

1. Place of Publication — Mysore City
2. Periodicity of the Publication — Bimonthly
3. Printer's Name — Dr. T. S. S. Rao (For and on behalf of AFST (I))
   Nationality — Indian
   Address — DFRL, Mysore-570 011
4. Publisher's Name — Dr. T. S. S. Rao (For and on behalf of AFST (I))
   Nationality — Indian
   Address — DFRL, Mysore-570 011
5. Editor's Name — Dr. N. Chandrasekhara
   Nationality — Indian
   Address — CFTRI, Mysore-570 013

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

T. S. S. Rao
Signature of the Publisher

Changes in Polyphenoloxidase and Other Endogenous Factors During Ripening in Some Banana Varieties

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Received 2 *April* 1986; *revised* 4 *August* 1986

Changes in the polyphenoloxidase (PPO) activity and level, polyphenol content, ascorbic acid and pH were studied during ripening in the pulp tissue of three banana cultivars and correlated them with their enzymatic browning potential. Generally, a combination of factors involving PPO activity and level, ascorbic acid content and pH influenced the browning potential at any particular stage of maturation and the total polyphenol content had no influence. Gel electrophoresis of the partially purified PPO and buffer soluble proteins during ripening revealed varietal differences in changes in PPO isoenzyme and protein patterns. Synthesis of new PPO isoenzymes and proteins and active changes in their pattern were apparent in the preclimacteric and climacteric tissues while the postclimacteric tissues were generally characterised by their breakdown.

Several endogenous chemical parameters are known to influence enzymatic browning in many fruits and vegetable tissues. These include polyphenoloxidase (PPO) activity and level, polyphenol content, ascorbic acid and pH. Besides being influenced by variety, all these controlling factors undergo significant changes during ripening even in the same variety. Further, isoenzyme patterns of some of the oxidative enzymes are reported to change during fruit maturation, with the disappearance of some isoenzymes and the subsequent appearance of new isoenzymes at various stages of ripening, thus suggesting a role for isoenzymes in the ripening process[1,2].

We had earlier reported significant differences in the susceptibility to enzymatic browning in five important varieties of ripe banana which were found to be influenced by the PPO activity and level, ascorbic acid content and pH of the pulp, while the total phenolic content had no influence.[3] In the present study, changes in the PPO and other important biochemical parameters were determined during ripening in the pulp tissue of three banana varieties and correlated with their browning potential. Changes in PPO isoenzyme patterns and soluble protein patterns were also studied by gel electrophoresis using the partially purified enzymes at various stages of ripeness in these varieties.

Materials and Methods

Raw materials: Following commercially important varieties of banana available in the local market were used in the studies: (i) 'Pachabale' or 'Dwarf cavendish' (*M. cavendishii*; genome AAA); (ii) 'Poojabale' or 'Poovan' (*M. paradisiaca*; AAB) and (iii) 'Rasabale' or 'Rasthali' (*M. sapidisiaca*; AAB).

Fully mature green fruits were procured and allowed to ripen at room temperature (25–30°C). Samples of pulp tissue were drawn at different stages of ripening based on skin colour, namely: green (raw), yellow green (half ripe), fully yellow (ripe), yellow with brown flecks (fully ripe) and yellow with black patches (over ripe).

Analytical methods: Soluble solids were measured as °Brix using a refractometer, total solids by drying in a vacuum oven, acidity (expressed as anhydrous citric acid) by titration with 0.1 N NaOH, pH using a pH meter, ascorbic acid by the 2, 6-dichlorophenol indophenol titrimetric method[4], total phenolics (expressed as tannic acid) by the method of Guadagni *et al.*[5], PPO by the method of Palmer[6], and protein by the method of Lowry *et al*[7]. Initial brownness and susceptibility of the tissue to enzymatic browning were determined by measuring the per cent diffuse reflectance of the ground pulp initially and after 1 hr with a reflectance meter using magnesium oxide standard to set the instrument to 100 per cent reflectance. The drop in reflectance ($\triangle$R) occurring in 1 hr was used as a measure of the browning potential.

Partial purification of PPO: Electrophoretic studies were conducted using the enzyme extracted from the pulp tissue at various stages of ripening and then partially purified by acetone precipitation as follows: Pulp (10 g) was homogenised at 0°C in a glass tissue grinder with a teflon pestle with 50 ml of 0.1 M potas-

sium phosphate buffer, pH 7.0 containing a nonionic detergent (Tween 80) at 1 per cent level. The homogenate was centrifuged at 20,000 ×g foı 15 min at 0°C. The enzyme was precipitated from the clear supernatant with 1.6 volumes (80 ml) of analar acetone at -20°C and stirred for about 10 min maintaining at -10°C using a bath containing salt-ice freezing mixture. The precipitate was collected by centrifugation at 15,000 ×g for 10 min at -10°C and dissolved in 25 ml of 0.01 M phosphate buffer pH 7.0. After standing overnight at 0°C, the solution was centrifuged at 20,000 ×g for 20 min to remove the inactive residue and the clear supernatant used for electrophoretic separation. It was maintained at 0°C until used.

Gel electrophoresis: Polyacrylamide gel electrophoresis was carried out by the method of Davis[8] using glass tubes of 5 mm inner diameter filled to 8 cm with 7.5 per cent polyacrylamide in 0.375 M Tris-HCl, pH 8.9 as the resolving gel[9] and 10 mM Tris-glycine buffer, pH 8.3 as the electrode buffer. The enzyme sample contained 10 per cent sucrose or glycerol, between 150 and 200 μg protein and bromophenol blue as tracking dye. It was layered directly on top of the resolving gel. Electrophoresis was carried out at 0-4°C initially with a current of 1.5 mA per tube for about 30 min and subsequently at 2.0 to 2.5 mA per tube for the remainder of the run with the anode at the bottom.

The gels were stained for PPO activity by first equilibrating with 0.1 per cent p-phenylenediamine solution for 30 min and then with 10 mM catechol in buffer for 30 min. Proteins were stained with Coomassie Brilliant Blue[9].

Results and Discussion

Soluble and total solids, acidity and pH: Soluble solids and moisture contents showed an increasing trend during ripening. Initially (in the raw stage) there was an increase in the acidity with a concomitant decrease in pH. As ripening progressed, there was a drop in acidity and increase in pH in 'Pachabale' and 'Poojabale' while in 'Rasabale' there was further steady decrease in pH (Table 1).

Ascorbic acid: Ascorbic acid content showed a different trend in 'Pachabale' as compared to the other two varieties. In the former, it was highest in raw and showed a steady decrease with ripening (Table 1). In

TABLE 1. CHANGES IN PPO LEVEL AND OTHER ENDOGENOUS FACTORS IN THE PULP OF SOME BANANA VARIETIES DURING RIPENING

Day No.	Ripeness*	pH	Ascorbic acid (mg/100g)	Total phenolics (mg/100g)	PPO concn. (units/g)	Browning potential (ΔR in 1 hr)
			'Pachabale'			
1	Raw	5.35	5.00	40	50.0	23
2	HR	4.90	3.00	66	47.5	24
4	Ripe	4.70	1.85	64	92.5	38
6	FR	5.20	1.23	64	105.0	31
8	OR	5.25	1.48	58	65.0	26
			'Poojabale'			
1	Raw	5.01	3.12	352	47.5	13
2	HR	4.40	6.75	536	70.0	14
4	Ripe	4.37	1.87	46	75.0	23
7	FR	4.67	0.90	48	60.0	18
9	OR	5.16	1.28	56	45.0	20
			'Rasabale'			
1	Raw	5.79	2.47	760	47.5	34
2	HR	4.48	9.01	880	62.5	29
3	Ripe	4.40	6.91	58	72.5	20
5	FR	4.12	4.66	61	65.0	12
7	OR	—	4.44	67	45.0	10

* Stage of ripeness judged by peel colour as follows:
Raw—green; Half ripe (HR)—Yellow green; Ripe—full yellow;
Fully ripe (FR)—yellow with brown flecks; over ripe (OR)—yellow with black patches.

'Poojabale' and 'Rasabale' it increased from raw to half ripe and decreased thereafter in agreement with the data already reported in literature.[10]

Total phenolics: Total phenolic content measured as tannic acid remained low and almost the same throughout ripening in 'Pachabale'. The trend was, however, markedly different in the other two varieties which recorded a several fold higher value in the raw and half ripe, then declined sharply to a significantly low value in ripe and remained almost constant thereafter (Table 1). The drop was to about one-eleventh of the half ripe value in 'Poojabale' and to one-fifteenth in 'Rasabale'.

There is no general agreement regarding the trends of tannin content in banana during ripening[11]. The "active tannin" presumed to be responsible for the astringency of the unripe fruit is reported to fall in the ripe fruit to 1/5th of its value in the green climacteric fruit.[12] The bulk of the tannins is leucoanthocyanin present in the unripe fruit as monomers or oligomers which are condensed to inactive high polymers at ripeness.[13]

PPO activity and soluble proteins: PPO activity and concentration in the pulp generally showed an increase from raw to ripe and declined in over ripe (Table 1). The increase was about 100 per cent in 'Pachabale', 58 per cent in 'Poojabale' and 55 per cent in 'Rasabale'. Buffer soluble proteins showed a steady increase from raw to over ripe. Young[14] had earlier reported no change in the activity of several banana enzymes as the fruit ripened while Montgomery and Sgarbieri[15] found the banana PPO activity, in general, to decrease with ripening in the pulp.

Susceptibility to browning: Susceptibility of the pulp to the enzymatic browning as judged by the drop in per cent reflectance after 1 hr was found to increase from raw to ripe and then decline with further ripening in 'Pachabale' and 'Poojabale' varieties while in 'Rasabale' it showed a steady decrease (Table 1).

Correlating with the changes in other biochemical parameters which influence browning, it was found that generally a combination of factors involving PPO, ascorbic acid and pH influenced the susceptibility of the pulp to browning at any one stage of maturation and no one particular factor could be attributed to exclusively influence browning. Thus, in 'Pachabale' and 'Poojabale' the maximum susceptibility at the ripe stage might be attributed to the maximum activity and level of PPO combined with a low level of ascorbic acid. Similarly, the maximum susceptibility of raw pulp in 'Rasabale' might be attributed to the exceptionally high pH of 5.8 in combination with a low ascorbic acid level and moderate PPO activity and level. The steady decrease in the susceptibility observed thereafter with maturation in the latter, in spite of the increase in PPO activity upto ripe, might be attributed again to the drop in pH of the pulp to about 4.5 at which the enzyme exhibits only 6.2 per cent of its activity at pH 7.0[3] and to a sharp increase in ascorbic acid in half ripe. Interestingly, total phenolic content, although noticed to be several fold high in raw and half ripe pulp in two varieties, did not appear to influence browning confirming our earlier findings.[3] These results differ from those of Weaver and Charley[16] who reported increase in brownness with ripening in bananas which had no correlation with PPO acitivity.

Changes in PPO isoenzyme and protein electrophoretic patterns: Changes in the PPO isoenzymes and the corresponding protein patterns were studied during ripening by electrophoresis using the partially purified PPO extract from the three banana varieties. Only the patterns obtained with "Pachabale" are given in Fig. 1.

The zymograms revealed some changes in PPO isoenzyme pattern from raw to ripe. Both the PPO isoenzyme and protein bands were generally sharp and clear in raw to ripe but showed tendency to get smudged and diffused with the disappearance of some of the bands and overlapping of others, as the maturation progressed to fully ripe and over ripe stages. This was possibly due to proteolysis and tissue breakdown associated with senescence. This was particularly obvious in 'Pachabale' and 'Rasabale' while in 'Poojabale' the activity and protein patterns were more stable and did not show much of breakdown in over ripe stage in conformity with its longer keeping quality observed.

Considering the isoenzyme patterns of 'Pachabale' PPO during ripening (Fig. 1(a)), it is seen that the fastest moving bands H and I on the zymogram were the only ones seen consistently throughout the maturation process. The bands A to G persisted with almost the same intensity from raw to ripe and then decreased in intensity progressively at fully ripe and over ripe stages. Band A could not be seen in fully ripe and over ripe while D and E showed considerable overlap and smudging in over ripe. An additional band C_1 of low intensity was seen in half ripe fruits which disappeared with further maturation. Similarly, in 'Rasabale' the bands D, E, F and H were seen consistently throughout. While A was absent in fully ripe and over ripe fruit, B and I were absent in raw but appeared with further maturation. There was overlapping and smudging of bands B and C and disappearance of bands A, G and 9I in over ripe indicating breakdown of some of the isoenzymes.

In 'Poojabale' the bands D to H were seen consistently throughout. Band B was not seen in raw but appeared to develop later while band I was present only in raw and disappeared with subsequent maturation. Band A was absent in half ripe and ripe.

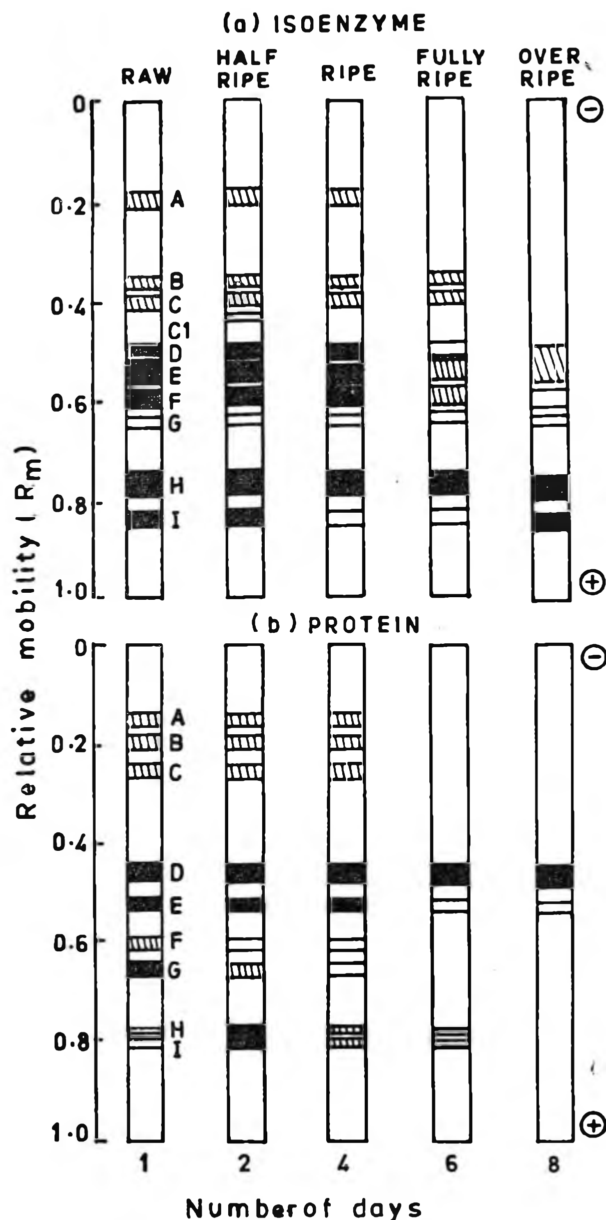

Fig 1. Gel electrophoretic (a) isoenzyme and (b) protein patterns of partially purified banana PPO during ripening.

Variety: "Pachabale" or Dwarf cavendish (*M. cavendishii*)
INTENSITY: High; Medium; Low

Considering the protein patterns of 'Pachabale' (Fig. 1(b)) the pattern was almost identical from raw to ripe after which there was considerable breakdown with disappearance of the bands A, B, C, F and G in fully ripe fruit and further loss of H and I in over ripe fruits. Bands D and E were the only ones seen consistently throughout the maturation process. In 'Rasabale' bands D and G were seen throughout the maturation with equal intensity, while bands A, B and E persisted with varying intensity. Band C was present only in raw and disappeared later while F was absent in raw and over ripe fruits. Band H increased in intensity in half ripe fruit and appeared as two close sharp and clear bands H and I with further maturation. Over maturation was marked by smudging of bands A and B and disappearance of F.

In contrast, the protein pattern of 'Poojabale' PPO extract showed considerable difference with appearance of new bands at E_1 and G_1 with maturation and no smudging indicative of breakdown could be seen in over ripe fruits. The pattern showed some further distinguishing features in that band C was seen with high intensity throughout the maturation while bands A and B were of high intensity in the raw and persisted with lesser intensity throughout.

As seen from the above data, there are varietal differences in the changes in PPO isoenzymes and soluble proteins in bananas during ripening and in their breakdown with senescence. Synthesis of new isoenzymes and active changes in their pattern were apparent in the preclimacteric (raw) and climacteric (half ripe and ripe) tissues while postclimacteric tissue was generally characterised by breakdown of the isoenzymes and tissue proteins.

Our results differ from those of Montgomery and Sgarbieri[15] who reported no change in the electrophoretic patterns of PPO isoenzymes of banana as it ripened, but are in agreement with those of Cash *et al*[2]., who found the PPO isoenzymes in the crude extract of concord grapes to change during ripening. The latter noticed appearance of new bands at late inception and verosion and disappearance of some at verosion to over ripe. Flurkey and Jen[17] likewise reported several apparent forms of PPO and constant interchanges of these forms during development of peach fruit.

There is evidence that in banana fruit pulp tissue, the rate of protein synthesis declines progressively from the initiation of the climacteric period[18]. This suggests that ripening in banana results primarily from a decline in intracellular organisation and the consequent action of enzyme systems preexisting in the cells[19]. Brady *et al.*[19] prepared pulp extracts from banana fruits at six stages of ripeness from preclimacteric (green) to yellow ripe and separated the proteins by gel electrophoresis. The gel pattern indicated a progressive change in the soluble proteins during the climacteric (ripe).

Acknowledgement

The authors are thankful to Dr. P. K. Vijayaraghavan, former Director, DFRL, Mysore and Prof. C. S. Vaidyanathan, Dept. of Biochemistry, Indian Institute of Science, Bangalore for their valuable guidance and helpful suggestions. They also thank Dr. T. R. Sharma, Director, DFRL, Mysore for kind permission to publish this paper.

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Studies on the Pectin Methylesterase Activity during Cold Storage of 'Patharnakh' Pear

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Received 26 *February* 1985; *revised* 5 *August* 1986

The effect of cycocel (2000 and 4000 ppm), gibberellic acid (100 and 200 ppm) Cacl$_2$ (4 and 6%) and wax emulsion (6 and 9%) treatments and date of harvest on pectin methylesterase activtty (PME) during cold storage of 'Pathernakh' pear fruits was investigated. Fruit firmness decreased whereas PME activity increased with the delay in harvest and during storage. Application of 200 ppm gibberellic acid and 4000 ppm cycocel increased the PME activity. The former treatment exhibited the highest PME activity. The date of harvest showed significant effect on fruit firmness and PME activity. Firmness was maximum and PME activity was minimum in the fruits harvested in the second and third week of July.

'Patharnakh' pear (*Pyrus pyrifolia* (Burm) Nakai) is the commercial cultivar of pear in Punjab and is very hardy. Hultin and Levine[1] reported that change in quality banana fruit including softening of the flesh is due to the activity of pectin methylesterase (PME). Nagel and Patterson[2] reported that PME activity decreased during development of pear. Decline in PME in pears was also observed throughout harvest and during storage[3].

The present investigations were undertaken to see

the effect of different treatments and date of harvest on PME and its relation to the softening of 'Patharnakh' pear fruit.

Materials and Methods

The fruits were collected from 14-year-old trees growing at the Regional Research Station, Bahadurgarh in Patiala. They were collected randomly from all sides of the trees at weekly intervals over a 22-day period beginning from July 22 in 1980 and July 15 in 1981. The fruits were kept in the cold storage, after giving the following treatments:

(1) Cycocel (CCC) 2000 and 4000 ppm, (2) gibberellic acid (GA) 100 and 200 ppm, (3) calcium chloride ($CaCl_2$) 4 and 6 per cent, (4) wax emulsion 6 and 9 per cent.

Thirty fruits were dipped separately for 2 min. in cycocel, gibberellic acid and wax emulsion, but only for 30 min in $CaCl_2$. Tween-80 was used as sticker. Ten fruits were packed in each polythene bag of 100 gauge having 35 cm×25 cm size with 30 perforations. These were punched (one hole in each of 2.5 to 3 sq.cm area) to facilitate gas exchange. Each treatment comprised 3 bags having 30 fruits and these bags were closed by stapling. These polythene bags were placed in ventilated wooden crates and were kept in a cold storage at temperature 0 to 3.3°C and relative humidity of 85-90 per cent. The fruits were removed after 100 days in cold storage and were analysed for PME activity.

Preparation of extract: For enzyme extraction, the fruit samples were stored immediately after harvest in a freezer at 0°C. Two g fruit pulp was subsequently ground in a chilled mortar with 10 ml of phosphate buffer (pH 6.5, 0.1 M). Polyvinyl pyrolidine was added along with the extraction buffer to avoid inactivation of the enzyme by the phenolic constituents. The extracts were centrifuged at 10,000 rpm for 10 min in a refrigerated centrifuge at 0°C and the enzyme activity determined immediately.

The method of Dingle *et al.*[4] was followed for the estimation of PME by measuring the increase in acidity after hydrolysis of pectin by the enzyme preparation. The PME activity was calculated as the micro or milli equivalents of methoxyl groups liberated per hr by one ml of the enzyme preparation under specified conditions of assay. Total soluble proteins were estimated by the method of Lowry *et al.*[5]

Firmness: Fruit firmness was measured with the help of "Fruit Tester" Penetrometer (made in Italy) after removing about one square inch of skin. The diameter of the tip of the punch was 0.8 cm.

Results and Discussion

Pectin methylesterase: The PME activity as affected by different treatments and date of harvest for two fruiting seasons is given in Tables 1 and 2. Post-harvest

TABLE 1. EFFECT OF DIFFERENT TREATMENTS ON THE PECTIN METHYLESTERASE (PME) ACTIVITY AND FIRMNESS OF FRUITS AFTER 100 DAYS OF COLD STORAGE

Treatment		PME (meq/hr/mg protein)		Firmness (kg/in^2)	
Chemicals	Concn (ppm / %)	1980	1981	1980	1981
CCC	2000	0.011	0.013	6.47	7.70
CCC	4000	0.013	0.016	6.30	7.64
GA	100	0.012	0.012	6.52	7.94
GA	200	0.015	0.016	6.26	7.40
$CaCl_2$	4%	0.010	0.012	5.90	7.58
$CaCl_2$	6%	0.012	0.014	6.19	7.67
Wax Emulsion	6%	0.011	0.012	5.90	7.35
Wax Emulsion	9%	0.007	0.009	5.21	6.99
Control		0.009	0.010	5.31	7.25
C.D at 5%		0.004	N.S.	0.38	0.37

TABLE 2. EFFECT OF DATE OF HARVEST ON THE PECTIN METHYLESTERASE (PME) ACTIVITY AND FIRMLESS OF FRUITS AFTER 100 DAYS OF COLD STORAGE

Harvest date	PME (meq/hr/mg protein)		Firmness (kg/in^2)	
	0	100	0	100
		1980		
July 22	0.002	0.005	8.85	6.39
July 29	0.004	0.008	8.70	6.01
August 5	0.007	0.013	8.50	5.86
August 12	0.008	0.018	8.27	5.76
C.D at 5% (d.f. 16)		0.003		0.25
		1981		
July 15	0.001	0.007	9.00	7.81
July 22	0.005	0.010	8.87	7.64
July 29	0.008	0.015	8.67	7.40
August 5	0.009	0.019	8.40	7.15
C.D at 5% (d.f. 16)		0.005		0.24

treatment of fruits with CCC, GA, $CaCl_2$ and 6 per cent wax emulsion resulted in more activity than in the control samples after 100 days of cold storage. The highest activity was recorded with 200 ppm GA followed by 4000 ppm CCC and 6 per cent $CaCl_2$ treatments whereas that with 9 per cent wax emulsion recorded lower activity than the control. The probable reason for low activity in the 9 per cent wax emulsion and control treatment is the rotting of the fruits under these treatments. A decrease in PME activity in the senescent tissue of 'Barlett' pear fruit was reported by Ahmed and Labavitch[6]. Nagel and Patterson[2] also reported similar results on pears. In the present study, the increase in PME activity during cold storage is, however, not in agreement with the results reported by Ben-Arie *et al*[3]. who observed 4–5 times increase in polygalacturonase activity but decrease in the PME activity in 'Spadona' pear after 15 weeks of cold storage.

The date of harvest significantly affected the PME activity during both years of the study. The PME activity increased with the delay in the date of harvesting during 1980 and as also after 100 days of cold storage during both years under study (Table 2).

Fruit firmness: The fruit firmness decreased with the delay in harvest and during cold storage with the increased PME activity (Tables 1 and 2). Reduction in the flesh firmness of 'Patharnakh' pear with the pre- and post-harvest application of $CaCl_2$ and post-harvest application of CCC, GA and wax emulsion was also observed by Bhullar *et al.*[7] Date of harvest exhibited a significant effect on fruit firmness which decreased with delay in the harvest during both years of the study. Keeping the fruit for 100 days in cold storage resulted in decreased fruit firmness. Similar results have been reported in case of 'Anjou' pear[8] and in 'Moltke' pear; the fruit firmness after removal from the storage decreased with the delay in harvest[9]. The best time for harvesting 'Patharnakh' pear for cold storage appears to be the second fortnight of July.

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Milk Scale Deposition on Heated Surface Under Laminar Flow

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Received 30 *September* 1985; *accepted* 10 *July* 1986

Milk scale deposition on heated surface under laminar flow conditions was studied. The experimental system consisted of an electrically heated stainless steel section of 22.86 mm diameter fitted concentrically to a 25.4 mm diameter glass tube. Experiments were conducted on fresh cow's milk. Results have shown that the deposition of milk began after an average induction period of 12.18 min. The rate of deposition decreased exponentially with increase in Reynolds number. The thermal conductivity and density of milk scale were also evaluated and the values were 0.0043 W/m-K and 1.3889 g/cm³ respectively. The milk scale contained 35–40 per cent protein, 12–13 per cent fat and 22 per cent ash.

When milk is heated in heat exchangers, deposits form on the hot surfaces. These deposits are of considerable interest to the dairy industry. If the routine cleaning is not adequate to remove the deposits, they

build up and form a hard layer, generally called scale. Removal of the scale is an important and difficult part of cleaning operation, and if not removed, it becomes the common source of high thermoduric count of pasteurised milk. Furthermore, scales are known to constrict the flow passage, thereby resulting in higher pressure drop and reduced product flow rate. In addition, the rate of heat transfer is greatly reduced. Quantitative relationship defining the deposition of milk solids and formation of scale as a function of operating parameters including time are, however, lacking. The effect of such deposits is usually neglected in designing milk processing equipment. This assumption may lead to poor performance of the equipment under most of the practical situations. All these effects of milk scale deposits on heating surface can, however, be better understood if the deposition rate kinetics is fully known. But it is difficult to study the rate kinetics and variables involved in deposition process in a commercial plant where thousands of litres of milk are processed everyday. Most of the studies conducted in this area pertain to static conditions. Information on milk solids deposition on heated surface under dynamic conditions of flow is limited[1-3]. As a first step towards a better understanding of the fouling process under dynamic conditions, an investigation was carried out and this paper describes the results of such an investigation.

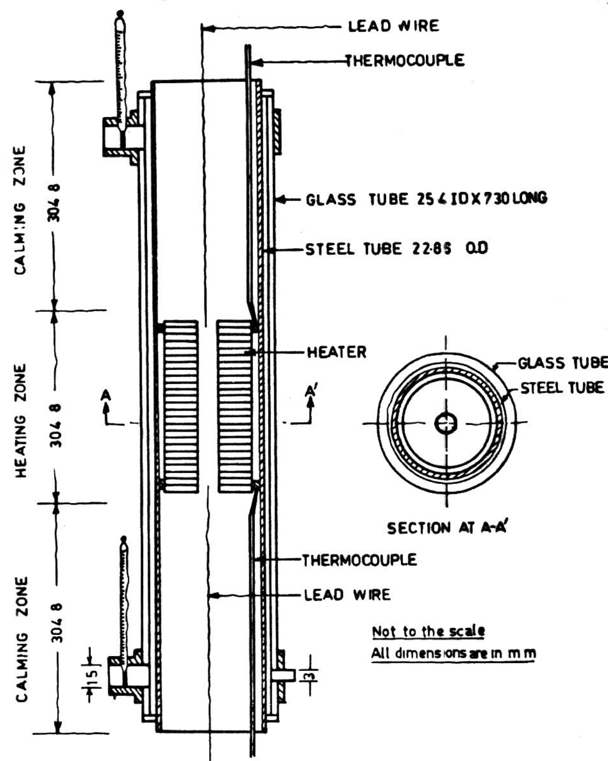

Fig 1. Detailed dimensions of the heating section

Materials and Methods

The experimental setup consisted of test section, feed tank, variable speed pump, dimerstat, voltmeter, microvotlmeter, thermoflask, manometer and a multimeter (Fig. 1). In most of the milk processing plants, plate heat exchangers are widely used. It is, however, difficult to fabricate the laboratory model of plate heat exchanger since it requires special type of facilities for making and assembling the plates. Therefore, experimental set up with annular heating section was preferred. The experimental set up was so designed that (i) thickness of the annular flow passage was equal to the gap generally maintained between two plates of a plate heat exchanger[3], (ii) calming zone with length greater than fifty times the equivalent diameter of the annulus was available to ensure fully developed flow conditions in the test section, (iii) the heating surface both for the test section and calming zone was made of stainless steel, and (iv) the system permitted easy dismantling and assembling and also visual observation of the flow in the test section.

The test section consisted of an electrically heated stainless steel tube of 22.86 mm outer diameter fitted on the central axis of a 25.4 mm inner diameter glass tube. This gave an annular clearance of 1.27 mm. Heating in the test section was done through an electric heating rod of length 22.6 mm placed concentrically inside the heating section.

All the experiments were conducted on fresh cow's milk. The system was first allowed to stabilize at the desired operating conditions by circulating distilled water through the system which took about 10–15 min. In the actual experiment, milk preheated to 343 K was used. The duration of each test run was in the neighbourhood of 100 min.

Milk inlet and outlet temperatures were measured using mercury-in-glass thermometers. Difference between the inlet and outlet temperatures varied from 1.1 to 4.8 K. Wall temperatures on the inner surface of heating section were measured through copper-constant thermocouples. The temperature of the surface in contact with milk was calculated using the relationship,

$$T_o = T_1 - \frac{P\left(\ln \frac{r_2}{r_1}\right)}{2LK\pi} \quad . \quad . \quad . \quad (1)$$

where,

T_o=outer surface temperature, K
T_1=inner surface temperature, K
P=power supplied, W
r_2, r_1=outer and inner radii of stainless steel tube, m
k=thermal conductivity of stainless steel, W/m–k
L=length of heating section, m.

The average difference between the heating surface and milk temperature in the test section ranged from 22.9 to 32.9 K. The mass flow rate of milk was regulated through a stainless steel valve fitted in the outflow line of the sanitary pump feeding the system. The rate of flow was measured by collecting the milk in a weighed glass container over a known period of time. Eight levels of milk flow rates in the range of 42 to 157 kg/hr were selected. The upper limit of the flow rate corresponds to a Reynolds number of 1555 which ensured that the flow was always in the laminar region. The pressure drop through the system was measured using a U-tube monometer. All observations were recorded at an interval of 10 min.

The deposits were collected after each test run and weighed. The length and thickness of deposit layer were also measured. The deposits were analysed by AOAC[4] methods.

Results and Discussion

Nature of deposits: The milk solids deposited on the heating surface formed a thin layer and were creamy white in colour. These deposits, however, did not form over the entire length of the test section. The length and thickness of deposit layer varied with the mass flow rate of milk (Table 1). Maximum thickness was in the middle and the variation in the deposit thickness was very small from the middle towards either end of the test section. The representative thickness of deposits was taken to be the average of the thickness measured at three points i.e. middle and at the ends. The deposits were collected after each test run and weighed. They were also analysed for chemical composition and contained (in per cent) 35–40 protein, 12–13 fat and 22 ash; ash contained 25 per cent calcium and 15 per cent magnesium.

Pressure drop: Milk deposits constricted the narrow flow passage and resulted in increase in pressure drop. For an initial period of 10 to 15 min the pressure drop remained constant. Beyond this, there was a progressive increase in the pressure drop, which was measured at constant mass flow rate. This is in agreement with the findings of Burton[5] and Thom[6]. An equation could be derived to calculate the deposit layer thickness from the pressure drop measurements. Using the expressions of Bird *et al*[7]. for average velocity and mass flow rate in steady state incompressible fluid flow through an annulus, the expression for Reynolds number in terms of mass flow rate is

$$Re=\frac{2\,Qm}{\pi R\mu(2-\epsilon)} \qquad \ldots\ldots (2)$$

Where,

Qm = mass flow rate
R = inner radius of outer tube
μ = viscosity of liquid
ϵ = a dimensionless parameter

The quantity $R\epsilon$ represents the gap thickness of the annulus. As deposits occur, this gap is reduced i.e. ϵ decreases. Thus, ϵ becomes a measure of deposit thickness. Clearly, $R\epsilon$ is a function of time because of ϵ.

For laminar flow, friction factor f, is a known function of Re i.e.

$$f=\frac{16}{Re}$$

and can be calculated using the following equation derived from the equations reported by Bird *et al*[7].

TABLE 1. CHARACTERISTICS OF MILK DEPOSITED INFLUENCED BY REYNOLDS NUMBER

	Reynolds number (Re_0)	Density of milk scale (g/cm³)	Thermal conductivity of milk scale ($K_d \times 10^{-3}$) (W/m-k)	Deposit layer		Induction period (min.)
				Thickness (mm)	Length (cm)	
	428.72	1.2640	5.77	0.220	12.7	11.77
	515.99	1.3491	3.95	0.175	12.0	12.29
	750.44	1.3912	3.71	0.125	11.2	12.31
	851.28	1.5000	4.90	0.095	10.5	17.28
	955.44	1.3041	5.16	0.085	10.2	10.66
	1073.38	1.3782	4.78	0.056	10.0	13.89
	1438.47	1.3711	3.89	0.023	9.5	9.51
	1554.37	1.5521	2.51	0.019	9.0	9.73
Mean		1.38891	4.33	—	—	12.18

$$f=\frac{R\epsilon\Delta P}{\rho V^2} \quad \ldots\ldots (4)$$

where,

ΔP=pressure drop per unit length, N/m^2

V=average velocity of milk flow, m/s

ρ=density of milk, kg/m^3

In terms of mass flow rate.

$$f=\frac{\pi^2\rho\Delta pR^5\epsilon^3(2-\epsilon)^2}{Q^2m} \quad \ldots\ldots (5)$$

For the initial condition when scale thickness is zero,

$$f_o=\frac{\pi^2\rho\Delta p_oR^5\epsilon_o^3(2-\epsilon_o)^2}{Q^2m} \quad \ldots\ldots (6)$$

The variation of friction factor f_o with Reynolds number for clean surface has been shown in Fig. 2. The data could be represented by eq. (3). This confirms that the flow was fully developed laminar flow.

Combining equations (3) and (5)

$$\Delta P=\frac{F}{\epsilon^3(2-\epsilon)} \quad \ldots\ldots (7)$$

Where F is defined as

$$F=\frac{8\mu Qm}{\rho\pi R^4}$$

Since for each experiment Q_m was fixed and temperature variation was small, F could be taken as constant. For initial conditions,

$$\Delta P_o=\frac{F}{\epsilon_o^3(2-\epsilon_o)}$$

$$\text{or } \frac{\Delta P_o}{\Delta P}=\frac{\epsilon^3(2-\epsilon)}{\epsilon_o^3(2-\epsilon_o)} \quad \ldots\ldots (8)$$

For clean surface, ϵ_o=0.1. The value of ϵ corresponding to the maximum deposit, was found to be 0.084. Since the factor $(2-\epsilon)/(2-\epsilon_o)$ is almost unity the eq (8) modifies to

$$\left(\frac{\Delta P_o}{\Delta P}\right)^{\frac{1}{3}}=\frac{\epsilon}{\epsilon_o} \quad \ldots\ldots (9)$$

The equation (9), therefore, gives an indirect measure of deposit thickness.

Deposition kinetics: From measurements on pressure drop, the time variation of ϵ/ϵ_o was known. The variation of $(1-\epsilon)$ R could be calculated. The average value of density of scale ρ_d was calculated from the known values of mass and volume of deposits. Knowing the average value of P_d, the variation of mass deposited per unit length on the surface could be represented by

$$M=\pi R^2\rho_d[(1-\epsilon)^2-(1-\epsilon_o)^2] \quad \ldots\ldots (10)$$

Where M is the mass deposited per unit length.

The quantity M when plotted against time yielded a linear relationship. It is clearly evident that upto an initial period of 10 to 15 min, there was no significant change in M with time. This period has been termed as induction period[8]. The induction period for the different mass flow rates was not the same. The values changed randomly from 9.51 to 17.28 min. An average value of 12.18 was used in subsequent analysis. Using this average value, a variable $Z=(\theta-\theta_o)$ was defined. In terms of Z

$$M=mZ \quad \ldots\ldots (11)$$

Where m is the mass rate of deposition per unit length.

The relationship is shown in Fig. 3. The effect of Re on mass rate of deposition per unit length has been shown in Fig. 4 which followed the relationship

$$m=0.0512\exp(-0.00188\,Re_o) \quad \ldots\ldots (12)$$

Mass flux: Mass flux of the deposits, per unit length i.e. Jm, which is an important parameter in quantitative estimation of scale deposits could be computed as follows. By fundamental definition

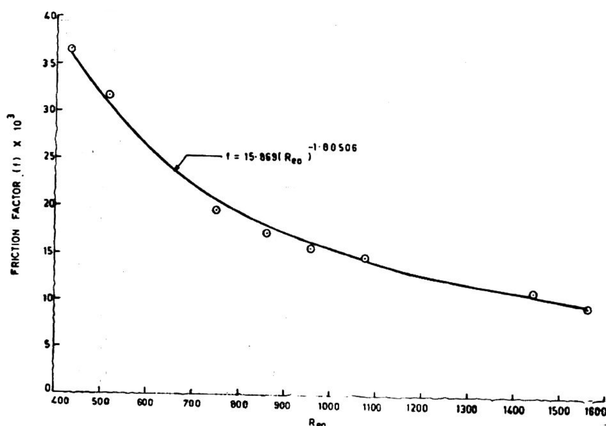

Fig 2. Variation of friction factor (f) with Reynolds number

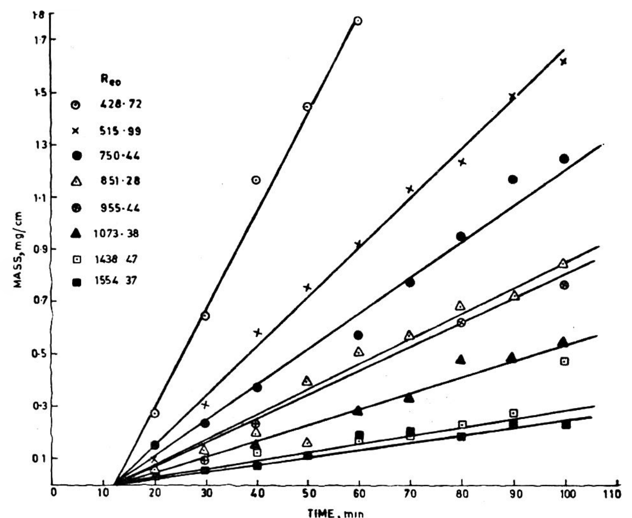

Fig 3. Kinetics of milk solids deposition at an average surface temperature of 365.11 K

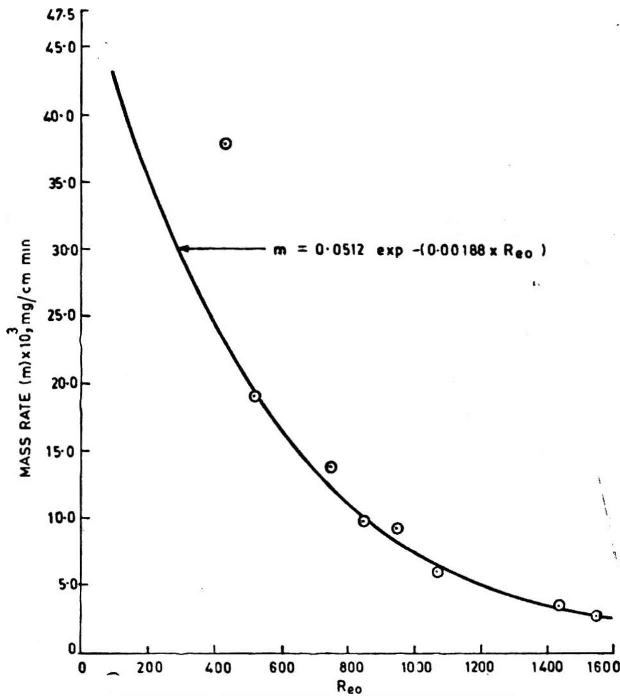

Fig 4. Variation of mass rate of milk solids deposit with Reynolds number

$$jm = \frac{\text{mass rate/unit length}}{\text{surface area of deposition}}$$

$$= \frac{m}{2\pi R(1-\epsilon)} \quad \ldots\ldots (13)$$

From equation (8) and (9), $R(1-\epsilon)$ becomes

$$R(1-\epsilon) = \left[\frac{mZ}{\pi \rho_d} + R^2(1-\epsilon_o)^2\right]^{\frac{1}{2}}$$

$$\text{or } jm = \frac{m}{(BmZ+C)^{\frac{1}{2}}} \quad \ldots\ldots (14)$$

where B and C are constants as given below

$$B = \frac{4\pi}{\rho_d} = 0.009$$

$$C = 4\pi^2 R^2 (1-\epsilon_o)^2 = 51.576$$

Thermal conductivity of milk scale.

An approach has been suggested for computation of the thermal conductivity of milk scale. For this, heat transfer coefficient was calculated using the basic heat transfer equation

$$h = \frac{q}{A(\Delta t)\ln} \quad \ldots\ldots (15)$$

where,

h = heat transfer coefficient, W/m^2–K

q = heat flow into milk, W

A = heat transfer surface area, m^2

$(\Delta t)\ln$ = logmean temperature difference, K

Due to formation of scale heat transfer coefficient, h increased with time. The time variation of h obeyed the equation

$$\frac{1}{h} = \frac{1}{h_o} + \frac{mZ}{2\pi R(1-\epsilon_o)\rho_d k_d} \quad \ldots\ldots (16)$$

where k_a is the thermal conductivity of milk scale W/m–k.

From plots of 1/h versus Z in terms of Eq (16), values of $1/h_o$ and k_d could be computed. The value of h_o being a function of Re, is different for different set. But k_d, being a property of material deposited, must be the same for all experimental runs. In practice, the calculated values of k_d fluctuated slightly with an average value of 0.0043 W/m–k. None of the earlier studies on milk scale formation have reported the thermal conductivity of scale. Therefore, the value of k_d could not be cross-checked.

Milk solids deposition kinetics on heated surface under laminar flow in the present case, has shown that the deposition begins after an average induction period of 12.18 min. The developed equation for evaluating the deposit layer thickness, which is based on pressure drop measurement, is very simple and can be tried for other types of surface configurations. The mass flux equation derived using the induction period concept gives a quantitative estimation of scale deposition on the surface. The estimated values of thermal conductivity provides information on one of the important input parameters for design of heat exchangers.

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Meat Yields and Quality Characteristics of Meat from Spent Hens of White Leghorn and Rhode Island Red Breeds

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Received 2 *December* 1985; *revised* 7 *July* 1986

Meat yields and quality characteristics of deboned meat from spent hens of 'White Leghorn' (WL) (light) and 'Rhode Island Red' (RIR) (heavy) breeds were compared. RIR yielded significantly higher deboned meat than WL (light) and WL heavy type (equal live weight as that of RIR). WL heavy type yielded significantly higher fat content than the other types. The most economic components, deboned meat and total edible meat could be well predicted from either live or dressed weights which are highly correlated in both the breeds. Deboned meat from RIR showed significantly higher water holding capacity, emulsifying capacity and lower collagen content than WL. Due to the higher meat yield and better quality, RIR spent hens should demand better price than WL in the market.

Availability of spent hens belonging to different breed types has increased in recent years due to rapid developments in Indian poultry industry. Development of further processed products from the spent hens' meat would be the most profitable way of utilizing this class of poultry with tough meat[1-3]. Meat yield and quality characteristics of meat is essential for utilization of any meat in further processed products. Besides, edible meat yield is an important criteria that determines the price of birds.

Effect of breed, strain and age on carcass components and meat yields in broiler was reported by many workers[4,5]. Dressing percentage was reported to be higher in heavy breeds than in light breeds[6]. Higher dressed yields and total meat yields were reported in broiler breed spent hens (twice heavier) than light breeds[7]. Very little information is available on the comparison of meat yields and quality characteristics of meat from spent hens of egg type breeds. In the present study, carcass components from spent hens of 'White Leghorn' (WL) and 'Rhode Island Red' (RIR) breeds and WL heavy type (similar body weight as that of RIR) were investigated for evaluating the comparative merit of the three breed types. Besides, the quality characteristics of hot deboned meat from both the breeds were analysed.

Materials and Methods

Spent hens: Spent hens of above 500 days old available from a research farm were utilized in the study. Data on various carcass components were collected on 12 birds from each of the 3 breed groups. White Leghorn (WL) and Rhode Island Red (RIR) spent hens were selected at random and WL heavy type (WL birds of similar body weight as that of RIR). The birds were fasted for 15 hr, live weight was recorded, slaughtered, hand dressed and dressed weight was recorded.

Carcass components: Weight of various components were recorded after hand deboning the dressed birds in hot condition within 3 hr of slaughter. Skin was removed from all over the body including shoulder part of the wing. Separable fat included abdominal fat and subcutaneous fat. Breast meat was from the breast and leg meat included meat from thigh and drumstick. Wing and back meat included meat from shoulder part of the wing and back of the carcass. Deboned meat was arrived at by adding breast meat, leg meat and wing and back meat. Total meat included deboned meat, skin, fat, gizzard, heart and liver. Deboned frames were pressure cooked to obtain cooked meat (meat left over when deboned meat was separated), chicken oil and cooked skin which were added to total meat to obtain total edible meat.

Meat quality evaluation: Deboned meat consisting of the meat from breast, legs, wings and back from 6 birds belonging to each of WL and RIR breeds was individually packed in polyethylene bags and frozen at −10°C for 6 days. The frozen meat thawed at 5°C for 15 hr and fine minced (once coarse mincing through 0.8 cm plate and then through 0.5 cm plate) was analysed for the physico-chemical characteristics as per

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the procedures reported earlier[8]. The data were analysed statistically and means were compared[9,10].

Results and Discussion

Carcass components: Carcass components and yields from the 3 breed groups are presented in Table 1. Significant differences between breed groups were observed for the components leg meat, deboned meat, deboned meat per cent of live and dressed weight and fat per cent of dressed weight. Deboned meat expressed as weight or per cent yield of live or dressed weights was significantly higher in RIR (heavy) breed. This showed that a unit weight of live bird or dressed carcass from RIR spent hen resulted in a significantly higher deboned meat yield than WL spent hen.

No significant difference was observed in deboned meat yield between the light and heavy types within the WL breed. However, the heavy type WL yielded significantly higher fat per cent of dressed weight than the other types. This has indicated that heavy type WL yielded relatively less deboned meat but more fat. Dressing percentage was relatively higher in RIR compared to WL and this is in agreement with the findings of Peters[6]. Yolk weight was relatively lower in heavy type of WL indicating a relatively lower level of egg production.

Breast meat weight did not differ significantly between the breed groups but leg meat weight was significantly higher in RIR. Breast meat when expressed as per cent dressed weight was significantly higher in light type WL compared to heavy type WL. Leg meat as per cent dressed weight was significantly higher in RIR than other types. This has shown that the increased deboned meat yield in RIR was due to the increased leg meat yield. And it was a desirable feature as dark meat was reported to have higher emulsifying capacity than light meat[11,12]. Total meat by weight or when expressed as per cent dressed weight or live weight was higher in RIR compared to both types of WL but the difference was not significant due to high variability. Deboned meat as per cent of meat total was also higher in RIR than other types.

TABLE 1. CARCASS COMPONENTS AND YIELDS FROM WHITE LEGHORN (WL), RHODE ISLAND RED (RIR) AND WL HEAVY BREED TYPE SPENT HENS

Components/yields	WL	RIR	WL heavy[1]
Live wt (g)	1547±70	1754±84	1754±87
Dressed wt (g)	1025±42	1208±64	1164±61
Liver wt (g)	41.17±2.29	32.17±1.83	35.92±3.23
Gizzard wt (g)	25.25±1.11	26.08±1.33	24.42±1.64
Heart wt (g)	7.67±0.33	8.50±0.45	7.58±0.36
Breast meat, (g)	158.33±4.70	172.83±8.75	158.50±4.90
Leg meat, (g)	157.42±6.61[a]	232.00±9.19[b]	176.67±6.19[a]
Wing+back meat, (g)	63.58±4.07	78.17±5.84	77.92±6.81
Skin wt, (g)	113.25±7.21	141.75±11.50	118.25±6.53
Fat wt, (g)	68.42±11.72	95.08±27.31	147.83±31.18
Ova wt ,(g)	40.75±7.62	32.33±5.81	24.63±4.39
Deboned frame wt, (g)	402±22.04	408±13.57	374±19.16
Dressing (%)	66.41± 0.85	68.82±1.19	66.29±0.92
Deboned meat, (g)	379±12.80[a]	483±19.55[b]	413±14.60[a]
Deboned meat % dressed wt.	37.18±0.79[a]	40.26±0.66[b]	35.95±1.15[a]
Deboned meat % live wt.	24.69±0.50[a]	27.67±0.51[b]	23.79±0.69[a]
Deboned meat % total meat	59.96±1.32	62.66±1.83	56.63±2.40
Total meat, (g)	636±27	786±56	747±49
Total meat % dressed wt.	62.09±0.73	64.59±1.23	63.90±1.17
Total meat % live wt.	41.26±0.84	44.55±1.37	42.31±0.73
Breast meat % dressed wt.	15.58±0.41[a]	14.38±0.40[ab]	13.84±0.49[b]
Leg meat % dressed wt.	15.41±0.36[a]	19.37±0.40[b]	15.44±0.64[a]
Skin % dressed wt.	11.16±0.34	11.37±0.74	9.90±0.37
Fat % dressed wt.	6.52±1.04[a]	7.01±1.52[a]	11.85±2.05[b]

[1] WL spent hens of similar live wt as that of RIR.
Figures with the same superscript in a row do not differ significantly ($P \leq 0.05$).

TABLE 2. CORRELATIONS AMONG SOME OF THE CARCASS COMPONENTS IN 'WHITE LEGHORN' (WL) AND 'RHODE ISLAND RED' (RIR) BREEDS

Carcass components	Live wt		Dressed wt		Deboned meat		Deboned meat % d. wt		Total edible meat	
	WL	RIR	WL	RIR	WL	RIR	WL	RIR	WL	RIR
Live wt	—	—	.95	.95	.92	.93	-.45	-.64	.92	.93
Dressed wt (d.wt)	.95	.95	—	—	.86	.96	-.62	-.71	.94	.98
Skin wt	.86	.62	.91	.79	.82	.69	-.51	-.75	.92	.81
Fat wt	.23	.88	.46	.93	.14	.86	-.67	-.71	.46	.96
Gizzard wt	.80	.50	.84	.45	.61	.40	-.72	-.42	.66	49.
Bone wt	.78	.53	.62	.34	.62	.40	-.24	-.07	.52	.30
Fat % (d. wt)	.10	.82	.32	.87	.04	.79	-.58	-.69	.33	.91

Correlation coefficients $>.576$ are significant at $P<0.05$; $>.708$ are significant at $P<0.01$.

Correlation: Correlations among some of the carcass components in both the breeds are presented in Table 2 Live weight and dressed weight were highly positively correlated with deboned meat and total edible meat in both the breeds but negatively correlated with deboned meat expressed as per cent dressed weight. This has indicated that per cent deboned meat yield increased relatively with decrease in live or dressed weights. This was due to the increased fat per cent with increased live or dressed weights. Total edible meat when expressed as per cent dressed weight was better correlated with live and dressed weights in RIR than WL showing that the waste was relatively lower in RIR with unit increase in live or dressed weights. This was confirmed from the fact that bone weight was significantly correlated with live weight, dressed weight and deboned meat in WL but poorly correlated in RIR.

Dressing percentage was negatively correlated with all traits in WL while it was positively correlated in RIR. But in both the breeds, poor correlations were observed. Fat weight and fat per cent dressed weight were highly correlated with all other traits in RIR. This fact could be utilized for predicting fat yield from live or dressed weights and similarly deboned meat and total edible meat from fat yield. Gizzard weight was significantly correlated with many traits in WL but poorly correlated in RIR. Skin weight was significantly correlated with most of the traits in both the breeds.

Meat quality: The physico-chemical characteristics of meat from both the breeds are presented in Table 3. Chemical composition (moisture, protein, fat and ash) of the meat was found to be nearly the same in both the breeds. No significant effect of breed was observed on pH, cooking loss, salt soluble proteins and extractable proteins. However, water holding capacity and emulsifying capacity were found to be significantly higher; and water soluble proteins and collagen contents were significantly lower in RIR meat than meat from WL. The higher emulsifying capacity in RIR meat could be due to the low collagen content and higher per cent of dark meat[13]. Emulsifying capacity was reported to be higher in dark meat than light meat[11,12]. A higher water holding capacity and emulsifying capacity in RIR meat indicated that RIR meat

TABLE 3. PHYSICO-CHEMICAL CHARACTERISTICS OF MEAT FROM WHITE LEGHORN (WL) AND RHODE ISLAND RED (RIR) SPENT HENS

Characteristics	WL	RIR
pH	5.98 ± 0.06	5.99 ± 0.04
Water holding capacity ml/100g	27.82 ± 6.12^{a}	54.38 ± 9.30^{b}
Cooking loss (%)	28.93 ± 0.68	31.11 ± 0.95
Emulsifying capacity ml oil/2.5 g	143 ± 5.32^{a}	168 ± 2.62^{b}
Water soluble proteins (%)	4.83 ± 0.15^{a}	3.86 ± 0.04^{b}
Salt soluble proteins (%)	11.51 ± 0.53	11.60 ± 0.36
Extractable proteins (%)	79.26 ± 3.11	75.28 ± 1.75
Collagen (%)	1.41 ± 0.05^{a}	1.14 ± 0.01^{b}
Moisture (%)	71.76 ± 0.73	72.20 ± 0.52
Protein (%)	20.66 ± 0.33	20.58 ± 0.31
Fat (%)	6.95 ± 0.77	7.04 ± 0.53
Ash (%)	1.06 ± 0.07	1.12 ± 0.05

Figures with same superscript in a row do not differ signficantly ($P \leq 0.05$)

was more desirable than WL meat for emulsion type sausage products. Due to the higher meat yield and better quality of meat, RIR spent hens should demand a better price than WL in the market.

Acknowledgement

The authors are thankful to Dr. P. N. Bhat, Director, IVRI for providing the necessary laboratory facilities.

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Studies on the Glycerolysis of Groundnut Oil and Cottonseed Oil

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Received 8 *July* 1985; *revised* 23 *June* 1986

Groundnut oil and cottonseed oil were glycerolysed at 180 and 190°C in the presence of 4, 8 and 12% glycerine using sodium carbonate as catalyst. The amount of alpha-monoglycerides in the glycerolysed oils ranged from 1.4 to 25.3% depending upon the oil, temperature of glycerolysis and amount of glycerine. Viscosity of the glycerolysed products decreased with increase in shear rates. The glycerolysed products exhibited flow behaviour of Newtonian liquids.

About 55 years ago in America, shortenings containing varying amounts of mono-and diglycerides were produced by adding glycerol to the fat during the refining process. These were termed as superglycerinated fats[1]. Choudhary[2] studied the production of monoglycerides by glycerolysis. Glycerolysis has also been suggested as one of the methods of obtaining higher yields of monoglycerides[3]. Since there is no information on the partial glycerolysis of oils for direct use in bread making, the present investigation on the glycerolysis of groundnut oil and cottonseed oil was undertaken.

Materials and Methods

Refined cottonseed and groundnut oils were purchased locally and contained 0.19 and 1.8 per cent free fatty acids, respectively. Glycerolysis was carried out with 4, 8 and 12 g of glycerine in 100 g of respective oils using anhydrous sodium carbonate as the catalyst. For cottonseed oil, 0.15 g sodium carbonate was sufficient whereas for groundnut oil, 0.3 g was necessary. The reaction was allowed to take place at 180 and 190°C for 1 hr with continuous mixing. The mixture was then cooled for 1 hr, transferred to a beaker and refrigerated. After 24 hr, the product was brought to

room temperature and the sediment, if any, was removed.

Free fatty acids and iodine value were determined by by AOAC[4] methods. Hanus iodine was used in the determination of iodine value. Glycerolysed oils were analysed for alpha-monoglycerides and hydroxyl value using AOCS[5] procedure. Average results of three replications are presented in Table 1.

Viscosity of glycerolysed oils at 35°C: Rheotest 2 (VEB, Berlin, West Germany) viscosimeter was used with S_1 coaxial cylinder system for determining the viscosity. Different cylinder speeds were used to evaluate the flow behaviour of the products. The following expression was used to calculate the viscosity expressed as centipoise (cp).

$$\eta = \frac{\alpha \times Z}{Dr}$$

where, η=viscosity; α =Torque; Z=Instrument constant, 5.56; and Dr=Shear gradient from the manual.

Results and Discussion

The results showing the effect of glycerolysis conditions on the properties of glycerolysed oils are presented in Table 1. The degree of glycerolysis depended mainly on temperature and on glycerine concentration. Groundnut oil was more amenable to glycerolysis than cottonseed oil under similar conditions. Glycerolysis of cottonseed oil at 180°C was poor as

TABLE 1. EFFECT OF GLYCERINE CONCENTRATION AND TEMPERATURE ON GLYCEROLYSIS OF GROUNDNUT AND COTTONSEED OILS

Glycerolysis temp. (°C)	Glycerine (g/100g)	Glycerolysed oil			
		Melting point (°C)	α-monoglycerides (%)	Hydroxyl value	Iodine value[a] (g/100g)
Groundnut oil					
180	4	30	7.0	48.0	84.8
	8	32	8.5	59.8	82.2
	12	32	9.5	65.5	83.8
190	4	32	10.4	85.2	83.1
	8	35	20.3	122.3	81.5
	12	35	25.3	140.4	81.2
Cottonseed oil					
180	4	L	1.4	26.3	96.9
	8	L	1.4	29.7	97.5
	12	L	1.8	34.7	91.5
190	4	30	5.9	62.0	95.2
	8	30	6.0	67.7	96.4
	12	30	7.3	74.4	93.0

[a]Iodine value: Groundnut oil, 89.5; Cottonseed oil, 98.0. L—liquid at 20°C.

TABLE 2. EFFECT OF GLYCEROLYSIS CONDITIONS ON THE VISCOSITY OF THE GLYCEROLYSED OILS

Oil/Gl Oil	Glycerolysis temp. (°C)	Glycerine (g/100g)	Viscosity (cP) at indicated cylinder (r.p.m.)						
			4.5	7.5	13.5	22.5	40.5	67.5	121.5
Groundnut oil (GO)	—	—	—	65.2	61.0	59.5	57.8	56.4	55.9
Glycerolysed GO	180	4	—	82.4	72.5	70.9	68.6	67.8	67.6
		8	—	103.0	99.1	96.1	93.5	93.4	—
		12	147.8	137.3	129.7	127.0	124.6	123.6	—
Glycerolysed GO	190	4	160.0	137.3	133.5	128.1	125.2	123.6	—
		8	219.1	208.0	198.3	193.0	188.1	—	—
		12	228.8	209.4	196.4	186.4	180.5	—	—
Cottonseed oil (CO)	—	—	—	61.8	61.1	54.9	53.4	53.0	52.8
Glycerolysed CO	180	4	—	61.8	61.0	57.2	57.2	55.9	55.1
		8	—	68.6	64.8	59.5	57.2	56.1	55.7
		12	—	68.6	64.8	59.5	57.9	56.1	55.4
Glycerolysed CO	190	4	—	68.6	61.0	59.5	58.5	57.9	57.6
		8	—	91.0	816.	81.6	79.1	77.8	77.7
		12	—	103.0	96.1	91.5	91.5	89.0	88.9

reflected by low alpha-monoglyceride content which varied from 1.4 to 1.8 per cent. However, by simultaneous comparison of hydroxyl value with alphamonoglyceride content of both the oils, it is reflected that there is an equilibrium stage after which the monoglycerides are formed at a faster rate. This stage is attained earlier in groundnut oil than in cottonseed oil. The miscibility of glycerine with oils has been reported to be the determining factor of the extent of glycerolysis[6]. The apparent miscibility of groundnut oil was greater than that of cottonseed oil during glycerolysis. Kawai[7] concluded that nature of the oil determines the extent of glycerolysis.

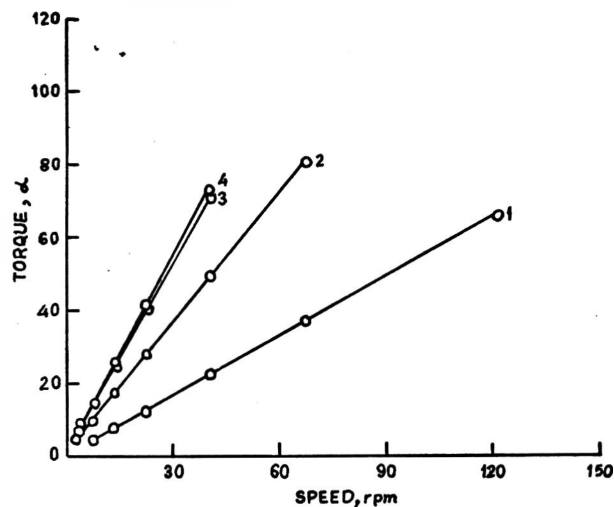

Fig 1. Flow behaviour of groundnut oil glycerolysed at 190°C having different alpha-monoglyceride contents.

Lines 1, 2, 3 and 4 represent glycerolysed groundnut oil with 0, 10.4, 20.3 and 25.3% ∝-monoglyceride contents

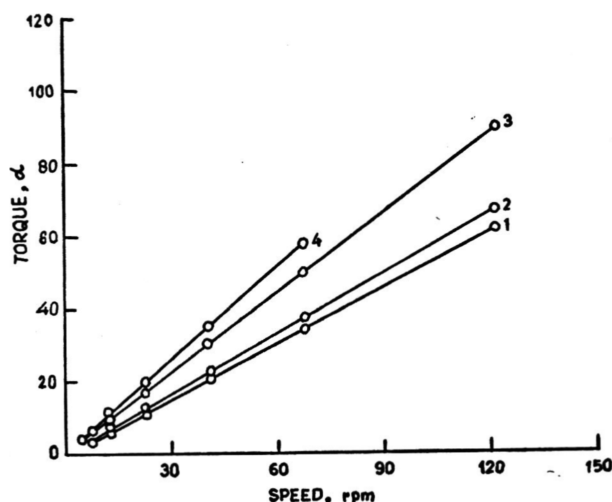

Fig 2. Elow behaviour of cottonseed oil glycerolysed at 190°C having different alpha-monoglycsride contents.

Lines 1, 2, 3 and 4 represent glycerolysed cottonseed oil with 0, 5.9. 6.0 and 7.3% ∝-monoglyceride contents.

The glycerolysed groundnut oils containing 20.3 and 25.3 per cent alpha-monoglycerides exhibited a melting point of 35°C (Table 1). However, with decrease in temperature, these passed through the stages of semi-solid to solid at refrigeration temperature. On the contrary, glycerolysed cottonseed oil with a maximum of 7.3 per cent of alpha-monoglycerides remained fluid at 30°C and semi-viscous at refrigeration temperature. This is in agreement with the findings of Mehta *et al*[8].

The values for the viscosity of the oils and their glycerolysed products are presented in Table 2. Groundnut oil was slightly more viscous than cottonseed oil but the viscosity of glycerolysed groundnut oil was considerably higher than that of glycerolysed cottonseed oil. The change in viscosity of groundnut oil with 7.5 r.p.m. of the coaxial cylinder decreased from 65.2 to 55.9 cp and that of the cottonseed oil from 61.8 to 52.8 cp as the speed increased to 121.5 r.p.m. under similar conditions. The viscosity of both the oils increased with increased in degree of glycerolysis.

The flow behaviour properties of oils and their glycerolysed products are presented in Fig. 1 and 2 The linear relationship of curves is indicative of the Newtonian nature of the products. The present data are in agreement with Muller[9].

From this study, it can be concluded that a product of desired composition and characteristics can be tailored by the manipulating the glycerolysis conditions.

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Storage Stability of Edible Oils and Their Blends

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Received 20 *May* 1986; *revised* 28 *July* 1986

Shelf-life of vegetable oil blends containing 25% raw edible oil and 75% refined oil was investigated. Free fatty acids (FFA) increase in expeller pressed edible oils was more rapid than in refined oils, during storage. Edible oil blends, on the other hand, showed a lower increase in FFA than did the individual oils. Peroxide value of raw edible oils and their blends tended to rise steadily to a maximum, declining gradually thereafter. For raw edible oils stored at 37°C in an incubator after 120 days, acceptability was poor at a peroxide value (m.eq./kg) between 5.9 and 16, for refined oils after 90 days at a peroxide value between 4.4 and 9, and for their blends at 4-45 after 90 to 120 days. Consumer acceptance trials indicated no strong dislike for these blended oils in comparison with traditional single oils, but a preference for the latter in certain instances was evident.

Domestic edible oil consumption in India is increasing steadily. A number of non-conventional edible oils are being made available to the consumer through imports, which touched 1.48 million M.T.[1] during the current year. These oils may not be acceptable to the consumer as thev are devoid of the regionally-preferred flavours of oils traditionally consumed in the country. In many countries[2], edible oil is often marketed as a blend of two or three oils, most of which are in raw form. There are reports both abroad[3,4] and within the country[5] to demonstrate that a blended edible oil, such as refined soybean oil with raw groundnut oil is better accepted by the consumer. The acceptability and, perhaps, keeping quality of non-conventional edible oils could be brought into line with familiar vegetable oils by suitable blending of the two. Since, the consumer flavour preferences for edible oils differ from region to region, oil blends containing various raw edible oils and refined oils were prepared, and their keeping quality is reported in the present work.

Materials and Methods

The edible oil blends prepared were as follows: mustard oil with refined rapeseed oil (1:3); sesame oil with refined cottonseed oil (1:3); groundnut oil separately with refined cottonseed oil (1:3); refined palm oil (1:3) and refined palmolein (1:3); and coconut oil separately with refined palm oil (1:3) and refined palmolein (1:3). Raw mustard oil and coconut oil were obtained from the local *ghanis*, while raw groundnut and raw sesame oils were expeller products. Refined oils of rapeseed, palm and cottonseed were obtained from the State Oil Seed Growers' Federation.

The keeping quality of these edible oil blends along with their ingredients as reference samples, was examined by filling them in a series of air-tight glass bottles of 100 ml each, and ageing them in an incubator at 37°C. All oils were initially analysed for free fatty acids (FFA), peroxide value, refractive index (R.I.) at 40°C and iodine value and saponification value according to the AOCS Official and Tentative Methods[6]. Their fatty acid composition was determined as methyl esters[7] on a Hewlett-Packard (402) gas-liquid chromatograph fitted with a flame ionization detector using a column (2 m × 3 mm I.D.) packed with 15 per cent diethylene glycol succinate on 80/100 dia to port 'S'. Column temperature was maintained at 200°C and the hydrogen gas flow was 30 ml/min.

Samples under storage were analysed every 30 days for FFA (as oleic acid) and peroxide value. Periodic sensory evaluation of odour and taste was done by a panel consisting of 8 people: they were asked first to arrange the oil samples by odour alone, so that the best odour was tasted first. Acceptability was classified as "Good" or "poor" when 70 per cent of the panel accepted or rejected the sample.

Results and Discussion

The refined oils had initially lower FFA (0.04 to 0.16 per cent) than the raw expeller and *ghani* oils (0.33 to 0.70 per cent). The initial peroxide value (m. eq/kg) of edible oils varied from 2.7 to 17.8, *ghani* oils being the lowest. Edible oil blends containing 25 per cent raw edible oil had a maximum FFA of 0.33 per cent, and a moisture content higher than that of refined oils (0.13 to 0.15 per cent) (Table 1).

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF EDIBLE OILS AND THEIR BLENDS

Oils used	Moisture (%)	Free fatty acid (%)	Refractive index at 40°C	Iodine value (Wij)	Sap. value
Raw mustard oil (MO)	0.20	0.33	1.466	112	172
Refined rapeseed oil (RRO)	0.10	0.09	1.465	118	190
25% MO+75% RRO	0.15	0.17	1.465	116	186
Raw sesame oil (SO)	0.20	0.66	1.465	111	192
Refined cottonseed oil (RCSO)	0.10	0.04	1.464	109	195
25% SO+75% RCSO	0.13	0.22	1.464	110	194
Raw groundnut oil (GO)	0.20	0.20	1.463	98	192
25% GO+75% RCSO	0.14	0.10	1.453	100	192
Refined palm oil (RPO)	0.10	0.16	1.457	53	199
25% GO+75% RPO	0.15	0.33	1.457	61	198
Refined palmolein (RPLO)	0.10	0.13	1.456	58	200
25% GO+75% RPLO	0.15	0.18	1.454	64	198
Raw coconut oil (CO)	0.20	0.70	1.448	10	250
25% CO+75% RPO	0.13	0.29	1.455	42	240
25% CO+75% RPLO	0.14	0.18	1.458	47	213

TABLE 2. THE FATTY ACID COMPOSITION (PER CENT) OF THE EDIBLE OILS AND THEIR BLENDS

Particulars	C-8	C-10	C-12	C-14	C-16	C-18	C-18.1	C-18.2	C-18.3	C-22.1
Raw mustard oil (MO)	—	—	—	—	1.9	1.1	10.6	13.8	8.7	52.3
Refined rapeseed oil (RRO)	—	—	—	—	4.4	1.5	64.7	21.0	8.3	—
25% MO+75% RRO	—	—	—	—	3.7	1.4	57.4	18.6	8.5	10.4
Raw sesame oil (SO)	—	—	—	—	10.2	5.3	43.3	41.2	—	—
Refined cottonseed oil (RCSO)	—	—	—	0.7	24.4	1.9	17.8	55.0	—	—
25% SO+75% RCSO	—	—	—	0.6	19.6	2.4	24.3	53.0	—	—
Raw groundnut oil (GO)	—	—	—	—	10.1	3.8	52.6	28.7	0.8	—
25% GO+75% (RCSO)	—	—	—	0.6	20.0	2.0	24.0	53.3	—	—
Refined palm oil (RPO)	—	—	—	1.2	46.9	3.6	39.0	9.1	—	—
25% GO+75% RPO	—	—	—	1.1	40.6	4.1	41.3	12.8	—	—
Refined palmolein (RPLO)	—	—	0.4	1.0	40.6	3.1	42.1	12.6	—	—
25% GO+75% RPLO	—	—	0.4	0.8	37.2	3.2	44.6	13.6	—	—
Raw coconut oil (CO)	8.9	7.8	45.5	17.4	8.5	1.7	5.5	2.8	—	—
25% CO+75% RPO	3.2	1.5	16.8	6.9	32.9	3.8	30.2	4.6	—	—
25% CO+75% RPLO	2.7	1.2	12.6	5.0	31.4	2.8	35.1	8.7	—	—

Cottonseed oil/sesame oil blends (Table 2) had a very much higher level of polyunsaturates (PUFA) (53 per cent) than coconut oil or mustard oil blends (4.6 to 18.6 per cent). The level of erucic acid in the blend containing mustard oil was 10.4 per cent of lauric acid in the blends containing coconut oil 12.6 to 16.8 per cent, and of palmitic acid in the blends containing palm oil and palmolein 31.4 to 40.6 per cent.

The FFA content of all edible oils increased steadily during storage at 37°C in an incubator (Table 3). This

TABLE 3. THE KEEPING QUALITY OF EDIBLE OILS AND THEIR BLENDS DURING STORAGE AT 37°C
CHANGES AT INDICATED DAYS OF STORAGE

Particulars	0		30		60		90		120		150	
	FFA (%)	PV (m.eq/ Kg)	FFA (%)	PV (m.eq/ Kg)	FFA (%)	PV (m.eq/ Kg)	FFA (%)	PV (m.eq/ Kg)	FFA (%)	PV (m.eq/ Kg)	FFA (%) Kg)	PV (m.eq/
MO	0.33	2.7	0.35	1.9	0.40	1.8	0.43	1.8	0.47	5.9	0.52	3.0*
RRO	0.09	14.8	0.13	8.9	0.14	8.7	0.18	9.0	—	—	—	—
25% MO+75% RRO	0.17	8.6	0.20	10.30	0.21	10.5	0.26	41.3	0.29	45.0	—	—
SO	0.66	7.4	0.71	8.2	0.81	7.0	0.70	6.8	0.80	6.4	0.98	6.0*
RCSO	0.04	6.4	0.06	5.3	0.09	4.5	0.09	5.3	0.11	3.0*	—	—
25% SO+75% RCSO	0.22	3.7	0.23	5.4	0.26	9.6	0.22	5.2	0.30	8.0	0.36	13.0*
GO	0.20	17.8	0.24	19.1	0.27	19.3	0.20	19.3	0.29	18.3	0.35	16.0*
25% GO+75% RCSO	0.07	13.2	0.09	17.7	0.12	10.4	0.13	12.1	0.18	12.0*	—	—
RPO	0.16	2.7	0.21	3.3	0.21	7.8	0.23	4.4*	—	—	—	—
25% GO+75% RPO	0.33	6.0	0.37	8.9	0.21	21.5	0.24	9.0*	—	—	—	—
RPLO	0.13	8.8	0.18	5.3	0.21	2.6	0.24	2.0*	—	—	—	—
25% GO+75% RPLO	0.18	7.4	0.20	15.0	0.22	7.1	0.24	10.0	0.24	13.0*	—	—
CO	0.70	tr	0.71	tr	0.84	tr	0.89	0.08	0.92	0.1	1.07	tr*
25% CO+75% RPO	0.18	3.0	0.36	7.0	0.36	5.4	0.25	5.0	0.48	5.0*	—	—
25%.CO+75% RPLO	0.29	2.6	0.31	3.5	0.33	4.4	0.34	4.3	0.40	4.0*	—	—

MO: Mustaed oil; RRO: Refined rapeseed oil; RCSO: Refined cottonseed oil; GO: Groundnut oil; RPO: Refined palm oil; PLO: Refined palmolein; CO: Coconut oil.
FFA: Free fatty acid; PV: Peroxide value.
*Poor acceptability.

increase was more conspicuous in raw edible oils (0.33 to 0.52 per cent), and was low in refined oils because of low initial values. Edible oil blends showed a lower increase in FFA (0.05 to 0.11 per cent) than did their ingredients. Among the various edible oil blends, sesame oil or groundnut oil blended with refined cottenseed oil showed the least increase in FFA.

Changes in peroxide values of edible oils stored at 37°C were not regular (Table 3). In most raw edible oils, there was a steady increase to a peak value and a slight decline thereafter. Refined oils showed irregular behaviour. Incorporation of 25 per cent raw edible oils in refined oils influenced the change in peroxide value (of the blends) in the direction of the raw oil component.

The acceptability of raw edible oils became poor when the peroxide value (m.eq/kg) reached 5.9 for mustard oil, 6.4 for sesame oil and 16 for groundnut oil, which occurred after 120 days of storage at 37°C. Coconut oil showed no appreciable increase in peroxide value during storage. In refined oils, acceptability was poor after 90 days of storage at 37°C, when the peroxide value (m.eq/kg) was 9 for rapeseed oil, 5.3 for cottonseed oil, 4.4 for palm oil and 2.0 for palmolein. Raw edible oils showed a longer shelf-life than refined oils probably due to the presence of natural antioxidant.

Blends containing 25 per cent of raw oils developed rancidity after 120 days of storage at a peroxide value (m.eq/kg) of 45 in the mustard oil and rapeseed oil blend, 12 to 13 in cottonseed oil blends containing sesame oil or groundnut oil, 9 in groundnut oil with palm oil, 13 in the groundnut oil and palmolein blend, and 4 to 5 in coconut oil blends containing palm or palmolein. The inadequacy of simple chemical indices like the peroxide, carbonyl and thiobarbituric acid values to corroborate sensory evaluation is well known[8].

These blended oils were subjected to consumer trial in selected parts of the country. Eastern areas like Calcutta and Bubhaneswar were covered with the rapeseed oil blend containing mustard oil, and in Kerala (Trivandrum) coconut oil blends containing palm oil and palmolein were tested About 1000 consumers

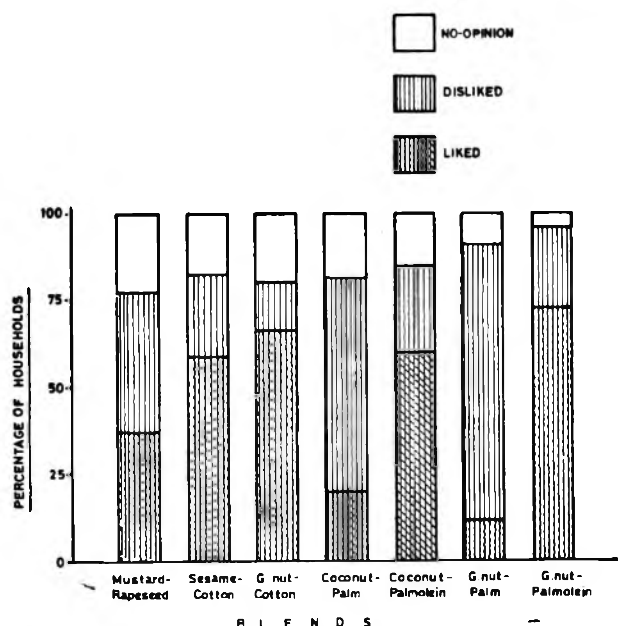

Fig 1. Consumer acceptance of edible oil blends

received a 250 g sample of the blend with a questionnaire. The consumer preference in various areas was for oil blends containing cottonseed oil with sesame oil or groundnut oil, coconut oil with palmolein, and rapeseed oil with mustard oil (Fig. 1). Blends containing palm oil were less acceptable to the consumer as a waxy solid mass separates out in these oils.

Incorporation of 25 per cent raw edible oil in refined oils prolonged the shelf-life over that of the refined oil alone by one or two months. Many traditionally-consumed oils, such as coconut, mustard and groundnut and refined oils such as palm oil and palmolein are low in polyunsaturates. Suitable blending can raise the level of these polyunsaturates. Edible oil consumption is mainly related to regionally preferred flavours and to the cost of oil. Through blending, consumer-acceptable flavours could be incorporated into non-conventional oils to make the latter more acceptable and stretch edible oil supplies. The present consumer acceptance trials indicated no strong rejection of an oil blend in comparison with the traditionally-liked oil though a preference for the latter was sometimes evident. Oil blends that have techno-economic and nutritional merits could thus ultimately be expected to receive prolonged acceptance given sufficient time for adjustment. A further advantage of using refined oils for blending is the high pesticide levels in raw oils, which the deodorization step in refining is known to bring down (e.g. DDT)[9].

Acknowledgement

The authors wish to thank the Joint Secretary, Ministry of Civil Supplies, New Delhi for financing the research project.

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RESEARCH NOTES

ETHANOL PRODUCTION BY *ZYMOMONAS MOBILIS*: EFFECT OF HIGH SUBSTRATE CONCENTRATION ON THE KINETIC PARAMETER

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Received 8 *February* 1986; *revised* 19 *July* 1986

Zymomonas mobilis **NCIM–2428 and NRRLB–806 were compared for ethanol production from glucose (100 g/l). The latter produced ethanol at higher specific rates of glucose uptake (6.41 g/g.h) and ethanol production (2.32 g/g.h). At higher glucose concentrations, parameters related to cell growth were more affected as compared to ethanol production indicating an uncoupling between growth and catabolism. Maximum ethanol yield was obtained by NRRL B–806 at 100 to 150 g/l concentration.**

Yeasts have been conventionally used in the manufacture of industrial alcohol and alcoholic beverages. A promising alternative to yeast as an ethanol producer is bacterium *Zymomonas mobilis*, which is a common micro-organism in sugar-containing juices in tropical countries and is found as a contaminant in spoiled beer, cider and perry[1]. Recently, Rogers *et al.*[2] have reported several advantages of ethanol production by *Zymomonas mobilis* as compared to yeast. Different substrates like glucose[2-4], fructose[5,6] and sucrose[7] have been used for ethanol production by *Zymomonas mobilis*. Skotnicki *et al.*[3] have observed considerable variation in different strains with respect to sugar or ethanol tolerance. While inhibition of growth and ethanol production by yeast on glucose have been widely studied, comparatively less is known about limiting step in alcoholic fermentation in *Zymomonas mobilis*. The present report deals with the comparison of two strains of *Zymomonas mobilis* and the effect of high glucose concentration on the kinetics of ethanol production.

Zymomonas mobilis NCIM–2428 and *Zymomonas mobilis* NRRL B–806 (obtained from National Chemical Laboratories, Pune and U. S. Dept. of Agriculture, NRRL, Illinois, USA respectively) were maintained on agar slants containing (g/1): glucose 20, yeast extract 10, potassium dihydrogen phosphate 2, ammonium sulphate 1, magnesium sulphate 0.5, agar–agar 20 and adjusted to pH 5.0 The fermentation medium was the same as above (except agar-agar) and the glucose concentration was adjusted at 100, 150 or 200 g/l as described in the text. Fermentation experiments were carried out in a laboratory fermentor (Bioengineering AG, Switzerland) equipped with control and recording facilities for pH, temperature, agitation, etc. For all experiments, 10 per cent (V/V) of inoculum was used to ferment 2.5 l medium with mild agitation (150 r.p.m.) at 30°C and pH 5.0 (controlled by Mostech AG regular MBB2N. Biomass was estimated by optical density (OD) measurement at 600 nm. Glucose was estimated by dinitrosalicyclic acid (DNS) method[9], and ethanol was estimated by the ceric ammonium nitrate method of Reed and Truelove[10] after distilling a neutralized sample of fermentation broth.

The experimental data for glucose, ethanol and biomass concentration, for the fermentation of 100 g/l glucose by both the strains were plotted. From these data, kinetic parameters during exponential growth phase were calculated and are shown in Table 1 for both the strains. It is seen that specific growth rate (μ), specific glucose uptake rate (q_s) and specific ethanol production rate (q_p) were much higher for the NRRL strain. However, final ethanol concentration and ethanol yields were not appreciably higher, but due to higher fermentation rate, the fermentation was almost complete in 16 hr (92 per cent of glucose was consumed). For NCIM strain, cell yield was more and the fermentation was slow and continued for 24 hr. These results

TABLE 1. COMPARATIVE KINETIC PARAMETERS FOR ETHANOL PRODUCTION BY *ZYMOMONAS MOBILIS* NCIM-2428 AND *ZYMOMONAS MOBILIS* NRRL B-806

Kinetic parameters	*Z. mobilis*	
	NCIM-2428	NRRL B-806
Specific growth rate, μ/hr	0.147	0.188
Specific glucose uptake rate, q_s (g/g hr)	3.01	6.41
Specific ethanol productivity, q_p (g/g hr)	1.18	2.32
Cell yield, $Y_{x/s}$ (g/g)	0.029	0.016
Ethanol yield, $Y_{p/s}$ (g/g)	0.42	0.43
Ethanol yield (% of theoretical)	83.7	84.9
Final ethanol concn (g/l)	41.5	42.5
Final biomass concn. (g/l)	2.9	1.59
Conversion of glucose (%)	97.0	98.0
Fermentation time (hr)	24.0	22.0

glucose, 100g/l; pH, 5.0; temp. 30°C

TABLE 3. EFFECT OF GLUCOSE CONCENTRATION ON THE KINETICS OF ETHANOL PRODUCTION BY *ZYMOMONAS MOBILIS* NRRL B-806

Kinetic parameters	Initial glucose concn (g/l)		
	100	150	200
Specific growth rate, (μ/hr)	0.188	0.152	0.145
Specific glucose uptake rate, q(g/g hr)	6.41	6.5	6.8
Specific ethanol productivity, q(g/g hr)	2.32	2.45	2.83
Cell yield, $Y_{x/s}$(g/g)	0.016	0.016	0.015
Ethanol yield, $Y_{p/s}$(g/g)	0.43	0.43	0.41
Ethanol yield, (% of theoretical)	84.9	84.9	80.9
Final biomass concn. (g/l)	1.59	2.20	2.53
Final ethanol concn. (g/l)	42.5	58.0	70.0
Final conversion of glucose (%)	98.0	89.2	88.5
Fermentation time (hr)	22.0	30.0	36.0

pH, 5.0; temp. 30°C

clearly demonstrate that NRRL strain is superior for ethanol production.

For such a strain, it would be interesting to ferment high glucose concentration (150, 200 g/l) to obtain high ethanol concentration in the broth. The kinetic parameters were evaluated according to the previous experiment, and are reported in Table 2. It is seen that with increasing glucose concentration parameters related to growth (specific growth rate, cell yield) are noticeably when compared to affected than those cennected with ethanol production (specific glucose uptake rate, specific ethanol production rate and ethanol yield). Laudrin–Seiller *et al.*[4] also made similar observations on the influence of initial glucose concentration (50–190 g/l) on the fermentation kinetics of *Zymomonas mobilis*. This suggests uncoupling between growth and catabolism as reported by Belaich *et al.*[11,12]. King and Hossain[3] reported maximal ethanol yield at an initial glucose concentration of 100 g/l, but specific growth and specific ethanol production rate were less than 10 per cent below the maximum observed at 75 g/l glucose level.

We obtained same ethanol yield (84.9 per cent) at 100 and 150 g/l glucose concentration, but specific growth rate is slightly reduced at the latter glucose concentration. At 200 g/l glucose, ethanol yield is reduced to 80.9 per with a decrease in specific growth rate and biomass yield indicating inhibition of growth and ethanol production. However, at all the three glucose concentrations, specific ethanol productivity remains nearly same, but final ethanol yields are reduced due to inhibition by increasing concentration of ethanol during the final stage of fermentation. At high initial substrate concentration, the intracellular water percentage falls during the fermentation cycle, which is responsible for inhibition of cell growth. Initial glucose concentration of 100 to 150 g/l appears to be optimal for maximum ethanol yield by *Zymomonas mobilis*.

Authors are grateful to Prof. A. K. Vasishtha, Director, H. B. Technological Institute, Kanpur, for the encouragement and the facilities provided during this work.

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MODIFICATION OF PONS'S METHOD OF ESTIMATING AFLATOXIN B_1 IN CORN, GROUNDNUT AND GROUNDNUT CAKE

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Received 24 *June* 1985; *revised* 29 *April* 1986

Pon's method for determination of aflatoxin B_1 has been modified by eliminating column clean up step and by introducing an additional TLC development in ether+hexane (1:1). Aqueous acetone extract is diluted to contain 25% acetone which expedites filtration and improves the clarity of the filtrate. Recovery of aflatoxin added to groundnut flake, groundnut cake and corn was 83-107%. Quantification of aflatoxin B_1 in naturally contaminated commercial groundnut cake by the modified method compared well with that by CB method.

With the outbreak of aflatoxicosis in turkeys in 1960 and the subsequent recognition of aflatoxin problem in food and feed, there have been constant efforts to evolve precise, efficient and simple methods of analysis for aflatoxins. As a result, many methods have been developed of which Best Foods (BF) and Contaminants Branch (CB) are accepted officially for the quantitative estimation of aflatoxin. BF[1] method is simple and rapid because it does not involve any purification steps. However, interference by pigments may complicate quantitation especially at low levels of aflatoxin. CB[1] and Pons's[2] methods arer ecommended by A.O.A.C.; in both, the precedures are lengthy and involve column chromatography. According to Pons[2], purification of the sample by column separation is not always essential. In this communication, a modified procedure which is simple but with recoveries comparable to any other method is described.

Aflatoxin B_1 reference standard: Crystalline aflatoxin B_1 prepared in this laboratory and whose purity was checked[1], was used.

Spiking of the sample: Known amounts of aflatoxin B_1 from a stock standard solution in choloroform was added to 50 g of groundnut (hand picked and sorted grade) flakes (0.25 mm size), corn and groundnut cake which were ground to pass through BSM-18. Chloroform in the samples was allowed to evaporate at room temperature and the samples were stored in a refrigerator until required for analysis.

TLC plates: 20×20 cm glass plates coated with TLC grade silicagel with binder (ACME Chemical laboratories) to get 300 μ thick layers.

Estimation of aflatoxin B_1 in the spiked samples was carried out as described by Pons[2-4], CB[1] and by the modified method as indicated in this paper. The steps involved are as follows: 50 g sample was extracted with 250 ml of acetone+water (85+15) by shaking for 30 min in a shaker; 50 ml filtrate was treated with 10 ml 20 per cent lead acetate and 50 ml water. The mixture was held for 5 min and filtered. The filtrate was again diluted with 100 ml water and extracted twice with 25 ml portions of chloroform[2-4] which was passed through a bed of anhydrous sodium sulphate. The chloroform extract was concentrated on a water bath to a suitable volume for TLC analysis.

Ultimate separation and quantification: Samples extracted by modified method were analysed by TLC as follows: The plate was developed either in ether alone or in combinations of ether: hexane (3:1 or 1:1) and subsequently developed in chloroform:methanol (97:3 v/v). In another experiment, the plate was directly developed in the latter solvent system, and after air drying, the plates were observed under long wave UV light for the appearance of bluish flourescent spots corresponding to those of aflatoxin B_1. Aflatoxin B_1 in the sample was quantified by dilution to extinction method of Coomes.[5]

It was observed that while partitioning toxin from aqueous acetone to chloroform, the following disadvantages were encountered in Pons's[1] method: (1) as a part of acetone goes into chloroform, the volume of the lower phase increased and (2) the filtration of chloroform extract through sodium sulphate bed was slow owing to moisture carried by acetone and the final extract was turbid. In the modified method, these difficulties were overcome by bringing down the acetone level to about 25 per cent. All the reported methods involve a column cleanup which is eliminated in the modified procedure, hence economising on chemicals and time.

The per cent recovery of aflatoxin B_1 from groundnut flakes on direct TLC in chloroform:methanol (97:3 v/v) solvent system was 143:76 which is significantly higher ($p < 0.05$), and if the plate was developed in ether alone prior to development in chloroform: methanol (97.3 v/v), the recovery was 65.64 per cent which is significantlylower ($p < 0.05$). On the other hand, if the plate was developed in ether:hexane (3:1 v/v) or in ether:hexane (1.1 v/v) the recoveries were 102.6 and 98.4 per cent respectively (n=8, SEM 28 df±6.02).

The recovery (per cent) of aflatoxin B_1 from groundnut flakes spiked at 240 p.p.b. level by the different methods was: Pons[2] 85.42; Pons[3] 96.52; Pons[4] 113.18; CB[1] 87.07 and the proposed method 97.92, [n=6; SEM (25 df)

TABLE 1. RECOVERY OF AFLATOXIN B_1 ADDED TO GROUNDNUT FLAKES, GROUNDNUT CAKE AND CORN BY THE MODIFIED METHOD

Aflatoxin added (ppb)	Per cent recovery in		
	Groundnut flake	Groundnut cake	Corn
15	89.08^{x}_{a}	106.67^{x}_{b}	90.68^{xy}_{ab}
50	99.52^{x}_{a}	104.00^{x}_{a}	105.20^{x}_{a}
100	89.20^{x}_{ab}	103.00^{x}_{a}	84.20^{y}_{b}

Mean of six estimations. SEM (36 df) ± 5.23

Means of the same column followed by different superscripts (x, y) differ significantly.

Means of the same row followed by different subscripts (a, b) differ significantly according to Duncan's new multiple range test ($P < 0.05$).

TABLE 2. AFLATOXIN B_1 IN NATURALLY CONTAMINATED COMMERCIAL GROUNDNUT CAKE SAMPLES BY C. B. AND MODIFIED METHODS

Method	Aflatoxin B_1 (ppb)
C. B.	328.33
Modified	295.00

Mean of six estimations; SEM (10 df) ± 44.65

Means do not differ significantly according to Duncan's new multiple range test.

I 4.4]. Recovery by the modified method was thus comparable to other standard methods.

Table 1 presents recoveries of aflatoxin B_1 by the modified methods for groundnut flakes, groundnut cake and corn spiked at 15, 50 and 100 p.p.b. levels. The recovery at 100 p.p.b. level was lower in case of corn. When a comparison among the three commodities is made, no significant variation was observed between groundnut flake and corn at 15 p.p.b. level but there was significant change in values between groundnut cake and corn at 100 p.p.b. levels. Though the differences observed are significant, it is relevant to point out that a 15 per cent variation is allowed in the visual method of estimation. While comparing recoveries (%) between modified method and CB method for naturally contaminated commercial cake a greater variance [SEM (10 df) ± 44.65] was noticed (Table 2), but there was no significant difference between the methods.

It is concluded that the modified method is simple and takes less time and chemicals is comparable to any other methods as far as per cent recovery of toxin is concerned.

The authors acknowledge Mr. B. S. Ramesh, for help in statistical analysis and Dr. V. Sreenivasamurthy for his encouragement throughout this work.

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L-MALATE CONTENT IN IRRADIATED ONIONS (*ALLIUM CEPA* L.) CV. VALENCIANA SINTETICA 14

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Received 1 *October* 1985; *revised* 24 *June* 1986

Results of L-malate evaluation in control and irradiated onions, (cv. 'Valenciana sintetica 14,') and its correlation with sprouting cumulative values are reported. It was concluded that if on the 150th day of storage, the malate content reaches a maximum value and the sprouting is 1% or less, then it would indicate that the samples have been irradiated. L-malate values are positively correlated to sprouting in control samples, while for irradiated ones correlation was negative.

L-malate has been determined, among a lot of other onion chemicals, in order to see if it could constitute a parameter of freshness in stored onions treated with maleic hydrazyde[1,2]. Gorin[2] reported that L-malate changed during storage in similar manner for control

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and treated batches, there being a pronounced difference from the 7th month until the end of the 9th month while in storage. No data are available in literature about evaluation of L-malate behaviour during storage of irradiated onions. This paper reports the results of an experiment on L-malate evaluation in control and irradiated onions, cv. 'Valenciana sintetica 14', and its correlation with sprouting cumulative values

Onion bulbs (medium size) of the 'Valenciana sintetica 14' cultivar packed in plastic net bags were obtained directly from the packaging line at a farmer's cooperative and stored under cover in an open shed for 9 months from 2 April to 26 December 1984. During storage, day temperatures ranged from about 33°C to about 15°C with minimum night temperatures ranging from about 18°C to 1°C. Relative humidity duiing this period were taken using a hair hygrometer; RH percentages from 60 to 85 were observed. Halft he batch was irradiated 30 days after harvest with a dose of .03 kGy (3 krad). Irradiation was carried out with a Phillips X-ray machine with a dose rate of 2.60 Gy/min.

One onion bag from each treatment was opened at regular intervals (Table 1) and the number of sprouted bulbs was recorded. Then, three sets of 5 bulbs from each sample were randomly withdrawn and analysed for L-malate. The onions from each set were sliced over a container with liquid nitrogen and dropped into it. The sliced onions were freeze-dried and ground in a cross-beater mill using a sieve with pores of 1.0 mm diameter. The resulting onion powder was kept undei nitrogen at –75°C. The mass fraction of moisture in the powders was 0.97–2.16 per cent, determined as in Gorin[3].

TABLE 1. SPROUTED BULBS IN IRRADIATED (.03 KGY) AND CONTROL ONIONS DURING STORAGE

Sampling date		Storage	Sprouted bulbs(%)*	
Day	Month	(days)	Control (%)	Irradiated (%)
2	APR	0	0.0	0.0
30	APR	28	0.1	0.0
30	MAY	58	0.3	0.0
2	JUL	91	1.1	0.1
30	JUL	119	4.0	0.1
30	AUG	150	12.2	0.2
28	SEP	179	30.7	0.5
30	OCT	211	47.1	1.2
26	NOV	238	63.4	3.3
26	DEC	268	80.0	5.8

*The values given are cumulative.

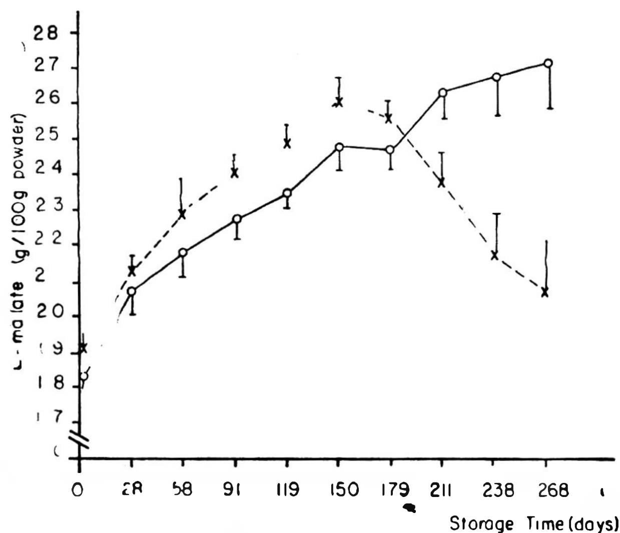

Fig. 1. Evolution of L-malate during storage of onions, cv. 'Valenciana sintetica 14', irradiated (x– – –x) with .03 kGy and untreated (0——0).

Values are mass fractions of g/100 g powder and vertical bars represent 1 S.D. (n=3). Values were not corrected for moisture in the powders that varied between 0.97% and 2.16%.

The enzymatic estimation of L-malate was carried out according to the procedure described by Gorin[2]: triplicates of 100 mg onion powder each were poured into Pyrex glass tubes. Ten ml distilled water, heated to 85°C, were added to each tube to achieve inactivation of natural enzymes. The tubes were hermetically closed, shaken on a Vortex mixer for one min and placed in a water bath (85°C) for 5 min. The suspensions were then centrifuged at 1,600 G for 45 min at 6°C in a refrigerated centrifuge.

The supernatant was poured into the cell for the enzymatic estimation of L-malate as desciibed in the literature.[4] Foi the reference cell, the same reagents were used, except that the malate dehydrogenase solution was replaced by double distilled water[2].

Table 1 shows the cumulative values of sprouted bulbs for both control and irradiated onions over the 9-month storage period. The percentage of sprouted bulbs in the irradiated batch (5.8 per cent) at the end of the experiment (268 days) was similar to that observed in the control onions (4.0%) after 119 days of storage.

The evaluation of L-malate during storage of control and irradiated onions, is presented in Fig. 1. The L-malate content increased during storage in a similar pattern in both the batches until the 179th day of storage. After this, until the end of the experiment (268 days), the control batch showed the same trend of

increase in L–malate values from 2.47 to 2.72 g/100 g powder. Inversely, the amount of L–malate in irradiated onions decreased from 2.57 to 2.08 g/100 g powder after reacting a peak at 150th day of storage.

In the light of these results, it can be concluded that a peak in the L–malate content near the 150th day of storage followed by a decline would suggest that onions had been irradiated, specially if at peak time less than 1.0 per cent of the bulbs show sprouting activity. Further research can confirm this hypothesis.

Thanks are due to Mr. L. Spadaro for his kind co-operation in this study and to Mrs. A. M. Graciani for onion powdeı preparation and enzymatic analysis.

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PREPARATION OF FRUIT FLAVOURED BEVERAGE FROM WHEY

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Received 17 *June* 1985; *revised* 18 July 1986

Whey beverage was prepared from whey free of proteins and fat with an acidity of 0.5% and 20° brix and flavoured with orange, pineapple, guava and mango fruit juices at 10, 15, 20 and 25% of whey. Sensory evaluation showed that whey beverage prepared from mango juice with 15% of whey was superior to others; maximum values for colour, sedimentation, turbidity and viscosity were also observed for this beverage.

In India, whey is obtained as a by-product in the preparation of *Chhana*, *Paneer*, cheese and casein. Generally, whey contains about 6.5 to 7.0 per cent solids, which is approximately half of the total solids of original milk and has a good nutritional value.

A large part of the whey produced in India is drained in to gutters which creates problems of pollution besides loss of valuable nutrients. A logical way to use it would be to return it to the human food chain in a palatable form. "Whevit" a nourishing soft drink was manufactured by using *chhana* whey, which was flavoured with orange, lemon, pineapple and mango essences.[1] Tomato whey drink was also prepared from cheese whey.[2] The present study was undertaken to utilize the whey in the preparation of fruit flavoured beverages and investigate their physico-chemical properties and acceptability.

The whey was obtained from standardised (4.0 per cent fat) cow's milk by simple acid (2 per cent citric acid) coagulation method. The precipitation of whey proteins was carried out at 0.5 per cent acidity by boiling for 15 min and allowed to settle the proteins for 5 to 6 hr. The small amount of fat present in the whey was separated by passing it through a cream separator. The nearly fat free, clear whey was used for the preparation of beverage base. The acidity of clear whey was adjusted with citric acid to 0.25 and 0.50 per cent. Sugar was added to get the four levels of brix i.e. 10°, 15°, 20° and 25° for each acidity level. These eight samples weıe subjected to sensory evaluation to select the whey beverage base.

The whey beverage base chosen was mixed thoroughly, strained and heated to boiling and held for 15 min. At the end of processing, the loss of volume was corrected by addition of hot water and allowed to cool. Canned orange, pineapple, guava and mango juices were added to separate batches of whey beverage base. Each fruit juice was added at 10, 15, 20 and 25 per cent of whey beverage base. The final product was adjusted to 0.5 per cent acidity and 20° brix, by adding citric acid and sugar respectively. Samples were stored at refrigeration temperature (4–5°C) till they were subjected to sensory evaluation by 9–point hedonic scale[3]. The evaluation was carried out by 5 selected judges. Four samples were given at a time. For each test, four replicates were used.

Sensory evaluation of whey beverage base (Table 1) showed that 0.5 per cent acidity with 20° brix sugar level was superior to other treatments. The effect of acidity level and concentration of sugar was found to be statistically significant. This was selected as a base for the preparation of fruit flavoured whey beverage. The results of the present study are in conformity with findings of Nelson *et al.*[4]

Highest scores were recorded in case of 10 per cent fruit juice of orange, 15 per cent of pineapple, 25 per cent of guava and 15 per cent of mango. The effect of fruit juice concentration was significant in case of orange and guava and non-significant with pineapple and mango. The fruit flavoured whey beverage adjudged as the best for each type of fruit was used for final comparison.

TABLE 1. AVERAGE SCORE OF WHEY BEVERAGE BASE UNDER DIFFERENT TREATMENTS

Sugar (°Brix)	Whey acidity (%)	Colour	Flavour	Consistency	Mouth feel	Mean score
10	0.5	81.25	73.75	75.00	72.85	75.71
15	0.5	83.75	78.75	81.25	81.42	81.29
20	0.5	86.25	83.75	80.00	87.14	84.28
25	0.5	82.50	78.75	80.00	80.00	80.31
10	0.25	72.50	67.50	68.75	67.14	68.97
15	0.25	71.25	71.25	73.75	70.00	71.56
20	0.25	73.75	77.50	73.75	75.71	75.71
25	0.25	73.74	75.00	75.00	78.57	78.58

Acidity: S.E.=0.05837; C.D.=0.1717
Sugar: 0.08255; 0.2428

TABLE 2. AVERAGE SENSORY SCORE OF FRUITS FLAVOURED WHEY BEVERAGE

Type of flavour	Whey beverage base concn. (%)	Colour	Flavour	Consistency	Mouth-feel	Mean score
Orange	10	7.3	6.6	7.1	6.7	6.92
Pineapple	15	7.3	6.7	7.1	6.8	6.97
Guava	25	7.1	6.4	7.1	5.9	6.62
Mango	15	7.8	7.7	7.1	7.1	7.42

S.E.=0.1450 C.D.=0.4636

TABLE 3. PHYSICO-CHEMICAL QUALITY OF FRUIT FLAVOURED WHEY BEVERAGE

Type of flavour	Whey beverage base concn (%)	Lactose %	Sucrose %	Proteins %	Ash %	Colour	Sedimentation %	Viscosity %
Orange	10	4.30	14.06	0.22	0.72	0.1R+0.3Y	1.5	3.5
Pineapple	15	4.18	14.16	0.26	0.78	0.41 Y	1.0	3.5
Guava	25	4.10	14.21	0.35	0.86	0.41 Y	1.0	3.5
Mango	15	4.20	14.15	0.28	0.50	0.7R+0.45Y	2.5	4.25

Total solids, 20%; turbidity, 96-98%; acidity, 0.5%; and pH 4.21-4.29.

As seen from Table 2 the highest score was recorded for mango flavoured whey beverage followed by pineapple, orange and guava. The highest score with respect to colour, flavour and mouthfeel was observed in case of mango flavoured whey beverage, while the consistency score was uniform with all the four fruits used.

The mango beverage maintained its physical characters. Physical quality attributes were also studied of the selected fruit flavoured whey beverages and results are given in Table 3. The maximum values for colour, sedimentation, turbidity and viscosity were observed with the mango flavoured whey beverage. Though sedimentation value was the highest in this product, it gets redispersed when shaken. Mango flavoured whey beverage with the highest turbidity and viscosity was liked by the panel.

It can be observed from Table 3 that all the beverages prepared by using orange, pineapple, guava and mango were similar in chemical composition with respect to the different parameters. Though whey proteins had been precipitated, the proteion content of the final product was due to proteins from fruit juice. Higher values were observed in guava fruit flavoured whey beverage due to use of a higher per cent of guava juice, which is rich in proteins and ash. The pH, total solids and protein content of the final product is similar to these reported by Guy[5], Nelson *et al.*[4] and Schuster.[6]

Thus, whey can be utilized in the preparation of acceptable and nutritious fruit flavoured beverages, as it considerably improved the sensory and chemical qualities of the final product.

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STUDIES ON DEHYDRATION AND DEEP FREEZING OF *PANEER*

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Received 7 *April* 1986; *revised* 21 *July* 1986

Even though the shelf-life of *paneer* can be extended from fifteen days to two months by dehydration, the reabsorption characteristics were poor and in addition, it lacked cohesive property. By deep freezing the shelf-life could be extended to eight days, but surface hardening was observed.

Paneer is an acid coagulated product of milk which is mainly used as a base for culinary preparations. According to the Prevention of Food Adulteration Act, of India, it should not have more than 70 per cent of moisture and not less than 50 per cent of fat on dry matter basis[1]. It has a limited shelf-life of three days at 10°C due to high moisture content.[2] The product goes bad with the development of off-flavours, growth of yeast and molds, case hardening, etc. An attempt was made to extend the shelf-life of *paneer* by dehydration and deep freezing.

The milk used in the experiment was procured from the National Dairy Research Institute, Bangalore. It was collected at the receiving point after dumping into the Batch Pasteuriser to obtain a composite sample which had the following composition: fat 4.2 per cent, total solids 12.5 to 13.2 per cent and acidity 0.15 per cent. *Paneer* was prepared by standard procedure[3,4], wherein five litres of milk was coagulated at 80°C with 1.5 per cent citric acid solution (pH 5.0). Curd was filtered and transferred into a wooden hoop (4″×4″×4″), and a pressure of 2 kg per sq.cm was applied to completely drain off the whey. The *paneer* was chilled to less than 5°C. The material was cut into 0.5, 1.0 and 2.0 cm cubes and dried in a hot air drier at 75°C. Using a Phila-Pa (U.S.A.) extruder with dies of dia 1, 2 and 4 mm, the *paneer* was extruded into a noodle like product to increase the surface area. *Paneer* was also deep-frozen at –9 and –15°C. Samples were drawn everyday to study the changes.

Fresh *paneer* had a proximate composition of fat 23.5 per cent, moisture 55.0 per cent and total solids 45 per cent. The effect of drying on composition of *paneer* is presented in Table 1. After 4 hr exposure to 75°C *paneer* cubes continued to have a moisture content of 15 to 18 per cent, which is due to case hardening. The surface hardening can be prevented by controlling the relative humidity (RH) and temperature of the circulating air. The extruded *paneer* retained less moisture of 5 to 9 per cent and drying was comparatively faster. Exposing for longer periods resulted in caramelisation and deepening of brown colour. It had poor rehydration characteristics. A longer contact with water resulted in disintegration and dispersion of the product, which lacked the cohesive property. Since casein is the principal milk protein responsible, it is already denatured by heat-acid coagulation involving unfolding of poly-peptides resulting in the loss of ability to bind, adsorb and absorb other food components. During drying, the proteins undergo further denaturation resulting in low water absorption capacity[5]. Through melting of surface fat during prolonged heat exposure, there was a fat loss ranging from 1.5 to 2.0 per cent in *paneer* cubes and 3.2 to 5.8 per cent in extruded *paneer*. The cubes did not keep well beyond 15 days and in all of them mold growth was seen. Extruded *paneer* kept well for two months, mainly due to reduced moisture content. The lipids being unstable undergo oxidation to produce off-flavours coupled with hydrolytic rancidity.

TABLE 1. EFFECT OF DRYING ON COMPOSITION OF *PANEER**

Cube size	Time needed (hr	min)	Moisture (%)	Fat (%)	Fat loss (%)
0.5 cm	3	00	15.0	41.50	2.0
1.0 cm	3	30	17.0	40.60	1.8
2.0 cm	4	00	18.0	40.25	1.5
			Extruder Paneer		
1.0 mm	1	45	5.0	44.60	5.8
2.0 mm	2	05	6.0	44.30	5.2
3.0 mm	2	25	8.0	44.60	3.8
4.0 mm	2	35	9.0	44.40	3.2

*Values are average of six trials.

Deep freezing of *paneer* to –9 and –15°C showed surface drying in eight days limiting its usage. Texture deterioration was not observed due to the formation of fine structured ice crystals by fast freezing. Significant colour changes were not noticed and flavour was acceptable. Appropriate packaging material which protects from desiccation might further help in extending the shelf-life. There was not much difference in the sensory attributes between the samples deep frozen at the two temperatures namely –9 and –15°C.

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PHYSICAL AND CHEMICAL COMPOSITION OF INDIAN COCOA (*THEOBROMA CACAO* L.) BEANS

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Received 17 *December* 1985; *revised* 24 *April* 1986

To know the regional variation in the quality of cured Cocoa (*Theobroma cacao* L.) bean samples from Kottayam, Puttur, Sakaleshpur and Chundale have been analysed. The ranges of variations being, weight per bean 0.78–0.89 g, violet beans 0–24%, salty beans 0–5%, insect damaged and fungus infested beans 0–20%, whitish beans 0–20%, rejects 0–32.5%, nibs 82.7–85.9% and shell 14.1–15.2%. The variations in chemical parameters are moisture 4.33–6.10%, total ash 2.97–3.14%, water insoluble ash 1.48–1.91%, alkalinity of water soluble ash 0.33–0.59% (asK_2O), pH of nibs 4.93–5.37, titrable acidity 2.17–2.88, volatile acidity 0.92–1.16, total sugars 0.96–1.00% lactic acid 0.27–0.57%, citric acid 0.56–0.88%, tannins 2.35–2.72% theobromine 1.32–2.04% and fat 48.30–52.63%.

In India, cocoa is grown in Karnataka, Kerala and some parts of Tamil Nadu. The cured cocoa beans of commerce[1] is obtained by subjecting fresh cocoa beans along with pulp to fermentation and drying. Although there are standards for quality of the beans with respect to physical parameters, no standards have been laid down for chemical parameters. Standards are prescribed for cocoa products such as cocoa powder and cocoa butter. A study of the chemical parameters provides useful indices for assessing the type of fermentation the beans have undergone and the regional and seasonal variations. It would also indicate the type of cocoa products that could be produced from the beans. With this in view, both physical and chemical parameters of cocoa beans processed at different regions have been analysed and data presented.

Cured beans (May–August 1983 season) procured from Kottayam (Kerala State), Puttur and Sakaleshpur (Karnataka State) and Chundale (Tamil Nadu State) were analysed. Size determination[2] was carried out on 100 beans randomly selected from the sample after discarding the damaged beans. The visual method for estimating the quality of fermented beans, namely, "cut test" was adopted to evaluate the beans. One hundred beans were cut longitudinally at the centre and the colour and texture of the cotyledons were taken as an index of fermentation.

AOAC methods[3] used for tea analysis were adopted for chemical analysis. For estimation of the alkaliod theobromine, Gerritsma and Koers[4] method was followed. Lactic acid was estimated by the method of Barker *et al.*[5] and citric acid by the method of Shaffron *et al.*[6]. The results are presented in Tables 1 and 2.

The physical analysis indicates the variations of weights of beans from 0.78–0.89 g which points out the tendency to harvest immature pods which should be avoided. The presence of violet beans (4–24%) indicates that some samples are underfermented. The presence

TABLE 1. PHYSICAL COMPOSITION OF COCOA BEANS

Source of samples	Samples analysed (No.)	Mean wt 100 beans (g)	Violet beans (% range)	Salty beans (%)	Insect damaged & fungus infected beans (%)	Whitish beans (% range)	Rejects (% range)	Nibs (mean) (%)	Shell (mean) (%)
Kottayam	19	77.95[a]	0–4	Nil	0–20	4–42	0–32.5	82.7 (65.61)[a]	17.3
Puttur	6	83.00[a]	0–4	Nil	Nil	0–20	0–18.6	85.9 (68.02)[a]	14.13
Sakaleshpur	7	89.14[a]	0–4	Nil	0–8	0–4	0–4.7	85.9 (68.04)	14.11
Chundale	4	80.75[a]	0–24	0–5	Nil	Nil	0–13.5	84.8 (67.04)	15.22
S.D.		±12.65 (32 df)	—	—	—	—	—	±3.15 (32 df)	

Figures in parentheses are the angular transformed variates.

Figures in the same column followed by same superscript do not differ significantly according to Duncans new multiple range test ($P < 0.05$)

TABLE 2. CHEMICAL COMPOSITION OF COCOA BEANS

Parameters	Kottayam	Puttur	Sakaleshpur	Chundale	S. D.
No. of samples	19	6	7	4	—
Moisture (%) @	5.76 (13.87)[a]	4.74 (12.55)[b]	6.10 (14.29)[a]	4.33 (12.00)[b]	±0.69 31 df
Total ash (%) @	3.14 (10.19)[a]	3.21 (10.35)[a]	3.17 (10.22)[a]	2.97 (9.92)[a]	±0.82 31 df
Water insoluble ash (%) @	1.64 (7.34)[ab]	1.48 (6.97)[a]	1.91 (7.94)[c]	1.73 (7.55)[bc]	±0.48 31 df
Alkalinity of water soluble ash (as K_2O/100g)	0.58[a]	0.59[a]	0.33[ab]	0.49[b]	±0.18 31 df
pH	5.16[a]	4.93[a]	5.53[c]	5.37[a]	±0.40 31 df
Titrable acidity (ml of 0.1 N NaOH/g)	2.88[ab]	3.52[a]	2.17[c]	2.51[ab]	±0.70 31 df
Volatile acidity (ml of 0.1N NaOH/g)	1.06[a]	1.16[a]	1.02[a]	0.92[a]	±0.38 31 df
Total sugars (%) @	0.96 (5.53)[a]	0.75 (4.85)[a]	0.80 (4.94)[a]	1.00 (5.66)[a]	±1.20 31 df
Lactic acid (%) @	0.57 (4.24)[a]	0.64 (4.34)[a]	0.27 2.92[a]	0.53 (3.98)[a]	±1.09 28 df
Citric acid (%) @	0.88 (5.38)[a]	0.62 (4.49)[b]	0.64 (4.56)[b]	0.56 (5.68)[b]	±0.53 30 df
Tannins (%) @	2.05 (8.13)[a]	2.37 (8.64)[a]	3.00 (9.95)[a]	2.72 (9.42)[a]	±1.46 31 df
Theobromine (%)	1.32 (6.55)[a]	1.54 (7.16)[b]	1.73 (7.55)[bc]	2.04 (8.22)[c]	±0.52 29 df
Fat (%)	49.29[a]	48.30[a]	52.63[a]	51.04[a]	±3.96 31 df

Figures in parentheses are the transformed variates.
Figures in the same row followed by different superscripts differ significantly according to Duncan's multiple range test ($P<0.05$).
@Statistical nanlysis was done on transformed variates (Angular-transformation).

of high percentage of whitish beans and rejects is attributed to harvesting of immature and under developed pods. The percentage of nibs varies from 82.8 to 85.9 per cent which is satisfactory.

The moisture content of cocoa beans is less than 6 per cent which indicates that they are well dried. The pH of the nibs varies from 4.93 to 5.53 indicating that some samples are acidic; accordingly the fermentation has to be controlled to produce beans of pH 5.2–5.6. Titrable acidity ranges from 2.17 to 3.52 and volatile acidity from 0.27 to 6.3 which quantifies the amount of acids in the beans. Tannins varied from 2.05 to 3.0 indicating reduction consequent to fermentation.

The results emphasize the point that only beans from well matured pods should be subjected to fermentation to obtain cured beans of uniform quality.

The authors thank Sri B. S. Ramesh, for statistical analysis of the data and Dr. B. L. Amla, Director of the Institute for his keen interest in this work.

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SOME PHYSICO-MECHANICAL PROPERTIES OF CHILLIES

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Received 12 *July* 1985; *revised* 29 *April* 1986

Physico-mechanical properties of chillies (*Capsicum*) (important in systematic design of transport, handling, packaging and storage systems) and shearing strength were determined. The bulk density, true density and bed porosity values of sound green chillies were 0.37 g/ml, 0.84 g/ml and 56.0% respectively, whereas for dried chillies the correspondingv alues were 0.13 g/ml, 0.58–0.62 g/ml and 77.6-79.0% respectively. Energy required to shear the dried chillies at different impact loads was measured and ranged from 0.052 to 0.199 joules.

Chilli (*Capsicum*) is an important cash crop and is mostly preserved in dehydrated form. In the absence of efficient mechanical drying system, currently almost all the chilli produced in the country is sun-dried. This results in considerable losses.[1] In addition, there are losses in transport, processing, packaging and storage. Unit technologies and technology systems for various phases such as transportation, handling, drying, packaging, and storage of chillies could not be developed systematically for want of adequate data on physico-mechanical properties. The present study was, therefore, undertaken to generate this data and included determination of physico-mechanical properties of green, sun-dried and mechanically dried chillies and shearing strength of mechanically dried chillies; initial moisture content was also measured in each case. The results are reported in this paper.

Chilli variety 'Pant C-1' (a common local variety of the Tarai region of North India) was selected for the study. For each experiment, one kg of ripened green chillies were hand picked from the field which formed the representative sample. Mechanically dried chillies were obtained by drying chilli samples in an experimental batch dryer by passing heated air at 50°C and at a velocity of 1.5 m/s.[2] Sun-drying was done by spreading the chillies in single chilli thick layers on concrete floor[3]. Bulk density was determined by measuring the volume of a known weight of chilli samples. True density was measured using toluene displacement method[4]. Each test was replicated thrice. The bed porosity was calculated using the experimentally determined values of bulk and true density. Moisture content was determined by drying the samples in a vacuum oven[5].

Shearing characteristics of the dried chillies were determined on an experimental shearing unit.[2] The unit employed was a compound pendulum with a cutting blade fitted to a hammer for shearing and had a provision to hold the chilli vertically at the central position under the axis of rotation of the pendulum. The blade attached with the hammer struck the chilli perpendicular to its position to shear it in horizontal direction. The equipment had a provision to drop the pendulum from any desired angle in the range of 0–90°. A pointer showed the location which the pendulum reached after shearing the chilli in the form of a residual angle which was used as a measure of residual energy of the pendulum. Energy spent in shearing the chilli was obtained by subtracting the sum of frictional and residual energy from the initial energy of the pendulum. Observations were taken for the values of initial angle of the pendulum in the range of 28 to 60°, based on the preliminary investigations which revealed that the shearing did not take place below 28°. For each pendulum angle, five replications were made. The dried chilli for shear test had a moisture content of 16.5 per cent (w.b.). Chilli samples for shear test were so chosen that the diameter of the selected chilli was about 1 ± 0.1 cm at the shearing plane and the length was 8 ± 0.5 cm.

The average values of bulk density, true density, and bed porosity of freshly harvested sound chillies and that of dried chillies are given in Table 1. This shows that the bulk density of dried chillies is approximately one-third while the true density is 70–74 per cent of the values for fresh sound chillies. Since the bed porosity has been estimated using the measured values of bulk and true density, the difference in values is obvious. The table also shows that the true density of dried chillies remains practically unaffected by the change in moisture content from 14 to 5 per cent (w.b.).

TABLE 1. PHYSICAL PROPERTIES OF FRESHLY HARVESTED SOUND CHILLIES AND DRIED CHILLIES

Type of chilli	Bulk density (g/ml)	True density (g/ml)	Bed porosity (%)
Freshly harvested sound chillies (moisture content: 75%w.b.)	0.37	0.84	56.0
Mechanical dried chillies moisture content: 14%w.b.)	0.13	0.62	79.0
Sun-dried chillies moisture content: 5%w.b.)	0.13	0.58	77.6

TABLE 2. ENERGY SPENT IN SHEARING THE CHILLI AT DIFFERENT PENDULUM ANGLES

Initial pendulum angle	Initial pendulum energy (joules)	Fictional energy loss (joules)	Striking velocity of pendulum (m/s)	Striking energy of pendulum (joules)	Residual pendulum energy (joules)	Energy for shearing chilli (joules)
60°	0.4957	0.1282	2.230	0.4316	0.1685	0.1990
50°	0.3541	0.1110	1.862	0.2986	0.1057	0.1374
40°	0.2319	0.0903	1.473	0.1868	0.0384	0.1032
35°	0.1793	0.0751	1.283	0.1418	0.0239	0.0803
30°	0.1328	0.0574	1.100	0.1041	0.0068	0.0686
28°	0.1160	0.0500	1.028	0.0910	0.0142	0.0518

The potential energy of the pendulum and the energy utilized, in shearing the sample and/or to overcome the frictional losses in the system, could be obtained with help of the following relationships:

$$E=0.9914\ (1-\cos\theta) \quad \ldots\ldots \quad (1)$$

$$\text{and, } E_f=E_i-E_r=0.9914\ (1-\cos\theta_i)-(1-\cos\theta_r)$$
$$=1.9828\ \sin\left(\frac{\theta_i+\theta_r}{2}\right)\sin\left(\frac{\theta_i-\theta_r}{2}\right) \quad \ldots\ldots \quad (2)$$

where, E=potential energy of pendulum, joules

F_f=Energy required for shearing and/or to overcome the frictional losses, joules

E_i=initial energy of pendulum, joules

E_r=residual energy of pendulum, joules

θ =pendulum angle, degrees

θ_i=initial pendulum angle, degrees

θ_r=residual pendulum angle, degrees

The frictional energy loss for different pendulum angles at no load (without sample) was calculated using equation (2). Frictional energy loss increased linearly with the pendulum angle, the relationshiop being:

$$E_f=0.002435\ \theta_i-0.013265\ (\text{Coeff. corr.}=98.9\%) \quad \ldots\ldots \quad (3)$$

In addition to the mechanical strength of the chilli, the shearing energy depended on the striking velocity and so the striking energy of the pendulum. The striking velocity of the pendulum was calculated using the relationship

$$V=\sqrt{2gR\,(1-\cos\theta_i)}=2\sqrt{gR}\left(\sin\frac{\theta_i}{2}\right) \quad \ldots\ldots \quad (4)$$

where, V represents striking velocity of pendulum, m/s; g stands for gravitational accleration constant, m/s^2, and R is the length of pendulum, m. The striking energy of the pendulum was estimated by subtracting half of the frictional energy loss value from the corresponding value of initial pendulum energy under no load. The values of striking velocity, striking energy of the pendulum and the shearing energy are given in Table 2. The dependance of shearing energy on striking energy was found to be linear, the relationship being:

$$E_{sh}=0.40705\ E_{st}+0.02165\ (\text{Coeff. corr.}=99.5\%) \quad \ldots\ldots \quad (5)$$

where, E_{sh} and E_{st} are the shearing and striking energies in joules, respectively. The energy required to shear the dried chillies at different impact loads varied from 0.052 to 0.199 joules.

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problem on stationary fits by using machinery adhesives. In contrast to mechanical devices, machinery adhesives are generally used in rigid cylindrical assemblies in shear or compressive mode to eliminated leakage and provide non cracking joints. The above book is well written to guide the designer, engineer or mechanic in selecting and using machinery adhesives effectively. This is a book of 'why', 'how' and 'what' for standard machinery adhesives. The book contains a lot of empirical and scientifically derived data. The data are very well organized and have been presented in tables and graphs for ready reference. The book contains 8 chapters.

The Ist chapter deals with the general information on machinery adhesives such as their definition, uses, selection, packaging and handling. An exhaustive table is given which provides information on trade names for most common generic plastics.

Chapter–2, provides the engineering data in respect of properties of machinery adhesives, proof load of steel bolts and stress area of threads, torque coefficient, viscosity, stress clearance, temperature, humidity, surface coverage, and the treatment for nuts and bolts, pipe fittings, sealing welds etc. The information is quite useful for design purposes where normal safety factors are used.

Chapter–3 deals with the environmental effects on machinery adhesives such as molecular breakdown by strong chemical reaction, solvation, absorption, stress cracking, mechanical stressing and desorption; all of which get accelerated by elevated temperature. A guide for the selection of anaerobic materials for sealing and locking in the presence of liquids and gases is also given in a tabular form.

Chapter–4 places emphasis on proper application of the machinery adhesives. The application considerations discussed include clearance of parts, proper applications, quality assurance and safety. The techniques, tools and instruments for application of liquid adhesives have been listed. A good description of systems and automation and the details of tools and instrument required for the same have also been provided.

Chapter–5 deals with the design of bolted joints loosening tendencies of bolted joints and prevention of premature loosening, applications for thread locking adhesives and securing of studs. Design equations and design data are provided for the guidance of designers and engineers.

Chapter–6 deals with the adhesives fitting of cylindrical parts. The information presented includes calculations, design data and other relevant design details.

Chapter–7 describes the traditional threaded systems, sealing techniques for such systems, design considerations and calculations and necessary design data on sealing.

Chapter–8 gives design hints for simplifying part manufacture, making use of standard machine parts, electrical insulation, machining etc.

In general, the book follows the systems approach to the problem of machinery adhesives for locking, retaining and sealing and is very informative and well designed, The book is an excellent reference work for designers. engineers and mechanics concerned with the problems of making sealed joints involved in the design and manufacture of various machines and equipments.

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

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

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

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