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(INDIA)

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3. To promote the profession of Food Science and Technology.

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—Editor

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Energy Requirement in Mechanical Chipping of Tapioca

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Chipping or slicing of tapioca with rotating knives was simulated by dropping a pendulum arm with knives of various sharpness at different cutting velocities. Cutting energy was determined at different velocities, bevel angles of knife and shear angles of cut in the pendulum. The cutting energy per unit cross-sectional area of the tuber decreased with increase in shear angle and bevel angle for all cutting velocities upto 60° to 75° and 30 to 45° respectively. The min. cutting energy per unit cross-sectional area of 3.10 kg, cm/cm^2 was found at 2.68 m/sec, 63° and 37.5° of cutting velocity, shear angle and knife bevel angle respectively.

Cassava or tapioca (*Manihot esculenta*) is the major root crop grown for the production of starch and allied products, animal feed and food. The tuber is cut into chips of desired thickness for drying and further processing. Cutting the tubers into the chips is by impact force. The optimised levels of the impact velocity or cutting velocity, sharpness of the blade and cutting angle will be more useful for the designers to develop the system for chipping of the tuber.

Prasad and Gupta[1] studied the mechanical properties of maize stalk as related to harvesting in a pendulum type impact shear test apparatus and reported that 23°, 35° and 2.65 m/sec of bevel angle, shear angle and cutting velocity respectively are optimum. The strength properties of forage crops and stalks of various crops have been studied by some of the research workers [2-5] Jindal *et al.*[6] and Parke[7] used pendulum type impacting device for applying impact load for the food materials. Kulshreshta *et al.*[8] studied the energy and peak force requirement in potato slicing in an Instron Machine at 0.25 to 1.0 m/min cutting speed and found the cutting speed was insignificant on cutting energy. In the reported study, the chipping of cassava with rotating blades was simulated by dropping the pendulum arm with a sharp knife. The objective of this study was to optimise the values of variables such as bevel angle of knife, shear angle and cutting velocity for cutting the tuber.

Theoretical considerations: Knife bevel angle (α): is the angle of the bevel edge or sharpness of the knife. The knife may be bevelled on one side or both sides (Fig.1).

Shear angle (β) is the angle, the plane of cut makes with the longitudinal axis of the tuber (Fig.1).

Cutting velocity (V_c) is the velocity of the knife when the pendulum is dropped from various desired heights to cut the tuber.

Determination of cutting energy: The theoretical approach to measure the cutting energy of plant, stalk and tuber is difficult as the process of cutting is complex[1]. The experimental approach to know the cutting energy is by the expression in terms of angular displacement of the pendulum arm. When the pendulum arm is in equilibrium as shown in Fig.1, the energy stored is zero and when raised to an angle θ with the equilibrium position, the potential energy stored E_s is given by

$$E_s = W_t h \quad \ldots (1)$$

$$h = R (1 - \text{Cos } \theta) \quad \ldots (2)$$

Where,

W_t = Weight of the pendulum arm, kg

h = Vertical component of the angular displacement of the pendulum arm corresponding to θ degrees, cm

R = The distance between the centre of swing and the centre of gravity of pendulum, cm

If the pendulum arm is released over a distance of θ, in the absence of cutting and moved over θ_o on the other side of the equilibrium position, the energy loss due to friction and air resistance E_f will be

$$E_f = W_t R \left[(1 - \cos \theta) - (1 - \cos \theta_o) \right] \quad \ldots (3)$$

when a sharp knife is attached to the pendulum arm it will cut the tuber, placed at the equilibrium position, during its downswing and take θ_c, the angular displacement in the upswing after cutting. Then the

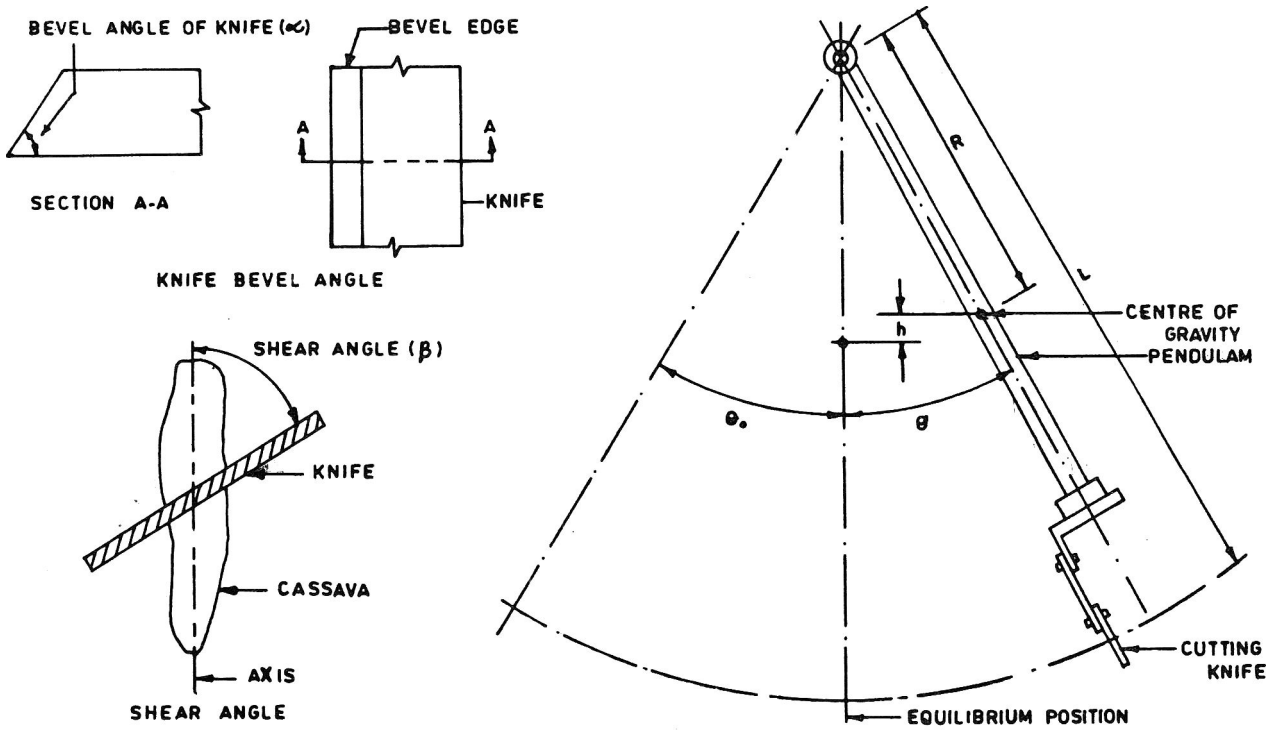

Fig.1. Schematics of pendulum arm and terminology

energy utilised in cutting the tuber E_c is the difference between the initial energy stored E_s and the total of the energy available in the pendulum arm after cutting and the energy lost in friction and air resistance E_f. This is expressed as

$$E_c = E_s - (E_f + E_o) \quad ....(4)$$

$$E_c = W_t R \left[(1 - \cos\theta) - \left\{ (1 - \cos\theta - (1 - \cos\theta_o) + (1 - \cos\theta_c) \right\} \right]$$

$$E_c = W_t R (\cos\theta_c - \cos\theta_o) \quad ...(5)$$

Using Eqn. (5), the energy required in cutting can be calculated experimentally.

Determination of cutting velocity of the knife: Theoretically, the velocity of the knife attached to the pendulum arm at the equilibrium position can be calculated from the work-energy method as follows:

$$W_t R(1 - \text{Cos}\,\theta) = \frac{I\omega^2}{2} \quad ...(6)$$

$$\omega^2 = \frac{2W_t R (I - \cos\theta)}{I} \quad ...(7)$$

The cutting velocity, V_c at the lowest position will be

$$V_c = \omega L = L\sqrt{\frac{2\Sigma W_c r_c (1 - \cos\theta)}{I}} \quad ...(8)$$

where,

ω = angular velocity of pendulum arm
I = Moment of inertia of pendulum arm
W_c = Weight of each component of the pendulum arm
r_c = The distance between the centre of swing and the centre of each component of the pendulum
L = Total length of the pendulum arm

Materials and Methods

A pendulum type impact shear test apparatus similar to the one used by Prasad *et al.*[1] was used to study the cutting energy requirement of cassava tuber. The experiments were conducted on local varieties of cassava bought from nearby market whose diameter ranged from 37 to 72 mm. In the pendulum arm of the apparatus, there was provision to hold and change the sharp knife and also to drop the pendulum from various heights by releasing a spring loaded lock. On the axis of the rotation of the pendulum arm, pointers were provided to indicate the angular displacement by travelling on the disc graduated in degrees. To obtain different cutting velocities of knife, the pendulum arm was released from different angular positions, calculated using equation (8). The cutting velocity was varied as 1.81, 2.68, 3.51 and 4.96 m/sec. The pendulum arm was made of mild steel rod of 15 mm

diameter weighing 1.693 kg per metre length. The effective length of the pendulum was 0.85 m. A dead weight of 2.4 kg was added to the pendulum to achieve higher cutting velocities by increasing the weight of the pendulum. The knives were made with 3 mm thick mild steel plate for 135 mm length and 35 mm width and sharpened on one side to the levels and bevel angles.

The various shear angles selected for the study are 30, 45, 60, 75 and 90°. The tuber was held between two clamps at the knife approach angle of 0°. The shear angle was varied by holding the tuber at various angles in the horizontal plane with the direction of motion of the knife. The sharpness of the knife varied as 15, 30, 45 and 60°. From the angular positions reached after cutting the tuber and in the absence of cutting by the knife in the pendulum arm, the energy requirement of cutting cassava was calculated with the equation (5) as done by Prasad *et al.*[1] The range of moisture content of the tuber during the experiment was 58 to 70 per cent, (wet basis)[9]. As the size of the tuber varies, the cutting energy calculated was expressed per unit area of cross-section of the tuber. The cross-sectional area of the cut tuber was determined by tracing on ordinary graph and counting the squares. The mean value of the three replications is taken for discussion.

Results and Discussion

Effect of knife bevel angle on cutting energy: The effect of knife bevel angle on cutting energy per unit cross-sectional area of tuber, E_{ca} is given in Fig.2. for the shear angle and cutting velocity of knife of 90° and 3.51 m/sec respectively. The relationship between the knife bevel angle and the cutting energy per unit area is found to be non-linear, decreasing from 15° to 37.5° and increasing beyond 37.5°. The energy requirement is a result of interaction among frictional, compression and shear force. With less bevel angle, the frictional forces are more as the sliding surface on the bevel edge is increased. The energy loss in friction is less when the slope of the knife bevel edge approaches the static frictional angle. For bevel angle more than 37.5°, it requires more energy to cut and penetrate through the tuber because of less sliding surface.

Effect of shear angle on cutting energy: Fig. 3 is the graphical representation of cutting energy per unit area with shear angle at 30° of bevel angle and cutting velocity of 3.5 m/sec. The results revealed that the cutting energy per unit area was minimum for the shear angle of about 63°. As the shear angle decreases from 90°, the area of tuber being cut increases thereby decreasing the cutting energy per unit area. When the shear angle is further decreased, the frictional force becomes significant resulting in increased cutting energy per unit area[1].

Effect of cutting velocity on cutting energy of cassava: The effect of cutting velocity on cutting energy is given in Fig. 4. The cutting energy per unit area was observed to be minimum corresponding to the velocity of 2.5 m/sec. The cutting energy per unit area increased sharply when the velocity increased beyond it. At lower velocities, the impact to cut and penetrate through the tuber is less and hence the energy requirement is increased. At higher velocities, the increase in the cutting energy per unit area may be due to the kinetic energy imparted by the pendulum is wasted as more energy is transmitted to the separated parts of the tuber after cutting.

It is concluded that the cutting energy per unit area for cassava was observed to be minimum at about 37.5° of the knife bevel angle. The studies on the effect

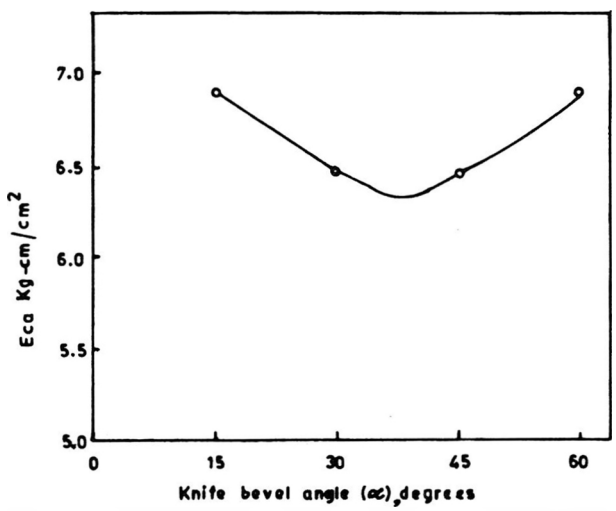

Fig.2. Relationship of knife bevel angle to cutting energy, Shear angle of cut; 90°; cutting velocity: 3.51 m/sec.

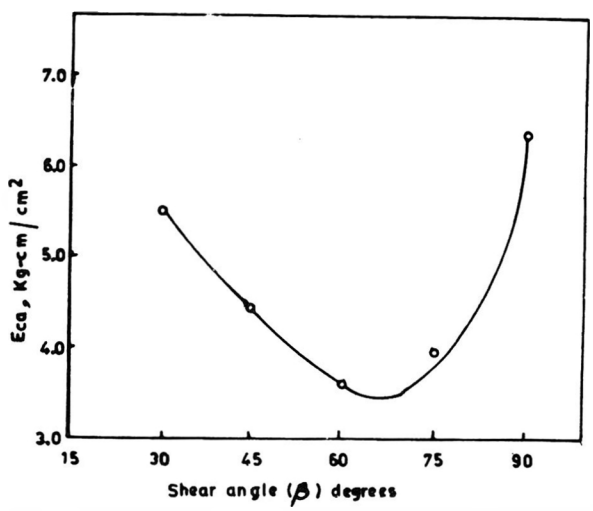

Fig.3. Relationship of shear angle to cutting energy. Knife bevel angle; 30°, cutting velocity; 3.51 m/sec.

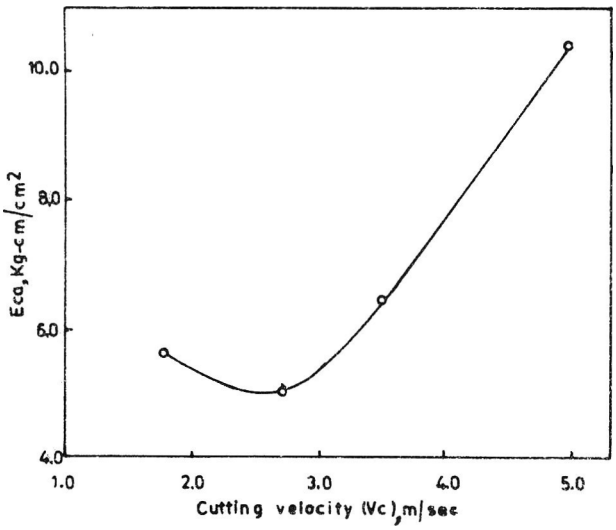

Fig.4. Effect of cutting velocity on cutting energy. Knife bevel angle; 30°; shear angle: 90 degrees.

of knife velocity indicated that the cutting energy per unit area was min. at a velocity between 2.25 and 2.75 m/sec and optimum shear angle was reported to be 63°.

References

1. Prasad J and Gupta C P, Mechanical properties of maize stalk as related to harvesting, *J agric Engng Res.* 1959, **20,** 79.
2. Feller R, Effect of knife angles and velocities on cutting of stalks without a counter-edge, *J agric Engng Res* 1959, **4,** 277.
3. Halyk P M and Hurlburt L W, Tensile and shear strength characteristics of alfalfa stems, *Trans ASAE* 1968, **11,** 256.
4. Pickett L K, Rheological properties of corn stalks subjected to tranverse loading, *Trans ASAE,* 1969, **12,** 392.
5. Prince R P, Measurement of ultimate strength of forage stalks, *Trans ASAE* 1961, **4,** 208.
6. Jindal V K and Mohsenin N N, Dynamic hardness determination of low kernels from impact tests, *J agric Engng Res* 1978, **23,** 77.
7. Parke D, The resistance of potatoes to mechanical damage caused by impact loading, *J agric Engng Res* 1963, **8,** 173.
8. Kulshreshtha M Pathak A K and Sarkar B C, Energy and peak force requirement in potato slicing *J Fd Sci Technol* 1988, **25,** 259.
9. *Approved methods. Moisture determination.* American Association of Cereal Chemists, Inc., St. Paul, Minnesota 1976, 44-15 A.

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Hydrolysis of Lactose in Whey Powder Solutions by *Aspergillus oryzae* Lactase

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In this study, the effects of pH and temperature on initial reaction rates and relative activities (%) for lactose hydrolysis in whey powder solutions by *Aspergillus oryzae* β-galactosidase were investigated. Optimum conditions were found as; pH = 4.5, T = 50°C and Michaelis-Menten Constant, Km = 52.78 mM lactose and maximum reaction rate, v_m = 1.25 mmole glucose l^{-1} min^{-1} for soluble lactase.

The disposal of whey, a by-product of cheese manufacture, is troublesome for dairy industry in recent years. Worldwide over 70 million t and in Turkey alone 850,000 t of cheese whey are produced every year. Being rich in proteins, lipids and lactose, whey has a high biochemical oxygen demand (BOD) of about 50,000 mg O_2/l. The great environmental hazards of whey as waste requires, large sewage disposal plants with high operating costs and expensive treatments.[1-3]

Numerous methods for whey treatment have been suggested in literature.[4-5] The most promising method seems to be the enzymatic hydrolysis of lactose to glucose and galactose by the enzyme lactase (β-galactosidase, β-D-galactoside galactohydrolase, EC. 3.2.1. 23). Lactases from bacterial, fungal and yeast sources have previously been used in the hydrolysis of lactose present in different solutions. Recent literature has been concerned with sources of lactase and immobilization for industrial application in hydrolysis of lactose containing sweet or acid whey.[6,7] A fungal β-galactosidase derived from *Aspergillus oryzae* was used in this study. The present investigation includes pH and temperature profiles for the soluble lactase; initial reaction rates and relative activities (percentage) for the determination of kinetic parameters for the hydrolysis of lactose in whey powder solutions in a batch reactor.

Materials and Methods

Spray-dried whey powder was obtained from Pinar Sut Mamulleri Sanayii A.S. Whey powder was reconstituted in water to the appropriate solids concentration and reconstituted whey was deproteinized by heating in a boiling water bath for 5 min. Whey powder solutions were treated with *Aspergillus oryzae* lactase (Sigma Chemical Company Ltd., USA; activity with lactose is 4.0 U/mg solid). Initial lactose concentration was determined by the method of Maeda.[8]

The activity of a preparation of β-galactosidase was determined using reconstituted whey powder solutions as substrate by measuring the amount of glucose produced in a specified period of time. One unit activity was defined as the liberation of 1 m mole of glucose in one min under specified reaction conditions (pH and temperature). For the soluble lactase activity determinations, 1.0 ml of a 146 mg/l solution of the enzyme (EL_0) in the acetic acid/acetate buffer (pH 4.5) was added to 700 ml of a lactose containing substrate solution of whey powder. The reaction mixture was stirred at 200 r.p.m. for 180 min in a batch reactor. The reaction was stopped by removing a 1.0 ml of aliquot from the reaction mixture and placing it in a boiling water bath for 5 min. The sample was filtered and glucose concentration of the clear filtrate was determined by the Sigma Glucostat Method.[9] This procedure involved the addition of 5.0 ml of assay solution to 0.5 ml of an appropriately diluted sample solution and holding the mixture at 37°C for 30 min. The absorbance of this mixture at 450 nm was read using a Bausch and Lomb Spectronic 20.

In order to obtain the pH and temperature profiles for lactase and calculate the Michaelis-Menten Constant (Km, mM lactose) and maximum reaction rate (Vm, m mole glucose. l^{-1}. min^{-1}) for the hydrolysis of lactose present in whey powder solutions, initial reaction rates (v, m mole glucose. l^{-1}. min^{-1}) were determined for different levels of initial substrate concentrations. This was accomplished by determining

the glucose concentration of sample aliquots removed at various intervals from the reaction mixtures. A 700 ml working volume of a reactor was used for enzymatic hydrolysis of lactose in whey powder solutions. In a typical run, 1 l of a substrate solution was first prepared. The substrate solution was a whey powder solution of a known lactose content changing in a range of 4.72-83.03 mM.

At time zero, reaction was initiated by the addition of a 1.0 ml of enzyme solution to substrate solution. Then 1.0 ml of samples were taken from the reaction mixture over a 0-180 min period. Each sample vial was immediately immersed in a boiling water bath and kept there for 5 min to inactivate lactase and filtered. The concentration of glucose of each clear sample was then determined.

Results and Discussion

As a first step in finding the best set of conditions for maximum initial reaction rate and for relative activity per cent, the pH profile of soluble *Aspergillus oryzae* lactase was determined. In the pH 3.0-6.0 range, the maximum initial reaction rate was observed at pH 4.5. Experiments were carried out by using 30g/l of a reconstituted whey powder solution (initial lactose concentration, SL_0 = 56.56 mM lactose) at 50°C. The activity of the soluble lactase measured at its optimum reaction conditions (pH = 4.5 and 50°C) and at the end of the reaction (180 min) was 1747.51 m mole glucose/l. min.g lactase. In each case, the activity is normalized relative to the maximum activity of the enzyme at the initial pH of the solution tested. Fig 1 shows that the maximum relative per cent activity was also obtained at pH 4.5. The temperature profile for soluble lactase was determined at several temperatures ranging from 30-65°C and a constant pH of 4.5 at 200 r.p.m. It was observed that the relative activity and the initial reaction rate of *Aspergillus oryzae* lactase increase with temperature up to 50°C, whereas they drop sharply after 50°C (Fig. 2).

A plot of the 1n (relative per cent activity) versus reciprocal temperature was fitted to two straight lines. From the slopes of these lines, the activation energies (Ea, cal/g mole) were calculated respectively as:

$Ea1 = 5053.91$ cal/g mole ($< 50°C$)
$Ea2 = -43247.41$ cal/g mole ($> 50°C$)

In order to obtain the Michaelis-Menten Constant and maximum reaction rate for the hydrolysis of lactose in reconstituted whey powder solutions by *Aspergillus oryzae* lactase, initial reaction rates were determined at the range of a 2.5-50.0 g/l of whey powder concentrations. Lactose concentrations of each substrate solutions were determined and initial reaction rates were calculated.

Next, a Lineveawer-Burk plot was prepared by plotting the reciprocal of the initial reaction rate against the reciprocal of the lactose concentration (Fig.3.). The Michaelis-Menten Constant and maximum reaction rate were calculated as Km = 52.78 mM lactose and $v_m = 1.25$ m mole glucose $.l^{-1}. min^{-1}$ respectively from the slope and intercept of the straight line.

The results obtained in this study will be the basis of *Aspergillus oryzae* lactase kinetics using whey powder as a substrate and also important for treatment of wastes of dairy industry.

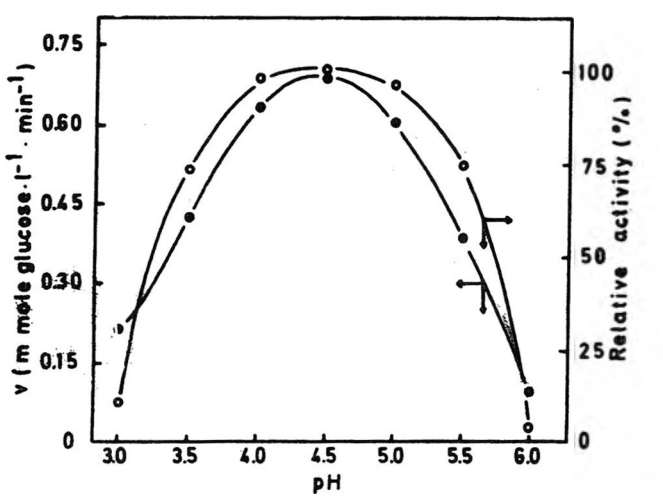

Fig. 1. Effect of pH on the initial reaction rate and on the relative activity (%) for the enzymatic hydrolysis of lactose in whey powder solution (SL_0 = 56.56 mM lactose, EL_0 = 146 mg lactase/l, T = 50°C, working volume = 700 ml, n = 200 rpm).

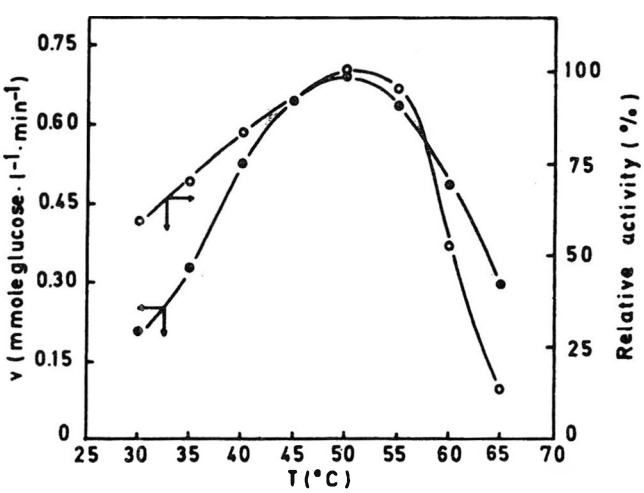

Fig. 2. Effect of temperature on the initial reaction rate and on the relative activity (%) for the enzymatic hydrolysis of lactose in whey powder solution (SL_0 = 56.56 mM lactose, EL_0 = 146 mg lactase/l, pH = 4.5, working volume = 700 ml, n = 200 rpm).

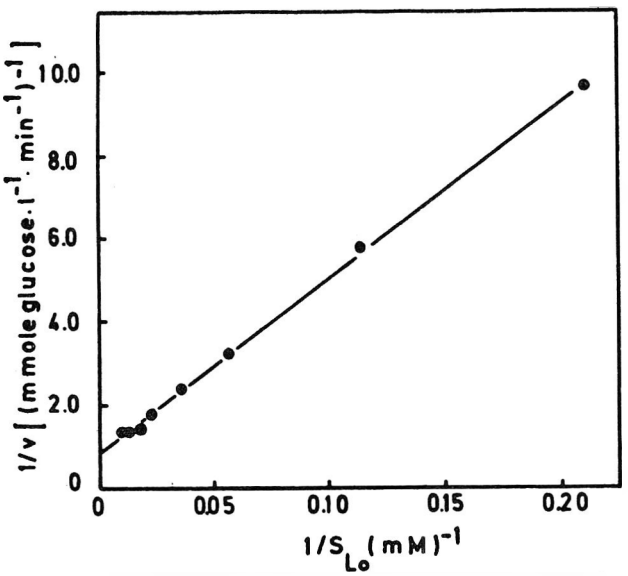

Fig. 3. Lineweaver-Burk plot of *aspergillus oryzae* lactase for the hydrolysis of lactose in whey powder solution (EL_o = 146 mg lactose/l, T = 50°C, pH 4.5, working volume = 700 ml)

References

1. Metin m, Sut sanayiinde peynir suyunun degerlendirilmesi, *Ege Univ. Munhendislik Fakult. Dergisi Seri: B Gida Muhendisligi*, 1983, **1** 151.
2. Prenosil J E Stuker E Hediger T and Bourne J R, Enzymatic whey hydrolysis in the pilot plant "Lactohyd", *Proceedings of 3rd European Congress on Biotechnology*, Munchen, 1984 a **2**, 557.
3. Prenosil J E Stuker E Hediger T and Bourne J R, Whey utilization enzymatic whey, hydrolysis in the pilot plant "Lactohyd", *Bio/Technology*, 1984 b, **2**, 44.
4. Kosikowski F, *Cheese and Fermented Milk Foods*, F V Kosikowski and Associates, New York, 1982, 711.
5. Kosaric N and Asher Y J, The utilization of cheese whey and its components, *Advances in Biochemical Engineering*, A. Fiechter (Ed.), 1985, Springer-Verlag, Berlin, Heidelberg, 1985, **32**, 25.
6. Richmond M L Gray J I and Stine C M, Beta-Galactosidase: Review of recent research related to technological application, nutritional concerns, and immobilization, *J. Dairy. Sci.* 1981, 64, 1759.
7. Roodpeyma S JR Hill C G and Amundson C H, Use of immobilized lactase in processing cheese whey ultrafiltrate, *Process Engng.* 1983, **6**, 113.
8. Maeda H, Preparation of immobilized β-galactosidase by poly (vinylprolidone) and the continous hydrolysis of lactose in acid whey, *Biotechnol, Bioeng.*, 1975, **17**, 1571.
9. Sigma Chemical Company, *Procedure No.* 510, St Louis, 1984, 12p.

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Evaluation of Some U.P. Hill Wheats for Their Physical, Chemical, Rheological and Bread Making Characteristics

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The Uttar Pradesh (U.P.) hill wheats had lower thousand kernel weight and higher hecto-litre weight with lesser extraction rate as compared to improved varieties of wheats. Maximum protein content was exhibited by the hill wheat 'Lalmisri'. Based on the values of Pelshenke, sedimentation and alveographic characteristics, the flours of 'Lalmisri' and 'UP 301' were found to be medium-strong gluten flours and the others weak gluten flours. In bread making characteristics such as loaf height, loaf weight and loaf volume, 'Lalmisri' was found to be superior to the others. However, in terms of crust colour, crumb softness, taste and eating quality, the bread made from 'UP 301' had an edge over that made from 'Lalmisri' and 'Sonalika'. 'Thumri' and 'Dutee' were rated as poor quality wheats for bread making.

The suitability of wheat for a particular end use is determined by the grain quality which is affected by genetic and environmental factors on the one hand and by milling techniques on the other[1,2] A few definitive studies are available on the physical and dough quality characteristics of indigenous Indian wheats and have been found to vary in their quality characteristics[3,4].

In the recent past, the fast growing milling and baking industry in India has been demanding segregation of wheat varieties on the basis of protein content to enable them to cater fully to the specifications of flour of different physical, chemical and rheological properties or by genetic improvement. Although there has been the advent of improved, high yielding varieties of wheat with the dwarf gene, there still appears to be a large number of pure strains of indigenous varieties of wheat being grown in specific regions of India.

Several indigenous varieties, especially those grown in the temperate climate of the northern hill tracts in India have been reported to possess superior bread making quality[5]. There are still many others about the qualities of which no information is available. To fill this gap in knowledge, the present investigation was undertaken.

Materials and Methods

Samples of 'UP-301' and 'RR-21' wheats from the year's harvest were obtained from the Crop Research Centre of this University, and the sample of the hill wheat 'Lalmisri' was obtained from the University's hill campus at Ranichauri (Garhwal hills). Samples of the two other hill wheats, namely, 'Dutee' and 'Thumri', were procured from the local growers in Almora district. All the wheat samples were cleaned free of chaff, grit, dirt and other foreign materials and were stored in airtight bins.

The wheat grains were inspected visually for their colour and vitreousness. On the basis of colour, the wheats were classified as white, amber or red wheat, and on the basis of vitreousness, they were classified as vitreous or opaque.

Hectolitre weight and thousand kernel weight were determined using the method described by Pomeranz[2]. Kernel hardness was measured by pressing ten average sized well filled grains at 12.5 ±

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0.5 per cent moisture from each wheat sample under the grain hardness tester (Manufactured by Kiya Seisakusho Ltd., Japan).

Cleaned samples of all the wheats were conditioned to 15 per cent moisture content for 48 hr with intermittent shaking once every 12 hr in airtight containers. After conditioning, each sample was milled on the Buhler Laboratory Mill (Model MLU - 202) by the standard procedure of AACC[6]. Milling yield was calculated on the basis of total recovered product (straight flour) of the wheat milled. Percent 'extraction rate' was also calculated on the same basis. The milled flour samples were packed into polythene bags and sealed until analysis.

Moisture, crude protein, total ash and crude fat were estimated by using standard methods of analysis[6] and the carbohydrate content was calculated by difference.

Colour grades were determined by the Kent-Jones and Martin Flour colour Grader (Henry Simon Ltd., Stockport, England). Pigment, gluten content, reducing and non-reducing sugars, and damaged starch were estimated by using standard procedures of AACC[6]. Pelshenke value was determined according to the method of Pelshenke and as modified by Villegas and Borlaug.[7] Sedimentation value was estimated by the method described by Pinckney *et al*[8]. Alpha-amylase activity was assayed colorimetrically based on the H-unit method proposed by Hagberg[9], with the revisions suggested by Perten[10]. Mixograph and Chopin Alveograph were used to study the dough characteristics of wheat flour.

Rheological characteristics: The 'remix' baking test procedure of Irvine and McMullan[11] was used for test baking. Bread was made from each sample of wheat flour, taking 100 g flour; 1.6 g active dry yeast; 2.4 g sugar; 1.0 g salt; 0.1 g ammonium biphosphate; 0.01 g potassium bromate; and water to make a dough of desired consistency, under the following conditions: mixing time, 3.0 min (Hobart mixer); bulk fermentation, 150 min at 30°C and 75 per cent RH; remixing, 2.0 min; recovery time, 25 min; proofing, 55 min; and baking at 450°F for 25 min.

The bread characteristics studied included loaf volume, loaf weight, loaf height and specific volume of loaf. The loaf volume was measured by the rape seed displacement method.

The following score card was used by a laboratory panel of ten members to evaluate the bread. The characteristics evaluated included loaf volume, loaf shape, crust colour and bloom, pile, crumb colour and sheen, crumb texture (grain), crumb softness, crumb stability, taste and eating quality, and keeping quality. Each charactersitic carried 10 points.

All the analyses have been done in duplicate and average values have been reported.

Results and Discussion

The results of visual observations indicated that 'Thumri' and 'Dutee' of the Kumaon hills were white and non-vitreous while 'Lalmisri' of Garhwal hills was amber coloured and vitreous as compared to the white colour and flintiness of the improved varieties of wheat.

The hill wheats were smaller in size and had lower 1000-kernel weights than the improved varieties (Table1). However, the reverse was true in respect of hectolitre weight. The values for 1000-kernel weight were in the range reported earlier whereas the values of hectolitre weight were greater than the reported range[12]. The hill wheat, 'Lalmisri' showed the highest hectolitre weight and kernel hardness and would be classified as a hard wheat when compared with medium hardness of two improved wheats.

The wheat 'UP 301' exhibited the highest values for flour yield, followed by 'RR-21' (Table 1). It was interesting to observe that flour yield was directly related with 1000-kernel weight and not with the test weight. This was in accordance with the earlier findings[13].

Flour yields of only 'UP 301' and 'RR-21' were comparable to the generally reported values of 70-72 per cent for hard wheats. On the other hand, 'Lalmisri' which was classified as a hard wheat did not show, as expected, a higher flour yield. The extraction rate for all varieties studied was 72 per cent or lower.

The moisture content of the hill wheats ranged from 11.3 to 13.2 per cent. 'Dutee' and 'Thumri' had protein contents in the ranges of soft wheats while 'Lalmisri' and two improved varieties showed protein contents of medium strong wheats. Interestingly, the hill wheat 'Lalmisri' had the highest gluten content among all the wheats (Table 2).

TABLE 1. PHYSICAL AND MILLING CHARACTERISTICS OF DIFFERENT WHEATS.

Variety	1000-kernel wt. (g)	Kernel hardness (Kg/grain)	Hecto-litre(wt) (kg)	Total break flour, (%)	Total reduction flour, (%)	Extra-ction rate, (%)
Thumri	32.0	7.71	92.1	17.73	48.69	66.42
Dutee	35.0	7.95	93.0	17.03	52.17	69.20
Lalmisri	28.6	10.43	94.5	14.70	51.56	66.26
Sonalika	38.9	9.16	87.9	16.00	54.27	70.27
U.P. 301	48.5	9.89	91.9	14.77	57.34	72.11

TABLE 2. CHEMICAL AND PHYSICO-CHEMICAL CHARACTERISTICS OF DIFFERENT WHEATS AND FLOURS

Variety	Wheats						Flours			
	Moisture (%)	Ash (%)	Crude protein (%)	Ether extract (%)	Gluten Wet (%)	Gluten Dry (%)	Colour grade value (KJ unit)	Pigment (carote-noid (p.p.m)	Pelshenke value (min.)	Sedimenta-tion value (cc)
Thumri	12.8	1.50	9.03	0.76	27.67	8.32	5.39	2.52	79.1	21.2
Dutee	12.7	1.30	8.50	1.38	26.94	8.22	4.35	3.17	81.3	22.4
Lalmisri	13.2	1.28	12.32	1.92	42.98	11.85	7.60	2.43	122.1	38.0
Sonalika	12.7	1.14	10.85	1.42	37.31	11.50	6.00	1.27	80.2	21.8
U.P. 301	11.3	1.44	11.59	0.94	35.27	11.46	6.90	1.12	89.1	26.5

The colour grade values of two improved wheats and the hill wheat 'Lalmisri', and the pigment content of all the hill wheats were in the ranges of 5.1 to 7.9, and 1.3 to 2.89, respectively. These values fell within the ranges reported for Indian wheats by Shurpalekar er al[14]. (Table 2). The amber coloured 'Lalmisri' had the highest colour grade value, Pelshenke value and sedimentation value, whereas the difference in the values of these attributes amongst other varieties is not very great.

Reducing sugars in terms of maltose units were highest in 'RR-21' and lowest in 'Thumri' (Table 3). 'UP-301' had the highest non-reducing sugars and alpha-amylase activity while 'Lalmisri' showed the lowest values for both these traits.

The damaged starch content of all the wheats ranged from 5.4 to 7.2 per cent. Flours from hard wheats have more damaged starch granules, and damage to starch generally increases with the protein content of wheat. It was observed that 'Lalmisri' had much lower starch damage than 'UP-301' whereas it was expected to be similar in medium strong wheats. The two other hill wheats, classified as weak wheats, had higher starch damage than 'Lalmisri' and no reason for these difference is being advanced at this stage.

TABLE 3. DAMAGED STARCH, SUGARS AND ALPHA AMYLASE ACTIVITY OF DIFFERENT WHEAT FLOURS

Variety	Damaged starch (%)	Sugars Reducing (mg of maltose/ 10g flour)	Sugars Non-reducing (mg of sucrose/ 10g flour)	α-amylase activity (A units)
Thumri	6.0	15.0	169.3	–
Dutee	7.2	27.0	134.3	6.7
Lalmisri	5.4	27.3	128.0	4.6
Sonalika	5.8	34.0	180.7	6.3
U.P. 301	6.8	27.0	221.7	7.6

The mixographic measurements of various wheats are presented in Table 4. The samples of 'Dutee' and 'Thumri' showed low degree of developing and weakening angle with higher mixing tolerances as compared to the remaining three wheats. Among the three hill wheats, the mixing time for 'Lalmisri' was lower than that for the two others, which might be due to the higher gluten content. The width and height of the curve at peak were lower for the two wheats of Kumaon hills than for the others. The peak height was maximum for 'Lalmisri' which might be due to highest protein content. The area under mixogram, which measures the energy input during dough development, was highest for 'UP-301' and lowest for 'RR-21' and hill wheats were intermediate in this respect.

The flours of 'Thumri', 'Dutee' and 'RR-21' showed the baking strength values less than 10 cm^2 along with lower values for stability, elasticity and deformation work. All the characteristics were positively related to the characteristics of weak flours[15]. On the other hand, remaining two wheats were classified as medium strong flours as the values for baking strength fell in the range of 10 to 30 cm^2 (Table 5). In further sub-classification, the dough of 'Lalmisri' was classified as balanced dough as the L/P ratio varied between 0.85 and 1.35, a characteristic range for balanced dough.

Bread made from the hill wheat, 'Lalmisri,' exhibited higher loaf height, loaf weight and loaf volume. The values for these characteristics for the bread made from 'RR-21' were comparable to those pertaining to 'Lalmisri' bread.

Scores for the different bread characteristics showed that the flour of 'Thumri' and 'Dutee' yielded poor quality bread whereas 'Lalmisri' and 'RR-21' produced acceptable bread with good crust and crumb colour.

From the results of this study, it is concluded that the

TABLE 4. MIXOGRAPH CHARACTERISTICS OF WHEAT FLOURS

Variety	Developing angle (X)	Weakening angle (Y)	Mixing tolerance 180° -(X + Y)°	Mixing time (min.)	Peak width (cm)	Peak height (cm)	Developing area, (cm^2)
Thumri	8	4	168	5.04	0.8	2.3	13.2
Dutee	5	3	172	4.47	0.7	2.3	15.0
Lalmisri	17	8	155	4.25	1.2	3.5	15.2
Sonalika	24	15	141	2.13	1.1	3.3	6.8
U.P. 301	10	7	163	5.53	1.2	3.3	23.4

TABLE 5. ALVEOGRAPH CHARACTERISTICS OF DIFFERENT WHEAT FLOURS

Variety	Exeten-sibility L (mm)	Stability P (mm)	Stability at rupture (mm)	Baking strength S(cm^2)	Elasti-city (cm^2)	L/P ratio	Swelling index, G (cc)	Defor-mation work, (erg.)
Thumri	24	42	20	7.3	4.5	0.57	12.5	62.6
Dutee	24	45	24	8.8	5.5	0.53	11.0	58.5
Lalmisri	55	52	22	18.6	11.8	1.06	17.0	128.7
Sonalika	35	38	11	8.5	4.3	0.92	15.0	72.7
U.P. 301	39	58	28	17.5	11.9	0.67	15.0	134.3

hill wheat, 'Lalmisri', is suitable for bread making whereas 'Thumri' and 'Dutee' are not, when compared to the improved wheat.

References

1. Majumdar S N, Wheat flour quality for bakery industry, *Wheat Research Workers Conference,* IARI, New Delhi, 1986.
2. Pomeranz Y, *Wheat Chemistry and Technology,* American Association of Cereal Chemists. St. Paul, Minnesota, 1971.
3. Singh B and Bailey C H, A biochemical and technological study of Punjab wheat varieties, *Cereal Chem,* 1940, **17**, 169.
4. Bains G S, Grain quality requirements for domestic consumption and export needs, *Wheat Research Workers Conferences,* Punjab Agricultural University, Ludhiana. 1986.
5. Kent-Jones D W and Amos A J, *Modern Cereal Chemistry,* Food Trade Press, London, 1967, 6th Edn.
6. *Approved Methods*, American Association of Cereal Chemists, St. Paul, Minnesota, 1976.
7. Welsh J R and Normann R M, Use of modified Pelshenke test for early generation wheat quality, *Crop Sci,* 1972. **12**, 80.
8. Pinckney A J, Greenway W T and Zeleny L, Further developments in the sedimentation test for wheat quality, *Cereal Chem,* 1957, **34**,16.
9. Hagberg S, A rapid method for determining alpha-amylase activity, *Cereal Chem,* 1960. **13**, 218.
10. Perten H, A colorimetric method for the determination of alpha-amylase activity ICC Method. *Cereal Chem,* 1966, **43**, 336.
11. Irvine G N and McMullan M E, The 'remix' baking test, *Cereal Chem,* 1960. **37**, 603.
12. Finney P L, Bains G, S Hoseney R C and Lineback D R, Quality of Indian wheats, *Cereal Sci, Today,* 1973. **18** 392.
13. Salunkhe D K, Kadam S S and Austin A, Quality of wheat and wheat products, *Indian Council of Agricultural Research Report,* New Delhi, 1986. pp. 1-6.
14. Shurpalekar S R, Kumar G V, Venkateshwara Rao, Ranga Rao G C P, Rahim A and Vatsala C N, Phisico-chemical, rheological, milling characteristics and bread and chapati making quality of Indian wheats, *J Food Sci Technol,* 1976. 13, 79.
15. Sangale P B, Kadam S S and Amaya A, Quality parameters of physical characteristics of flour and dough. *Quality of Wheat and Wheat Products,* (Salunkhe, D.K., Kadam, S.S., and Austin, A., Eds.) Indian Council of Agricultural Research. 1986. 220.

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Studies on the Effects of Germination and Drying Conditions on the Cyanide Content of Sorghum Sprouts

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The effect of germination and drying on the cyanide content of sorghum sprouts were investigated by germinating sorghum of local red variety (LRV) at temperatures of 25°C and 30°C for 6 days and drying them at 55°C for 24 hr. The toxic hydrogen cyanide content of the sorghum sprouts increased linearly as the number of days of germination increased. The highest amount of hydrogen cyanide was got from sorghum sprouts germinated at 30°C for 3 days. From the fourth day, the cyanide content started to decrease giving the least value on the sixth day of germination. Hydrogen cyanide in sorghum sprouts germinated at 25°C increased linearly with germination time, the highest value was got on the sixth day of germination. The cyanide content of the sorghum samples decreased as the drying time increased, giving the least value after 24hr drying. Germinating at 30°C produced higher amount of hydrogen cyanide in sorghum sprouts than germinating at 25°C.

With the growing awareness in Nigeria of the need to utilise local resources in industrial ventures due to dwindling economic resources, both the private business entrepreneurs and government departments are embarking on large scale industries allied to agriculture. The brewing sector and some food industries are using malted sorghum in the preparation of beverages and baby foods.

Dry sorghum seed is reported to contain low or undetectable amounts of dhurrin, a cyanogenic glycoside which yields hydrocyanic acid (HCN) upon hydrolysis[1]. However, the sorghum plant contains exceptionally high levels of the cyanide[2,3]. It has also been found that drying sorghum sprouts at 50°C and grinding the sprouts to produce a meal did not reduce the hydrogen cyanide content.[4] Some breweries in Nigeria use sorghum sprouts in lager beer production. The objective of this study was to determine the effects of germination and drying times on the cyanide content of sorghum sprouts.

Materials and Methods

The sorghum grain used in this investigation was of a local red variety designated 'LRV'. The grain had a good germinative energy and was not water sensitive (Table 1). The sorghum sample was germinated according to the method of Morall, *et al.*[5]. Samples of grain (1 kg) in nylon bags were surface sterilized by immersion for 5 min. in a 0.2 per cent (w/v) solution of wescodyne, a commercial disinfectant and detergent containing 1 - 6 per cent available iodine. The grains were then washed and drained three times in running tap water (3 min, 25 - 28°C). This sterilisation and washing procedure was then repeated. The grains were then steeped in running tap water (25 - 28°C) with a cycle of 3 hr wet, 1 hr dry (15 - 28°C) for 24 hr. The grain was then immersed in a 0.19 per cent (v/v) solution of commercial bleach containing 5 per cent sodium hypochlorite for 10 min, then washed in running tap water for 3 min and drained. The washing procedure was repeated twice to avoid contamination by moulds and bacteria. The excess surface moisture was removed by gently centrifuging the grain for 1 min at 300 **g** in a centrifuge. The imbibed grain was removed from the nylon bags and germinated in shallow stainless steel trays with fine mesh bottoms.

TABLE 1 GERMINATIVE ENERGY AND WATER SENSITIVITY OF SORGHUM GRAIN (LRV) (% GRAINS GERMINATION)*.

Germination period (hr)	Germinative energy 4.0 ml (%)	Germinative count (%)	Water sensitivity
24	94.0	95	0
48	95.0	97	0
72	0	98	0

* Mean of two experiments

Samples were germinated for 6 days at temperatures of 25°C and 30°C. The grains were covered with moistened filter papers to avoid excess evaporation. At 6 hr intervals, 5 ml water was sprayed on the germinating grains using a perforated water can while at the same time, the grains were turned. Each time-temperature parameter was repeated twice. Higher germination temperatures yielded over mature sprouts. At 24 hr intervals before spraying of water, some quantity of the sorghum sprout (50g) was taken from the malt bed and kept in a refrigerator to determine the effect of germination temperature on the cyanide content of sorghum sprouts, while a second sample (50g) was taken and dried in a forced draught oven at 55°C with some samples (10g) being removed at 4 hr intervals to determine the effect of drying times on the cyanide content of sorghum sprouts. Drying lasted for 24 hr.

Determination of cyanide content of ungerminated sorghum and sorghum sprouts: Dry ungerminated sorghum seed was prepared for distillation by grinding 20 g to 20 mesh in a Wiley mill and transferring the powder to the distillation flask with sufficient water to bring the volume to 200 ml. Dried and refrigerated sorghum sprouts (15 g) were each blended with 100 ml of water at high speed for 3 min in a waring blender, and the homogenate was transferred to a 1 litre distillation flask with sufficient water tc bring the volume to about 200 ml. The apparatus and procedure of Honig *et al*[6] were used with minor modifications for distilling hydrogen cyanide from the homogenate. A drop of Dow-corning antifoams B, 10 ml 10 per cent lead nitrate and 10 ml 10 per cent tartaric acid were added to the homogenate in the distillation flask, and stirred magnetically during steam distillation in nitrogen atmosphere. Two hundred ml of distillate was collected in a 250 ml Erlenmeyer flask which contained 30 ml of 0.25 N sodium hydroxide. Care was taken to immerse the end of the distillation tube below the surface of the sodium hydroxide solution in the collecting flask before beginning the distillation. After 200 ml distillate has been collected, the receiver was replaced with another and 20 ml 1 N sulphuric acid was added to the distillation flask through a separatory funnel fitted at the too of the flask, and a second portion of 175 ml of distillate was collected. The first 200 ml portion of distillate was filtered through 0.45 micron Millipore filter and diluted to 250 ml with distilled water. Two 100 ml aliquots were titrated with 0.02 N silver nitrate using official methods of analysis of Association of Official Analytical Chemists (AOAC) recommendation for cyanide in beans . The second 175 ml portion of the distillate was filtered as described above, diluted to 200 ml and titrated as above. The combined titration values were used to calculate the amount of hydrogen cyanide generated from sorghum sprouts or dry ungerminated seeds. Determinations were carried out on blanks containing only water and reagents and controls containing known amounts of added cyanide to ensure that there were no interfering substances and that the recovery of cyanide was complete.

Results and Discussion

It was found in this study that relatively small amount of hydrogen cyanide (HCN) was derived by pulverising, digesting, and steam distilling sorghum seed. The hydrogen cyanide (HCN) in the sorghum seed was 16 p.p.m. The effect of germination time and temperature on the cyanide content of sorghum sprouts is shown in Table 2. At a germination temperature of 25°C, the hydrogen cyanide content of the sorghum sprouts increased linearly as the number of days of germination increased. The highest amount of hydrogen cyanide (580 p.p.m.) was got on the 6th day of germination. Six days was chosen as the maximum time for germination to allow for full development of diastase enzymes and also to control malting loss[8]. At a germination temperature of 30°C, the hydrogen cyanide content of the sorghum sprouts was only up to 3rd day and the highest amount of hydrogen cyanide obtained was 671 p.p.m. There was a sharp decrease from the fourth day up to the sixth day of germination. Similar results are reported by Panasuik and Bills[4]. This observation might be explained by the fact that from the fourth day of germination the mature sorghum sprouts had developed leaves and the respiration of these sprouts through the leaves might have led to the decrease in the amount of hydrogen cyanide. This observation was not made when sorghum was germinated at 25°C. Germination temperatures of 25°C and 30°C were chosen because development of maximum diastatic power has been reported in this range of tempe-

TABLE 2. EFFECT OF GERMINATION TIME AND TEMPERATURE ON THE HYDROGEN CYANIDE CONTENT OF SORGHUM SPROUTS.

Germination period (days)*	HCN content (p.p.m) at germination temp* 25°C	30°C
1	110	150
2	160	584
3	390	671
4	529	450
5	540	390
6	580	350

* Mean of determinations on duplicate sorghum sprout samples.
** Time from steep out Hydrogen cyanide content of ungerminated sorghum was 16 ppm

rature[8]. It was also observed in this study that germinating at a higher temperature yielded higher hydrogen cyanide at a shorter germination time than at a lower temperature but as the germinating time increased the cyanide content decreased drastically.

The effect of length of drying on the cyanide content of sorghum sprouts germinated for different number of days at 25°C and dried at 55°C is shown in Table 3. For each germination time, the hydrogen cyanide content of the sorghum sprouts decreased with drying times. Minimum values were obtained after 24 hr drying. This trend was also observed when sorghum sprouts germinated for different number of days at 30°C were dried at a temperature of 55°C (Table 3). HCN value which was as low as 35 p.p.m. was got from malt germinated for six days and dried at 55°C for 24 hr. This might be explained by the fact that the endogenous autolytic enzymes β - glucosidase and hydroxynitrile lyase which are responsible for the breakdown of the cyanogenic glycoside became more active at 55°C, the hydrogen cyanide evaporated with the moisture during drying, thereby leading to the decrease in hydrogen cyanide content of the sorghum sprouts.

TABLE 3. EFFECT OF DRYING TIME ON THE HYDROGEN CYANIDE CONTENT OF SORGHUM SPROUTS GERMINATED.

Germination period (days)**	HCN (p.p.m.) at drying periods indicated (hr)*						
	0	4	8	12	16	20	24
				25°C			
1	110	100	92	88	85	70	60
2	160	150	145	130	120	116	100
3	390	385	370	360	345	320	242
4	529	510	431	420	385	370	350
5	540	525	490	451	425	386	370
6	580	560	540	510	480	445	420
				30°C			
1	150	147	140	132	125	120	110
2	584	540	520	470	360	240	200
3	671	526	416	396	350	320	280
4	450	325	250	230	170	120	80
5	390	280	260	170	120	90	75
6	350	270	165	80	60	50	35

* Mean of determinations of duplicate sorghum sprout samples.
** Time from steep out.

The hydrogen cyanide content of sorghum sprouts grown at 30°C for three days was 671 p.p.m. in terms of the original dry weight of ungerminated seeds. The average fatal dose of HCN for humans is 50 - 60 mg[10] and this amount was readily obtained when sorghum was germinated at 30°C for 3 days, since the yield was equivalent to 67.1 mg of HCN per 100 g seed. Even when sorghum was germinated at 25°C the hydrogen cyanide content was still high.

Sorghum sprouts germinated at 30°C for six days and dried at 55°C for 24 hr which contain acceptable levels of HCN can be used in lager beer brewing.

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References

1. Akazawa T, Mijanich P and Cohn E E, Studies on cyanogenic glycoside of *Sorghum Vulgare*, *Pl. Physiol*, 1960, 35, 535.
2. Gorz H J, Haag W L, Specht J E and Haskin F A, Assay of P-hydroxybenzaldehyde as a measure of hydro cyanic acid potential in sorghum, *Crop Sci*, 1972, **17**, 578.
3. Conn E E, *Cyanogenetic glycosides* In: *International Review of Biochemistry, Biochemistry of Nutrition* by A. Neuberger and T H Jukes (Eds), Univ. Park Press, Baltimore, M.D., 1979, 1A, 27, p.21.
4. Panasuik O and Bills D D, Cyanide content of sorghum sprouts, *J Fd Sci*, 1984, **45**, 79.
5. Morall P, Boyd H K, Taylor J R N and Van Der Walt W H, Effect of germination time, temperature and moisture on malting of sorghum, *J Inst Brew*, 1986, **92**, 439.
6. Honig D H, Hockridge M E, Gould R M and Rachis J J, Determination of cyanide in soy beans and soybean products, *J Agric Fd Chem*, 1983, **31**, 272.
7. *Official Methods of Analysis*. Association of Official Analytical Chemists, Washington, D C. 1980, 13th Ed, Sect. 26, 134 and 26, 136.
8. Okafor N and Aniche G N, Brewing a larger beer from Nigerian/Sorghum, *Brew Distil Int*, 1980, **10**, 32.
9. Wang Y Y D and Fields M L, Germination of corn and sorghum in the home to improve nutritive value, *J Fd Sci*, 1978, **43**, 1113.
10. Windholz M, *The Merck Index*, Merck and Co. Inc. N.J., 1976, 9th Edn. 633.

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Production of Wax Gourd Candy by Using High Fructose Syrup

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This study was carried out to determine the effect of substitution of sucrose with high fructose syrup (HFS) on the chemical composition and sensory qualities of wax gourd *(Benincasa hispida)* candy. Candy was prepared by using sucrose and HFS in the proportions of 100:0, 75:25, 50:50, 25:75 and 0:100 in syrups at 30, 45, 60 and 75 °Brix. Chemical analyses of the samples revealed that total soluble solids, reducing sugars and non-reducing sugars increased, while pH and moisture decreased with increase in the concentration of the syrup (°Brix). On the other hand, reducing sugars increased with increase in HFS at all the Brix levels. Protein and ascorbic acid were unaffected by the level of HFS in the syrup. Candy prepared with 25% replacement of sucrose with HFS at 75°Brix proved the best as far as overall sensory quality and storage life of the product were concerned. The least acceptable product was the one prepared with 100% household sugar.

The wax gourd (*Benincasa hispida,* Cogn.) also known as wintermelon, gourd melon or petha, serves as a vegetable in diets and as raw material in candy, beverages, and jam production. Petha candy is popularly prepared in Pakistan and is used as a sweet as well as an additive in certain bakery products. It is normally prepared by cutting the gourd into suitable sized pieces, treating with lime water and immersing in heavy sucrose syrup[1]. Candy thus obtained is usually dull in appearance and coated with sugar crystals and is not much liked by the consumer.

Corn sweeteners have been found useful in the production of confectionery[2-4], canned fruits, jams, marmalades[5,6], ice cream[7] and in products that do not require a crystalline structure[8]. Improvements in the physical properties such as viscosity, prevention of crystallization, decreased hygroscopicity and minimized exudation in foods prepared by the use of corn sweeteners have been reported[9-12]. Crocco[13] reviewed the advantages of replacing sucrose with high fructrose corn syrup in product formulations and noted that increased fructose level resulted in reduced crystallization, increased stability to further inversion and better compatibility with fruity flavour than sucrose. Consumption of corn sweeteners has, therefore, steadily increased during the past few years[14].

This study was undertaken to elucidate the use of high fructose syrup (HFS) in the manufacture of wax gourd candy and to determine the suitability and level of HFS in its preparation.

Materials and Methods

Wax gourd candy was prepared according to the slightly modified method of Girdhari Lal *et al.*[1]. Firm and mature fruits were thoroughly washed, cut into slices, peeled and the inner fluffy portion removed. The remaining edible portion was cut manually into cubes measuring approximately 1.5 cu cm. Clarified lime water (500 g unslaked lime in 7.5 l of water) was prepared and the cubes soaked (1 kg cubes per 2 l lime water) overnight in it to harden the texture. Four to five rigorous washings were given before blanching in boiling water for 5 to 8 min, followed by treatment with green colour for 30 min and draining. These were immersed for 24 hr in syrups of 30, 45, 60 and 75°Brix after which the strength of the syrup was adjusted to the original level and the process repeated. Each syrup contained the following proportions of cane sugar/HFS by weight:-

Treatment	Cane Sugar (99.9% sucrose)	HFS
T_1	100	0
T_2	75	25
T_3	50	50
T_4	25	75
T_5	0	100

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The cubes were drained for six hours, packed in polyethylene bags, sealed and stored at ambient temperature for further studies.

Moisture, pH, T.S.S. and proteins were determined by the methods described in A.O.A.C.[15]. Sugars were determined according to the method described by Ruck[16], while ascorbic acid was analysed by titration against 2,6-dichlorophenol indophenol according to Pearson[17].

Sensory evaluation was done by a panel of 6 judges comprising of the academic staff and students, using a 9-point Hedonic Scale as described by Larmond[18].

The data collected were statistically analysed by using analysis of variance technique and Duncan's Multiple Range Test. Five per cent probability level was used to test the significance among the treatment means[19].

Results and Discussion

The data for chemical analyses of fresh and blanched wax gourd fruit as well as candy prepared in syrup at 75°Brix are presented in Table 1. An increase in the pH of blanched fruit is due to the treatment with lime water. The fall in pH in candy samples T_1 through T_5 is due primarily to the increase in HFS content of the samples. The HFS used in this study had a pH of 4.3. A similar decrease in pH is observed with an increase in °Brix of the syrups (Table 2).

Fresh wax gourd fruit had a high moisture content (97.0 per cent) which further increased to 98.1 per cent after blanching due to absorption of water (Table1). In the prepared candy, an increase in moisture content is observed as the level of HFS is raised in the samples (Table 1). This is primarily due to the hygroscopic nature of and the presence of additional moisture in the HFS[12,20]. The fall in moisture content with an increase in °Brix of various sugar syrups (Table 2) is natural. This is due primarily to the absorption of sugar by the fruit to replace the moisture.

A decrease in T.S.S. from 4.2 to 2.2 per cent in blanched wax gourd fruit is due to the blanching treatment with boiling water resulting in the absorption of moisture by the fruit pieces and partial leaching of soluble solids. An increase in T.S.S. in the candied samples is due to the imbibation of sugar by the fruit through osmosis. On the other hand, a gradual decrease in T.S.S. is observed as the level of HFS is raised in the samples (Table1). This difference in T.S.S. in samples having more HFS is due to the hygroscopic nature of HFS and the presence of additional moisture in it[12].

TABLE 1. AVERAGE COMPOSITION OF FRESH AND BLANCHED WAX GOURD FRUIT AND CANDY PREPARED USING DIFFERENT LEVELS OF SUCROSE AND HIGH FRUCTOSE SYRUP IN SYRUP AT 75° BRIX

Wax gourd treatment	pH	Moisture (%)	T.S.S. (%)	Sugars		Protein (%)	Ascorbic acid (mg/100g)
				Reducing (%)	Non-reducing (%)		
Fresh	5.3	97.0	4.2	3.4	0.2	0.65	22.0
Blanched	8.9	98.1	2.2	0.02	0.4	0.59	13.0
Candied							
T_1	7.9	22.5	74.0	3.6	69.5	0.56	11.9
T_2	7.8	22.7	73.7	25.9	46.9	0.57	11.9
T_3	7.9	23.2	73.3	36.9	36.4	0.53	11.9
T_4	7.4	23.7	72.7	51.0	20.8	0.56	11.9
T_5	7.0	24.3	72.0	65.9	6.1	0.55	11.9

T_1-T_5 descriptions as in text.

TABLE 2. EFFECT OF °BRIX OF THE SYRUP ON THE AVERAGE COMPOSITION OF WAX GOURD CANDY

°Brix	pH	Moisture (%)	T.S.S. (%)	Sugars		Protein (%)	Ascorbic acid (mg/100g)
				Reducing (%)	Non-reducing (%)		
30	8.4	72.8	23.8	13.6	9.3	0.38	9.7
45	8.1	57.9	38.7	21.3	16.6	0.42	10.5
60	7.8	42.5	54.2	29.0	24.4	0.47	11.2
75	7.5	23.3	73.1	36.4	35.9	0.56	11.9

A substantial increase in reducing sugars and a decrease in non-reducing sugars was observed within the treatments. The results revealed that reducing sugars in candied fruits prepared with 100 per cent cane sugar (T_1) were low as compared to samples prepared with increasing amounts of HFS (T_2 through T_5). The reverse is the case with non-reducing sugars (Table 1). This increase/decrease in reducing and non-reducing sugars is due to the increasing/decreasing amount of HFS in the prepared samples. A similar increase in reducing as well as non-reducing sugars in samples prepared by using syrups at various °Brix (Table 2) seems to be the result of increase in sugar concentration of the syrup.

The average protein content of edible portion of fresh wax gourd fruit was 0.65 per cent which decreased to 0.59 per cent after blanching (Table 1). A further decrease in protein content was observed in the candied samples which remained almost unaffected by the changes in sucrose:HFS ratio, However it was slightly affected by the concentration of syrup used for candying.

Treatment of fresh fruits with lime reduced the ascorbic acid content by 32 per cent which further fell after blanching to 41 per cent. In the prepared candy samples, ascorbic acid content remained almost constant irrespective of the treatment (Table 1). The fall in ascorbic acid content as a result of lime treatment or blanching is a normal phenomenon. It is well documented that this vitamin is most sensitive of the vitamins and is lost during blanching from as low as 10 per cent to almost complete destruction[21].

Sensory evaluation: Preliminary studies had revealed that candy samples prepared with syrups of upto 60 °Brix became mouldy almost within one week of the preparation. Hence samples prepared in syrups at 75 °Brix, packed in polyethylene bags and stored at ambient temperature inside a laboratory shelf were evaluated for colour, flavour, taste, texture (mouth feel) and overall acceptability. This evaluation was carried out soon after preparation and at 7 days of interval for upto 4 weeks. The effect of substitution of sucrose by HFS and storage on sensory evaluation of the candy are presented in Tables 3 and 4.

The results obtained revealed that wax gourd candy prepared with 100 per cent house hold sugar (T_1) was unacceptable after storage for 28 days. The colour, taste, texture and flavour were adversely affected by crystallization of the sugar in the product. A slight change in appearance of the candy prepared with 100 per cent HFS (T_5) was noticed after storage for 4 weeks but the product remained acceptable even though it appeared sticky due to the absorption of HFS on the surface. There was a highly significant effect of treatments on colour. taste, flavour, texture and overall acceptability of the product. The candy prepared by incorporating 75 per cent house hold sugar and 25 per cent HFS (T_2) ranked superior to all the other treatments owing to better retention of taste, flavour and texture throughout the storage period. As regards the effect of storage on sensory attributes, the results

TABLE 3. EFFECT OF SUBSTITUTION OF HOUSEHOLD SUGAR BY HIGH FRUCTOSE SYRUP ON SENSORY CHARACTERISTICS OF CANDY PREPARED IN SYRUP AT 75 °BRIX

Characteristics	Treatment				
	T_1	T_2	T_3	T_4	T_5
Colour	4.6 (b)	7.3 (a)	7.3 (a)	7.4 (a)	7.3 (a)
Taste	4.8 (c)	7.7 (a)	6.8 (b)	6.8 (b)	6.5 (b)
Flavour	4.9 (c)	7.5 (a)	6.6 (b)	6.7 (b)	6.3 (b)
Texture	4.5 (d)	7.5 (a)	6.9 (bc)	7.1 (b)	6.6 (c)
Overall acceptability	4.3 (c)	7.5 (a)	6.9 (b)	6.8 (b)	6.5 (b)

Letters within parentheses denote ranking according to DMR test. Any two means not having the same letter in parentheses differ significantly at 5% level.

TABLE 4. EFFECT OF STORAGE ON THE SENSORY CHARACTERISTICS OF CANDY PREPARED IN SYRUP AT 75 °BRIX

Storage period (days)	Sensory characteristics				
	Colour	Taste	Flavour	Texture	Overall acceptability
0	7.2 (a)	6.9 (a)	6.7 (a)	6.8 (a)	6.9 (a)
7	7.0 (a)	6.8 (a)	6.7 (a)	6.9 (a)	6.6 (ab)
14	6.9 (a)	6.6 (ab)	6.4 (ab)	6.5 (b)	6.4 (bc)
21	6.5 (a)	6.2 (b)	6.2 (ab)	6.2 (bc)	6.2 (bc)
28	6.4 (a)	6.1 (b)	6.0 (b)	6.1 (c)	6.0 (c)

Letters within parentheses denote ranking according to DMR test. Any two means not having the same letter in parentheses differ significantly at 5% level.

showed a gradual decline in the score (Table 4). There was a highly significant effect of storage on colour, taste, flavour, texture and overall acceptability of the product.

References

1. Girdhari Lal, Siddappa G S and Tandon G L, *Preservation of Fruits and Vegetables*, Indian Council of Agricultural Research, New Delhi, 1960.
2. Richmond W, *Choice Confections, Manufacturing Methods, Formulas*, Manufacturing Confectioner Pub. Co., Oak Park, Ill.
3. Colquohoun J M, New machines and methods in jam industry *Fd Manuf*, 1961. **36**, 195.
4. Coleman P E and Harbers C A, High fructose syrup: Replacement for sucrose in angel cake, *J Fd Sci*, 1983, **48**, 452.
5. Pangborn R M, Leonard S J, Simone M and Luh B S, Effect of sucrose, citric acid and corn syrup on consumer acceptance, *Fd Technol*, 1959. **13**, 444.
6. Jersch C., Some applications of fructose, *Conf Manuf Market*, 1984. **21**, 11.
7. Horner C, Justifying HFCS in ice cream mix *Dairy Rec* 1981. 89. 93.
8. Thor P K and Carman H F, High fructose corn syrup; An important new sugar substitute, *Calif, Agric*, 1979. **33**, 13.
9. Tressler D K, The use of high conversion corn syrup in making jellies, jam and fruit butter, *Fruit Prod, Vinegar Ind*, 1940, **19**, 228.
10. Meeker E W, Confectionery sweeteners, *Fd Technol*, 1950. **11**, 361.
11. Leighton A E, Influence of modern corn syrups on candy processing, *Confect Prod*, 1964. **30**, 185.
12. Beck B and Franta R, Sweeteners: 3. Alternatives to cane and beet sugar. *Fd Technol*. 1986. **40**, 116.
13. Crocco S, A closer look at the new 'higher' fructose syrup *Fd Engg Int*, 1977. **2** 41.
14. Fronta R, Sweeteners: 1. Consumption trends, *Fd Technol*, 1986. **40**, 112.
15. *Official Methods of Analysis*. Association of Analytical Chemists, Washington, DC. 1984.
16. Ruck J A, *Chemical Methods for Analysis of Fruit and Vegetable Products*, Res. Sta. Summerland, Res. Branch Canada, Dept. of Agric. Publ. No. 1154. 1969.
17. Pearson D, *The Chemical Analysis of Foods*. Churchill Livingstone, Edinburgh, 1976.
18. Larmond E, *Methods of Sensory Evaluation of Foods*, Canada Dept. Agric. Publ. No. 1637, 1977.
19. Steel R G D and Torrie J H, *Principles and Procedures of Statistics. A Biometrical Approach*. 1980. McGraw Hill Book Co. Inc., New York, 1980.
20. Wardrip E K, High fructose corn syrup, *Fd Technol*, 1971. **25**, 47.
21. Harris R S and Loesecho H V, (Eds.) *Nutritional Evaluation of Food Processing*, John Wiley and Sons Inc., New York, 1960.

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Instron Texture Profile Parameters of *Khoa* as Influenced by Composition

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An increase in total solids (TS) was accompanied by a considerable increase in Instron hardness, gumminess and chewiness, but decrease in cohesiveness of *Khoa*. Water dispersible protein (WDP) had the opposite effect although its effect on cohesiveness was non-significant. Milk acidity and free fat content of *Khoa* did not show any significant correlations with texture profile (TP) parameters. However, their inclusion in regression analysis made a small but perceivable improvement in predictability of cohesiveness and chewiness. Significant interrelationships among TP parameters, particularly between hardness and cohesiveness, gumminess and chewiness, and also between cohesiveness and all other parameters indicated that one or two most important TP parameters could probably serve as an index of the texture profile of *Khoa*. Adhesiveness and springiness of *Khoa* generally showed poor correlations with compositional characteristics as also with other TP parameters..

Instrumental devices such as the General Foods Texturometer, Ottawa Texture Measuring System, Universal Food Rheometer and Instron Universal Testing Machine have been found useful in texture assessment of various foods for research and quality control purposes[1]. Recent developments in texture testing equipments particularly with respect to test cells that contain the sample, transducers (devices to convert forces and deformation into electric signals for recording) and microprocessor controls have greatly enhanced the applicability of these instruments in food texture measurement[2]. Thus, in spite of certain limitations such as high cost, the instrumental approach remains most promising for the reasons of reproducibility, sensitivity and versatility. Bourne[3] applied the Instron for texture profile analysis of pears. Subsequently, many workers have found this technique useful for various food products[4-9]. The present paper discusses Instron texture profile parameters of *Khoa* as affected by certain compositional characteristics viz., solids content, free fat, water dispersible protein and milk acidity.

Materials and Methods

Preparation of Khoa: Pooled buffalo milk obtained from the Experimental Dairy of the Institute and standardized to a fat-SNF ratio of 0.6 (approx. 15 per cent total solids) was used for *Khoa*-making employing a stainless steel steam kettle and an iron stirrer (Khunti). The titratable acidity of milk was varied between 0.15 and 0.17 per cent LA by allowing it to stand for 0.5 - 2.5 hr at ambient temperature for spontaneous acid development to different degrees. The extent of dehydration was so regulated that the resulting product contained (in per cent) 56.2 - 71.9 total solids, 20.8 - 28.0 fat, 3.2 - 13.5 free fat, 14.9 - 18.9 total protein and 8.5 - 28.7 (of total protein) water dispersible protein (WDP).

Texture profile analysis: The Instron Universal Testing Machine having a sample holder, deforming mechanism, transducer and a recorder, all put together in a sophisticated form records the stresses developed within the sample during its deformation through compression or tension (elongation). It thus provides a very versatile means of studying food texture. Cylindrical samples of *Khoa* measuring 1.9 mm in dia and 20 mm in height were subjected to uniaxial compression to 80 per cent of the initial sample height,[10,11] using Instron Model 4301, fitted with a 100 N load-cell. The force – distance curve obtained for a two – bite deformation cycle employing a cross-head speed of 50 mm/min and chart speed of 100 mm/min was used to determine various texture profile parameters of *Khoa* (at 30°C), as described by

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Bourne[12]. Each measurement was made in duplicate. Data for 22 different lots of *Khoa* were subjected to correlation and regression analyses[13] in order to develop interrelationship as under,

$$I = k_0 + k_1 C_1 + k_2 C_2 \ldots\ldots + k_n C_n \text{ and}$$

$$I = k_0 . C_1 k_1 . C_2 k_2 \ldots\ldots C_n k_n$$

where,

I = Instrumental parameter.
C_1, C_2, C_n = Chemical parameters.
k_0, k_1, k_2, k_n = Constants.

Results and Discussion

The range of numerical values for each of the TP parameters is presented in Table 1. The coefficients of correlations between various TP parameters of *Khoa* and compositional characteristics presented in Table 2 indicate that Instron hardness of *Khoa* was highly correlated ($P<0.001$) with TS, increasing TS resulting in increasing hardness. Water dispersible protein (WDP) showed a slightly lower but equally significant negative correlation with hardness. The log-linear relationship exhibited greater correlations as compared to linear relationship for all chemical parameters affecting hardness. Thus, TS alone accounted for 78 per cent variation in hardness whereas WDP showed a much lower effect (Equations 1,2 Table 3). Milk acidity added to TS in the regression analysis did not better the prediction value of the equation ($R^2 = 0.785$, log-model). The combined effect of TS and WDP was nearly same as that of TS, WDP, milk acidity and free fat (Equations 3,4), these regressions providing slightly better prediction of hardness than that based on TS alone. Obviously, the solids in *Khoa* contributed to the strength of the solid network of the coagulated mass in the semi-solid product. Higher WDP, on the other hand, meant that a smaller portion of the total protein was transferred to the solid net-work which resulted in correspondingly lower strength of the latter and hence in less hard *Khoa*.

Cohesiveness of *Khoa* tended to decline with increasing TS and so with increasing fat and protein contents (Table 2). The positive correlation of

TABLE 1. TEXTURE PROFILE PARAMETERS OF KHOA*

	Range
Hardness, N	0.244 – 9.081
Cohesiveness	0.350 – 0.738
Adhesive Force, N	0.043 – 0.327
Springiness, mm	4.5 – 8.3
Gumminess, N	0.176 – 4.961
Chewiness, N. mm	0.880 – 32.246

*Prepared in 22 different lots.

TABLE 2. COEEFICIENTS OF CORRELATIONS# BETWEEN COMPOSITION AND INSTRON PARAMETERS OF KHOA

Compositional variable	Instron Parameter					
	Hardness	Cohesiveness	Adhesiveness	Springiness	Gumminess	Chewiness
TS	0.816*** (0.886***)	−0.710*** (−0.699***)	0.299 (−0.241)	−0.193 (−0.176)	0.758*** (0.831***)	0.669*** (0.748***)
Milk acidity	0.085 (0.112)	0.191 (0.193)	0.019 (−0.062)	0.320 (0.301)	0.160 (0.169)	0.217 (0.087)
Fat	0.837*** (0.871***)	−0.657*** (−0.647**)	0.181 (0.116)	−0.125 (−0.108)	0.802*** (0.826***)	0.728*** (0.760***)
Free fat	0.007 (−0.020)	−0.131 (−0.069)	0.018 (−0.034)	0.033 (0.063)	−0.035 (−0.038)	−0.057 (−0.064)
Total protein	0.815*** (0.892***)	−0.653*** (−0.640**)	0.286 (0.216)	−0.135 (−0.120)	0.766*** (0.851***)	0.688*** (0.728***)
Water dispersible protein	−0.584** (−0.682***)	0.274 (0.194)	0.100 (0.235)	−0.344 (−0.329)	−0.621** (−0.718***)	−0.618** (−0.660***)

*$P<0.05$ **$P<0.01$ ***$P<0.001$
#Figures in parentheses are coefficients of correlations for log-linear relationships

cohesiveness with milk acidity and WDP were much smaller and non-significant. Regression analysis indicated that TS contributed about 50 per cent cohesiveness, and TS together with WDP accounted for 58 per cent cohesiveness (Equations 5, 6; Table 3). While TS in combination with milk acidity could predict cohesiveness to the same extent (Equation 7) as TS and WDP, all four variables including free fat could account for a somewhat greater (63 per cent) variation in this parameter (Equation 8). It thus appeared that increasing TS and, to some extent, free fat caused a decline in the interparticle binding in *Khoa* structure whereas WDP and milk acidity had the opposite effect.

TABLE 3. REGRESSION EQUATIONS FOR INSTRUMENTAL TEXTURE PROFILE PARAMETERS AS RELATED TO CHEMICAL COMPOSITION OF KHOA

Sr. No.	Equation[#]	Coeff. of correlation ($R^{\#\#}$)	Coeff. of determination (R^2)
1.	$H = 4.060.10^{-20}.TS^{10.933}$	0.886**	0.784
2.	$H = 6.983.10^{2}.WDP^{-2.072}$	0.682**	0.465
3.	$H = 3.003.10^{-16}.TS^{9.241}\ WDP^{-0.681}$	0.903***	0.816
4.	$H = 4.974.10^{-16}.TS^{9.176}.MA^{-0.197}.FF^{-0.218}$ $WDP^{-0.731}$	0.907***	0.823
5.	$C = 1.509 - 0.015\ TS$	0.710**	0.504
6.	$C = 1.666.10^{4}.TS^{-2.330}.WDP^{-0.231}$	0.758***	0.575
7.	$C = 1.002 - 0.015\ TS + 3.362\ MA$	0.758***	0.574
8.	$C = 8.454.10^{4}.TS^{-2.343}.MA^{0.864}.FF^{-0.017}$ $WDP^{-0.215}$	0.794**	0.630
9.	$Ad = 8.490.10^{-11}.TS^{4.351}.WDP^{1.064}$	0.541*	0.293
10.	$G = 3.304.10^{-17}.TS^{9.176}$	0.831***	0.690
11.	$G = 2.727.10^{2}.WDP^{-1.952}$	0.718***	0.515
12.	$G = 4.996.10^{-12}.TS^{6.912}.WDP^{0.911}$	0.872***	0.760
13.	$G = 1.623 + 0.211\ TS - 83.317\ MA$	0.924***	0.854
14.	$G = 18.946 - 89.216MA - 0.17WDP$	0.837***	0.701
15.	$G = 4.197.10^{-11}.TS^{6.833}.MA^{0.667}.FF^{-0.235}$ $WDP^{-0.946}$	0.880***	0.774
16.	$Ch = 6.877.10^{-17}.TS^{9.418}$	0.748***	0.560
17.	$Ch = 2.002.10^{3}.WDP^{-2.043}$	0.660***	0.435
18.	$Ch = 3.307.10^{-11}.TS^{6.934}.WDP^{-1.000}$	0.791***	0.625
19.	$Ch = 3.210.10^{-11}.TS^{6.828}.MA^{-0.825}.FF^{-0.369}$ $WDP^{-1.096}$	0.803***	0.645

\# H = Hardness, C = Cohesiveness, Ad = Adhesiveness, Spr = Springiness, G = Gumminess, Ch = Chewiness, TS = Total solid, MA = Milk acidity, FF = Free fat, WDP = Water dispersible protein

\#\# * $P<0.05$ ** $P<0.01$ *** $P<0.001$

TABLE 4. COEFFICIENTS OF CORRELATIONS# AMONG DIFFERENT INSTRON PARAMETERS OF KHOA

	Hardness	Cohesiveness	Adhesiveness	Springiness	Gumminess	Chewiness
Hardness	1.000 (1.000)					
Cohesiveness	−0.537•• (−0.590••)	1.000 (1.000)				
Adhesiveness	−0.006 (−0.116)	−0.448• (−0.424•)	1.000 (1.000)			
Springiness	0.011 (0.123)	0.384 (0.448•)	−0.500• (−0.460•	1.000 (1.000)		
Gumminess	0.971••• (0.983•••)	−0.360 (−0.432•)	0.110 (0.033)	0.146 (0.240)	1.000 (1.000)	
Chewiness	0.914••• (0.861•••)	−0.245 (−0.424•)	−0.232 (0.009)	0.344 (0.249)	0.974••• (0.866•••)	1.000 (1.000)

• $P<0.05$ •• $P<0.01$ ••• $P<0.001$

Figures in parentheses are coefficients of correlations for log - linear relationships.

Adhesiveness of *Khoa* did not bear any significant correlation with any individual compositional characteristic (Table 2). However, the combined effect of TS and WDP was appreciable ($P<0.05$) but still much lower (Equation 9) than in the case of hardness and cohesiveness. Springiness also did not seem to be influenced by the compositional variables studied, although its correlations with acidity and WDP, positive and negative respectively, appeared to be appreciable. Gumminess and chewiness of *Khoa* were greatly dependent upon TS and WDP as observed also for hardness, the correlation coefficients being higher for the log model. Relevant regression equation (Nos. 10-12- Table 3) showed that TS alone could account for 69 per cent gumminess whereas WDP for 52 per cent, the combined effect of the two being perceivably higher (76 per cent). However, TS and milk acidity appeared to determine most of the gumminess (85 per cent) which was apparently higher (Equation 13) than the cumulative effect of TS and WDP (Equation 12), or milk acidity and WDP (Equation 14), or even all chemical variables taken together (Equation 15). Further, chewiness showed more or less similar dependence on TS and WDP as seen in the case of gumminess, though their individual as well as combined effects were somewhat lower (Equations 16-19). Combination of all four variables was perceivably better than that of TS and WDP in predicting chewiness of *Khoa*.

The significant impact of compositional variables on the TP parameters of *Khoa* as noted above provides an indication of the profound role of these factors in determining the product's texture. While no literature reports are available indicating such relationships in *Khoa*, Keller *et al.*[14] observed significant correlations with the moisture content and certain rheological properties of mozzarella cheese. Desai[9] noted high correlations between moisture content and various Instron texture profile parameters for *Chhana*.

Negative correlation observed between hardness and cohesiveness of *Khoa* (Table 4) indicated that as hardness increased, there was a significant decrease in cohesiveness ($P<0.01$). Further, gumminess and chewiness bore the opposite relationship with hardness ($P<0.001$). Cohesiveness exhibited significant positive correlation (in log-model) with springiness and negative correlations with other parameters. Another significant ($P<0.05$) correlation was between adhesiveness and springiness, a more adhesive *Khoa* being less springy. Derived from gumminess and springiness, chewiness showed a direct correlation with the former but not with the latter, pointing thereby to the larger contribution of gumminess to it. Gupta *et al.*[6] found significant correlations between Instron hardness and other parameters except cohesiveness of different types of processed cheese foods. These observed interrelationships between instrumental parameters indicate the possibility of using a single, relatively simple parameter as an index of the texture profile of the product so as to facilitate the quality control programmes.

References

1. Voisey P W, Recent advances in texture test instrumentation and their application. *In Food Texture and Rheology* by Sherman P, (Ed), Academic Press, London, 1979, 65.
2. Timbers G E and Voisey P W, The Ottawa Pea Tenderometer

and the Ottawa Texture Measuring System: The Evolution of a system for Quality Control and Food Texture Research. *In Food Texture: Instrumental and Sensory Measurement* by Moskowitz H R, (Ed) Marcel Dekker, Inc., New York, 1987, 65.

3. Bourne M C, Texture profile of ripening pears, *J Fd Sci*, 1968, **33,** 223.
4. Breene W M, Application of texture profile analysis to instrumental food texture evaluation, *J Texture Studies*, 1975, 6, 53.
5. Sherman P, The textural characteristics of dairy products. *In Rheology and Texture in Food Quality* by de Man J M, Voisey P W, Rasper V F and Stanley D W, (Eds), The AVI Publishing Co., Westport, USA, 1976, 392.
6. Gupta S K Karahadian C and Lindsay R C, Effect of emulsifier salts on textural and flavour properties of processed cheeses, *J Dairy Sci*, 1984, **67,** 764.
7. Green M L, Marshall R J and Brooker B E, Instrumental and sensory texture assessment and fracture mechanisms of cheddar and cheshire cheeses, *J Texture Studies*, 1985, **16,** 351.
8. Peleg M The basics of solid foods rheology, *In Food Texture : Instrumental and Sensory Measurement* by Moskowitz H R, (Ed), Marcel Dekker, Inc., New York, 1987, 3.
9. Desai H K *Rheological Properties of Heat-and-Acid Coagulated Indian Milk Products*, 1988, Ph.D. thesis, Kurukshetra University.
10. Mohsenin N N and Mittal J P, Use of rheological terms and correlation of compatible measurements in food texture research, *J Texture Studies*, 1977, **8,** 365.
11. Shinin J M, *Measurement of Rheological Properties of Cheese by Instrumental Methods*, 1987, M.Sc. Thesis, University of Guelph, Canada.
12. Bourne M C, Texture profile analysis, *Fd Technol*, 1978, **32,** 62.
13. Croxton F E, Crowden D J and Klein S, *Applied General Statistics*, 3rd ed. Prentice-Hall of India, New Delhi, 1975, 389, 419, 453.
14. Keller B, Olson N F and Richardson T, Mineral retention and rheological properties of Mozzarella cheese made by direct acidification, *J Dairy Sci*, 1974, **57,** 174.

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Inhibition of Growth of Pathogenic Micro-organisms During Production and Storage of Cultured Milk

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Lactobacillus acidophilus **strains LBKV$_3$ and LBKI$_4$ were inoculated in sterilised skim milk along with enterotoxigenic *Staphylococcus aureus* in one set while the other set was inoculated with mastitic *Escherichia coli*. Many lactic cultures have been reported to be antagonistic to pathogenic bacteria, so a comparison was made of the acidophilus cultures with mixed strain lactic cultures, LF-40 and yoghurt cultures (*Lactobacillus bulgaricus*-LBW and *Streptococcus thermophilus*-CH$_1$). Staphylococcal and coliform counts were estimated along with titratable acidities, at selected time intervals upto 72 hr during production and storage at 37°C and subsequent storage at 15°C for 60 hr. It was observed that both the *L. acidophilus* strains LBKV$_3$ and LBKI$_4$ were able to restrict growth of *Staph. aureus* only after 16 and 20 hr, respectively, while they reduced the growth of *E.coli* after 48 and 72 hr at 37°C. However, when the samples were stored at 15°C there was no significant decrease in coliform counts throughout the storage periods, while it showed continuous lowering down of *Staph. aureus* count when it was associated with LBKV$_3$ and yoghurt starters. Inhibition of both the pathogenic organisms was more at 37°C than at 15°C. Rates of acid production of all four cultures were different at both the temperatures studied.**

The possibility of contamination of milk with *Staphylococcus aureus* and *Escherichia coli* and their subsequent growth and enterotoxin production in fermented products like yoghurt and acidophilus milk is mentioned[1]. However, the presence of inhibitory substances in fermented milks reduced their danger[1-4]. Recently, the inhibitory effect of yoghurt and acidophilus-yoghurt cultures on a pathogenic strain of *Staph. aureus* introduced under simulated production conditions is attributed to lactic acid, hydrogen peroxide and possible bacteriocin production[1]. Price and Lee[5] have reported that the inhibition of *Bacillus*, *Pseudomonas* and *Proteus* spp. is due to the presence of H_2O_2 in lactobacilli. Shahani *et al.*[3] have obtained a lactic acid free preparation called 'acidophilin' and concluded that the inhibition is due to the combined effect of lactic acid and certain metabolites. Commercial preparations of yoghurts, acidophilus milks and other fermented milk products showed no pronounced antimicrobial activity and the activity is attributed to lactic acid[6]. Inhibition of *Salmonella typhimurium* in yoghurt has been attributed to intracellular dissociated moiety of lactic acid and also factors other than lactic acid[8], which have been indicated as low pH and O-R potential. Casein fraction in whole yoghurt is indicated to provide partial protection to this organism[9]. From the studies on factors affecting the viability of pathogens in fermented milks the factors like lower fatty acids and diacetyl have been indicated to contribute to the inhibition[10].

The present study was planned to investigate the influence of two human strains of *L. acidophilus*, a mixed lactic culture LF-40 and a yoghurt culture on enterotoxigenic strain of *Staph. aureus* and a pathogenic strain of *E. coli* when they are grown with association in milk.

Materials and Methods

Source of cultures: *L.acidophilus* strain LBKV$_3$ is a vaginal isolate from a adult woman and strain LBKI$_4$ is an isolate obtained from human faeces. *Staphylococcus aureus* and *Escherichia coli* used in the study were obtained from Department of Veterinary Bacteriology of Gujarat Agricultural University, Anand Campus, Anand.

Yoghurt cultures - *Streptococcus thermophilus* C-1 and *Lactobacillus bulgaricus* LBW were taken from the culture collection of the department.

LF-40 - The mixed lactic Streptococci culture is a culture combination procured from Hansen's Laboratory, Denmark.

All lactic cultures were activated through two consecutive transfers in sterilized skim milk at 37°C for 24 hrs. The lactic cultures were then inoculated into sterilized skim milk flasks containing 300 ml milk at the rate of 2 per cent (v/v). Subsequently, one set of flasks was inoculated with nutrient broth cultures of pathogenic strain *Staph. aureus* (24 hr old culture) in 0.1 ml quantity to give a count of approximately 10^3 c.f.u/ml of milk. Initial staphylococcal and coliform counts were recorded on Staphylococcus (S_{110}) and MacConkey's agars, after incubating the plates for 36 and 24 hr respectively at 37°C.

The contents of the flasks were thoroughly mixed by Cyclomixer and aseptically distributed into sterilized test tubes in equal quantities of 15 ml per tube. After 12 hr of incubation at 37°C, half the number of the tubes from both the sets were stored at 15°C and the rest were stored at 37°C.

At selected time intervals during preparation and during storage at 37°C and 15°C of upto 60 hr, 1 ml quantity was taken from each of the four cultures and appropriately diluted using phosphate buffer (IS:1479, part III)'. Suitable dilutions were poured into petri dishes and then melted Staphylococcal agar (S-110, Difco)[12] was added to plates in which dilutions of cultures containing *Staph. aureus* were placed. Those plates after solidifications were incubated at 37°C for 36 hr and the number of typical staphylococcal colonies were counted. In other set of plates in which dilutions of cultures containing *E.coli* were placed melted MacConkey's agar was poured and allowed to set. After solidification, they were overlayered with 4-5 ml uninoculated MacConkey's agar and after setting plates were incubated at 37°C and typical coliform colonies counted after 24 hr. Titratable acidity of the inoculated milk samples as per cent lactic acid was determined at the various periods of production and storage according to method specified in part I of IS:1479[13] using 10.0 g sample.

Results and Discussion

It could be seen from Fig.1 that there was steady increase in staphylococcal counts upto 4 and 8 hr respectively in the presence of *L.acidophilus* $LBKV_3$ and $LBKI_4$ at 37°C and thereafter there was gradual decline in the counts upto 48 hr and 60 hr of storage, respectively. In skim milk inoculated with LF-40 and yoghurt cultures, increase in staphylococcal counts were observed, upto 12 and 4 hr respectively. Thereafter the counts showed decline and the decline was faster in yoghurt cultures than in LF-40.

Maximum inhibitory activity was observed after 36 to 48 hr of storage in both the acidophilus milks. These

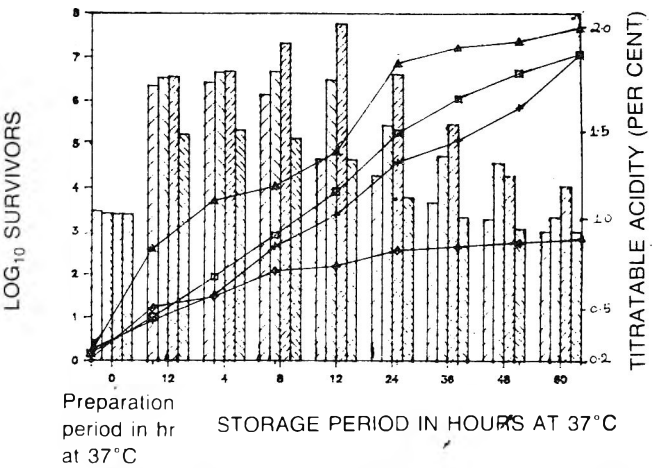

Fig.1. Inhibition of *Staph. aureus* during the production of cultured milks at 37°C and subsequent storage at 37°C. Log_{10} survivors of *Staph. aureus* in cultured milks consisting strain of $LBKV_3$ (⧄), $LBKI_4$ (⧅), LF-40 (⧄), CH_1 + LBW (⧅) and titratable acidity in $LBKV_3$ (□), $LBKI_4$ (+), LF-40 (◇), CH_1 + LBW (△).

results match well with our cup-well assay observation done with these cultures. There was a steady increase in acidity in all the cultures, however, their inhibitory effect varied suggesting that the inhibition was not completely due to increase in acidity in case of acidophilus milks. Konecny[15] made similar observations. For LF-40 and yoghurt cultures there was better inverse relationship between acidity and staphylococcal counts.

It could be observed from Fig.2 that the milk inoculated $LBKV_3$, $LBKI_4$ yoghurt culture and LF-40 had shown reduction in staphylococcal counts after 8, 24, 8 and 36 hr, respectively, on storage at 15°C after an initial incubation for 12 hr at 37°C. In this case, the rate of inhibition and acid production in all the cultures

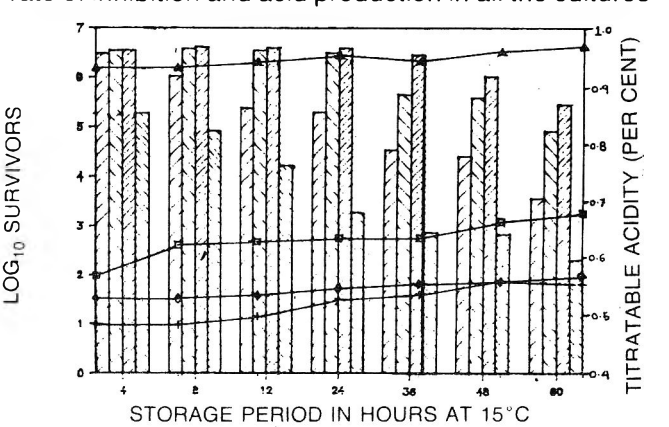

Fig.2. Inhibition of *Staph. aureus* during the production of cultured milks at 37°C and subsequent storage at 15°C. Log_{10} survivors of *Staph. aureus* in cultured milks consisting strain of $LBKV_3$ (⧄), $LBKI_4$ (⧅), LF-40 (⧄), CH_1 + LBW (⧅) and titratable acidity in $LBKV_3$ (□), $LBKI_4$ (+), LF-40 (◇), CH_1 + LBW (△).

were significantly different. At both the temperatures viz., 37°C and 15°C, $LBKV_3$ had higher antibacterial activity against *Staph. aureus* than $LBKI_4$ and yoghurt culture was superior to all other lactic cultures.

It could be seen from Fig.3 that there was a steady increase in coliform counts upto 48, 36, 48 and 24 hr of storage at 37°C, respectively in *L.acidophilus* $LBKV_3$ and $LBKI_4$, LF-40 and yoghurt cultures. Thereafter, the coliform counts showed a decline. In case of milk inoculated with yoghurt cultures the inhibitory activity increased linearly with acidity.

It could be seen from Fig.4 that except in *L.acidophilus* $LBKV_3$ which has shown inhibition after 60 hr of storage at 15°C, none of the remaining cultures were able to reduce the coliform counts upto the end of 60 hr storage at 15°C. Extent of inhibition and rate of acid production of $LBKV_3$ was higher than that of $LBKI_4$ and yoghurt cultures showed maximal effect among all the lactic cultures tested.

Results of inhibition of *E.coli* by the yoghurt culture are in agreement with those reported by earlier workers[15]. The results obtained with regard to inhibitory activity of *L.acidophilus* observed by earlier workers[4] among various lactic cultures tested is in agreement with the results of present study. Results obtained on effect of different temperatures on inhibitory activity are similar to those of earlier study, where it has been indicated that the higher storage temperature had a greater inhibitory effect than lower one[14]. It is because of higher acidity due to continued growth and activity of the cultures at 37°C. The acidity at 37°C exceeded 2 per cent with lactobacillus cultures whereas at 15°C it is never more than 1 per cent.

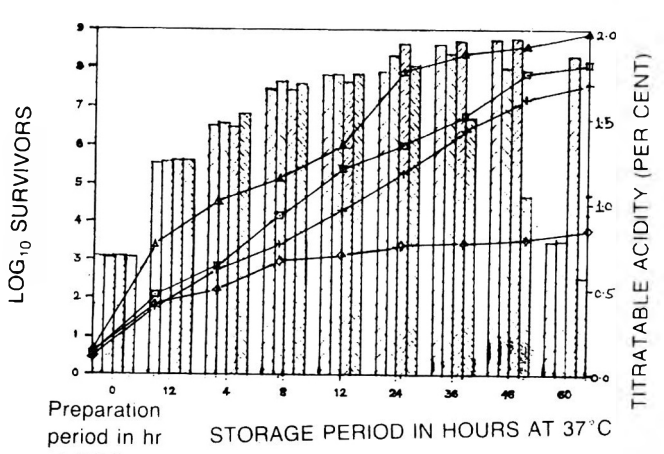

Fig.3. Inhibition of *E.coli* during the production of cultured milks at 37°C and subsequent storage at 37°C
Log_{10} survivors of *E.coli* in cultured milks consisting strain of $LBKV_3$ (⧄), $LBKI_4$ (⧅), LF-40 (⧄), CH_1 + LBW (⧅) and titratable acidity in $LBKV_3$ (□), $LBKI_4$ (+), LF-40 (◇), CH_1 + LBW (△).

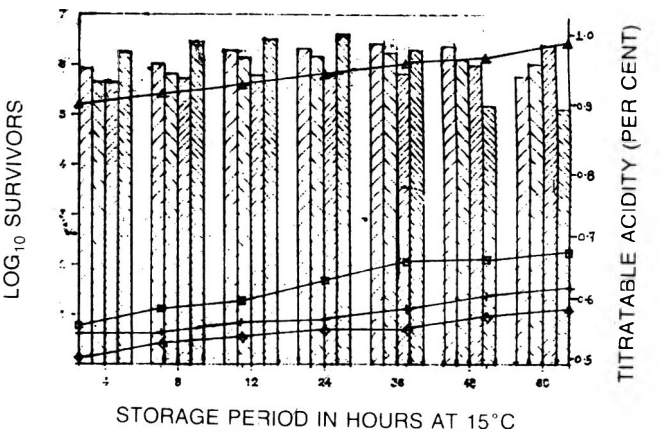

Fig.4. Inhibition of *E.coli* during the production of cultured milks at 37°C and subsequent storage at 15°C.
Log_{10} survivors of *E.coli* in cultured milks consisting strain of $LBKV_3$ (⧄), $LBKI_4$ (⧅), LF-40 (⧄), Ch_1 + LBW (⧅) and titratable acidity in $LBKV_3$ (□), $LBKI_4$ (+), LF-40 (◇), CH_1 + LBW (△).

In the present study, $LBKI_4$ strain showed slower rate of acid production and lower inhibition compared $LBKV_3$. Variations between strains have been indicated by several workers[1-4] and this observation is in agreement with our observations. Shahani *et al*[3] indicated that inhibitory influence is due to combined effect of lactic acid and other metabolites and if strains are not selected, there may be less pronounced antimicrobial activity as observed with commercial preparations. It is interesting to note that from the observations in Fig.4 where acidity was lower than 0.7 per cent the inhibitory influence observed with $LBKV_3$ and $LBKI_4$ was greater than LF-40 which further confirms that these strains produce inhibitory substances other than lactic acid and this needs to be confirmed by further studies. Present study also indicates that *Staphylococcus aureus* and *Escherichia coli* grow to $10^{5.7}$ cfu/ml and 10^8 cfu/ml respectively, before they are inhibited, which may prove to be dangerous in toxigenic cultures. This study further indicates the need for selection of proper cultures for giving the therapeutic benefits to the consumer[16].

References

1. Attaie R, Whalen P J, Shahani K M and Amer M A, Inhibition of growth of *Staphylococcus aureus* during production of acidophilus yoghurt *J Fd Prot*, 1987, **50**, 224.
2. Shahani K M, Vakil J R and Killara A, Natural antibiotic activity of *Lactobacillus acidophilus* and *L. bulgaricus*. I. Cultural conditions for the production of antibiotics, *Cult Dairy Prod J*, 1976, **11**, 14.
3. Shahani K M, Vakil J R and Killara A, Natural antibiotic activity

of *Lactobacillus acidophilus* and *L.bulgaricus*. II. Isolation of acidophilin from *L.acidophilus*. *Cult Dairy Prod J*, 1977, **12**, 14.

4. Dahiya R S and Speck M L, Hydrogen peroxide formation by lactobacilli and its effect on *Staphylococcus aureus*, *J Dairy Sci*, 1968, **55**, 1568.
5. Price R J and Lee J S, Inhibition of Pseudomonas species by hydrogen peroxide producing lactobac lli, *J Milk Fd Technol*, 1970, **33**, 13.
6. Spillmann H Puhan Z and Banhegyi M, Antimicrobial activity of thermophilic lactobacilli, *Milchwissenschaft*, 1978, **33**, 148.
7. Rubin H E, Thomas N and Vaughan F, Lactic acid inhibition of *Salmonella typhimurium* in yoghurt, *J Dairy Sci*, 1982, **65**, 197.
8. Park H S and Marth E H, Behaviour of *Salmonella typhimurium* in skim milk during fermentation of lactic acid bacteria, *J Milk Fd Technol*, 1972, **35**, 482.
9. Rubin H E and Vaughan F, Elucidation of inhibitory factors of yoghurt against *Salmonella typnimurium*, *J Dairy Sci*, 1979, **62**, 1873.
10. Northolt M D, Growth and inactivation of pathogenic microorganisms during manufacture and storage of fermented dairy products, *Neth Milk Dairy J*, 1984, **38**, 135.
11. *Methods of Test for Dairy Industry*, *IS*:1479-1962 *Part III, Analysis of Milk*, Indian Standards Institute, New Delhi.
12. *Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures*, Difco Laboratories INc., Michigan, 1977, 12th Edn.
13. *Methods of Test for Dairy Industry*, *IS*: 1479-1960 *Part I, Rapid Examination of Milk*, Indian Standards Institute, New Delhi.
14. Konecny S, Inhibition of some pathogenic and potentially pathogenic bacteria in cultured milk products, *Prumysl Potravin* 1986, **37**, 427 (*Dairy Sci Abstr*, 1986, 49, 7357.
15. Goel M C, Kulshrestha D C, Marth E H, Francis D W, Bradshaw J G and Read R B Jr, Inhibition of coliforms in fermented milks, *J Milk Fd Technol*, 1971, **34**, 54.
16. Sandine W E, Muralidhara K S, Elliker P R and England D C, Lactic acid bacteria in food and health: A review with special reference to enteropathogenic *Escherichia coli* as well as certain enteric diseases and their treatment with antibiotics and lactobacilli, *J Milk Fd Technol*, 1972, **35**, 691.

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Effect of Heat Treatment on the Quality of Mozzarella Cheese from Buffalo Milk

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Mozzarella cheese was made from raw, pasteurised (63°C/30 min) and h gh temperature heated (71°C/30 min) buffalo milk standardised to 4.0% fat. Cheeses prepared from raw and pasteurized milk were superior to those prepared from milk heated to high temperature. The flavour characteristics of pasteurized milk cheese was superior to that of raw milk cheese whereas the body and texture characteristics were similar. The melting and stretching characteristics of cheese made from milk heated to high temperature were also inferior to the cheeses made from raw and pasteurised milks. The rheological characteristics of cheese made from milk heated to high temperature improved with the addition of 0.01% calcium chloride.

Mozzarella cheese is traditionally manufactured from raw buffalo milk in Italy. It is difficult to predict the shelf life and quality of the finished product due to the presence of different micro-organisms. Now a days pasteurized milk is used for the preparation of cheese and other dairy products. The heat treatment of cheese milk is an important process variable as it affects the coaguability, yield, rate of ripening and sensory characteristics of the product. In this paper, the effect of heat treatment on the sensory and physico - chemical properties of Mozzarella cheese prepared from buffalo milk is discussed.

Materials and Methods

Cheese manufacture: Cheeses were made from standardised raw, pasteurised (63°C/30 min) and heated to high temperature (71°C/30 min) buffalo milk containing 4.0 per cent fat. The total solids (TS) content of milk varied from 13.48 to 14.20 per cent. In all the three cases, milk was brought to 35°C before the addition of 2 per cent starter culture consisting of *S. thermophilus* and *L. bulgaricus* in the ratio of 1:1. In the case of milk subjected to high heat treatment, calcium chloride at 0.01 per cent level was added after dissolving it in potable water. After ripening of milk for 80-90 min, Meito rennet (obtained from Meito Sangyo Co. Ltd. Japan) was added @ 1.0 g/100 lit of milk. The curd was cut by ½" knives and allowed to stand undisturbed in the whey for 10-15 min. The cooking was then started gradually reaching final temperature of 41 ± 1°C in 40 min. Nearly ⅓ of the whey was removed till the curd became visible. The mixture of whey and curd was then held at this temperature with occasional stirring till the titratable acidity of the whey reached 0.40 - 0.43 per cent. The whey was then completely drained and the progress of acidity development was monitored. In the meantime "stretch test" of the curd as recommended by Kosikowski[1] was carried out. When the titratable acidity of fresh whey reached 0.75 per cent, sufficient hot water at 80-83°C was added to the vat to cover the curd. After 2-3 min of warming up, the curd was kneaded, stretched and moulded by hand. The hot plastic curd was then moulded into balls or cubes (½ - 1 kg) and then dipped into chilled water (5-8°C) for a min. of 2 hours. The cheese was removed from the water and kept in the refrigerator for the draining out of the water.

Milk: Fat, solids-not-fat, titratable acidity (TA), casein and total solids (TS) were determined as described in IS:SP:18 (Part-XI)[2].

Cheese. Samples were evaluated organoleptically for different quality attributes like appearance, body and texture and flavour by a select panel of trained judges using an 18 point score card developed by Duthie *et al.*[3].

Moisture and fat were determined as per the methods outlined in Laboratory Manual,[4] while TA was measured by the method recommended by AOAC[5].

Meltability was determined by the method of Nilson and LaClair[6] with suitable modification. A cylindrical

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sample of cheese was taken with the help of a cork borer from the cheese block. The area of the base of the bored samples was 2.43 cm^2. It was sliced into discs of 10 mm height. Three discs of each sample were placed on Whatman No.42 filter paper and placed on the Corning glass petri-dish and then covered with another plate. The whole assembly was then kept for 15 min. in an atmospheric oven pre-heated to 140°C. The area of the melted cheese was then traced on a paper with the help of carbon and pencil after taking it out from the oven. The area was measured with a planimeter (Zero setting device polar compensation planimeter milk optical tracer No.75167, made in Japan). From the data, the meltability was calculated. Meltability = A/B where, A = Area of melted disc, B = Area of original disc.

Stretchability test was carried out as per the principles of 'Stretch test' described by Kosikowski[1]. About 10 g of cheese was taken in 250 ml beaker containing 3/4th of its volume of hot water maintained at 80-83°C in a water bath. It was kept in the beaker for about 3 min. A glass rod was inserted into the molten cheese sample and then pulled out slowly after providing few turns by hand. This ensured proper adherence of the product to the glass rod. Cheese thread formation was observed when the rod was being gradually lifted. The length of thread was assumed as the stretchability parameter. Longer threads indicated better stretching characteristics. The stretchability was graded on a 5 point arbitrary scale where 5 represented the best stretchable characteristics.

Results and Discussion

Sensory evaluation: The mean flavour scores of Mozzarella cheese differed significantly ($p < 0.01$) due to the heat treatment while the appearance and body and texture scores of the high heat treated milk cheese were significantly lower than the cheeses made from raw and pasteurised milks (Table 1). It was observed that pasteurized milk resulted in a very good product. In the case of raw milk, the development of acidity during cheese manufacture was erratic. The flavour of raw milk cheese was also inferior due to the presence of off-flavours which may be due to feed or certain adventitious micro-organisms. Pasteurization helped in removing some of the absorbed off-flavours and also created desirable heated flavour. The product obtained from highly heated milk was also inferior which could be due to higher moisture content of the cheese and more TS losses during kneading in hot water.

Physico-chemical properties: The moisture content increased with increase in heat treatment indicating significant ($p < 0.01$) effect of heat treatment on the moisture content of the cheese. The cheese made from high heated milk had significantly (CD = 2.02) higher moisture than the other cheeses. This possibly resulted in a significantly higher yield (Table 2). Another reason for the higher yield could be due to the denaturation of whey proteins resulting in the retention of larger quantities of whey proteins. The difference in moisture content between the cheeses made from raw and pasteurized milks was not significant whereas it was significant when raw and high heated milk was used. Fat content (16.26 per cent) and its recovery (73.97 per cent) was lowest in the cheese made from high heated milk. This could be due to the higher TS loss during kneading in hot water. The recovery of TS was highest in the cheese made from pasteurized milk and was lowest in the cheese made from high heated milk similar to the fat recovery.

The moisture retention, yield, fat and TS recovery were significantly higher in the case of cheese made from pasteurized milk compared to raw milk, whereas more moisture retention, yield, less fat and TS recovery were observed in high heated milk. This could be due to the heat induced changes resulting in the

TABLE 1. SENSORY EVALUATION OF CHEESE

Treatment	Flavour	Body & Texture	Appear-ance	Comments
Raw	7.71	4.36	2.85	Foreign flavour
Pasteurized (63°C/30 min)	9.25	4.54	2.89	–
High heat (71°C/30 min)	8.53	2.82	2.04	Lacks flexibility, weak, rough surface, coarse texture.
C D	0.29	0.20	0.23	–

Excellent score; Flavour-10, Body & Texture-5, Appearance-3.

TABLE 2. PHYSICO-CHEMICAL PROPERTIES OF CHEESE

Attributes	Raw	Pasteurized (63°C/30 min)	High heated (71°C/30 min)
Moisture (%)	52.37 ± 0.46	53.59 ± 0.20	59.87 ± 0.82
Yield (%)	15.60 ± 0.20	16.37 ± 0.33	18.18 ± 0.55
Fat (%)	22.31 ± 0.44	21.71 ± 0.41	16.26 ± 0.55
Fat recovery(%)	87.14 ± 2.01	88.85 ± 2.31	73.97 ± 3.29
TS recovery(%)	52.69 ± 0.56	53.38 ± 0.55	48.59 ± 1.66
TA (%)	0.53 ± 0.02	0.57 ± 0.02	0.43 ± 0.04
Meltability	3.62 ± 0.12	4.03 ± 0.16	1.82 ± 0.23
Stretchability	5.0	5.0	1.0

The meltability and stretchability of the cheese made from the milk heated to high temperature with the addition of 0.01% $CaCl_2$ were 2.07 ± 0.26 and 2.0 respectively.

denaturation of whey proteins[7] and interaction between casein and whey proteins. The changes vary with the time and temperature of heat treatment. Thus, the whey proteins which might have gone to whey in the case of raw milk cheese must have been retained partially with the curd in case of pasteurized and high heated milk cheeses. Also, due to heat treatment some of the soluble minerals could have been converted into colloidal form and thus retained in the cheese. In addition to this, there may be some interaction between fat and protein leading to higher recovery of fat in cheese. However, higher losses of fat and TS were observed during kneading and stretching in hot water resulting in reduced recovery of fat and TS in the high heated milk cheese. This could be ascribed to alteration in physico-chemical equilibrium of minerals and proteins as a result of which a soft curd will be formed during manufacture with high heat treated milk[8]. Higher fat and TS loss in moulding water was also observed by Schafer and Olson[9] in case of Mozzarella cheese made from ultra high temperature treated milk.

The acidity development in raw milk was observed to be inconsistent as observed earlier in cheddar cheese[10]. The acidity development was almost identical in the case of pasteurized and high heat treated milks. Further, the low acidity of the high heat treated milk cheese could be due to the increased losses of fat and SNF and retention of higher moisture in the cheese.

The heat treatment resulted in a significant effect ($p<0.01$) on the melting characteristics of cheese (Table 2). However, no significant difference in the melting characteristics was observed between raw and pasteurized milk cheeses (CD = 0.79). The melting of high heated milk cheese was significantly inferior to the raw and pasteurized milk cheeses. The melting of pasteurized milk cheese was higher than the raw and high heat treated milk cheeses. This indicated that higher heat treatment caused an inferior melting characteristic. Similarly, the stretching characteristics of high heated milk cheese was also observed to be inferior to other cheeses (Table-2). The inferior melting and stretching characteristics of high heated milk cheese could be due to the lower amount of calcium in ionic state in milk as the heat treatment results in the shift of calcium from soluble to the colloidal state[11]. In our experiment also, it was observed that the melting and stretching characteristics of the cheese made from milk heated to high temperature improved with the addition of 0.01 per cent calcium chloride confirming the role of ionic calcium. The improvement in these characteristics could also be ascribed to the breakdown of the casein micelle due to the heat treatment resulting in the formation of smaller micelle which may form larger aggregation depending upon the temperature attained.[12]

References

1. Kosikowski F V, *Cheese and Fermented Milk Foods,* Edwards Brothers. Inc. Michigan, U.S.A, 1982, 179.
2. *ISI Hand book of Food Analysis : IS:SP:*18-1981 *Part XI. Dairy Products.* Indian Standards Institution, New Delhi.
3. Duthie A H, Lemaire J T, Nilson K M, Partridge J A and Atherton H V, Scorecard for Mozzarella cheese, *Cult Dairy Prod J,* 1980, **15,** 5.
4. *Methods of analysis of milk and its products, Laboratory Manual,* Milk Industry Foundation, Washington 1959.
5. *Official Methods of Analysis. Association of Official Analytical Chemists.* Washington, DC, 1980.
6. Nilson K M and LaClair F A, A National survey of the quality of Mozzarella cheese, *Am Dairy Rev,* 1976, **38,** 18A.
7. Marshall R J, Increasing cheese yields by high heat treatment of milk, *J Dairy Res,* 1986, **53,** 313.
8. Wilson G A and Wheelock J V, Factors affecting the action of rennin in heated milk, *J Dairy Res,* 1972, **39,** 413.
9. Schafer H W and Olson N F, Characteristics of Mozzarella cheese made by direct acidification from ultra high temperature processed milk, *J Dairy Sci,* 1975, **58,** 494.
10. Scott R, *Cheese Making Practice,* Applied science publishers Ltd, London, 1981, 127.
11. Webb B H, Johnson A H and Alford J A, *Fundamentals of Dairy Chemistry,* AVI Pub. company Inc, 1983, 3rd Edn, 662.
12. Hostetter H, Imhof K and Stein J, Studies on the effect of heat treatment and lyophilisation on the state of distribution and physiological properties of milk proteins with special consideration of heat treatment conditions applied in uperization. I. Effect on the state of distribution of milk proteins, *Milchwissenschaft,* 1965, **20,** 189.

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A Comparative Study on Heated Adrenal and Immunoglobin G Antigen for Identification of Cooked Meats of Cattle and Sheep

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A comparative study was undertaken to find out suitability of boiling resistant, ethanol precipitable (BE) adrenal and heated IgG antigens of cattle and sheep to raise species specific antibody for identification of cooked meats of cattle and sheep. The antibodies raised in rabbits against BE antigens of cattle and sheep could be rendered species specific by absorption with cross reacting antibodies. These species specific sera could detect adulterant cooked cattle or sheep meat as the case may be upto 1% level. No species specific antibodies could be detected in sera produced in rabbits using heated IgG antigens of cattle and sheep. The cross immunisation of buffaloes and goats with heated BE antigens of cattle and sheep respectively was not successful.

Fresh meat antigens are successfully used to produce species-specific antibodies for the identification of meat of different species animals. However, the quest for an antigen to be used to produce species specific antibodies for identification of cooked meats still continues because the solubility properties and antigenic competence of the proteins are altered considerably on heating. Recently, heated IgG[1], myoglobin[2] and adrenal gland[3-5] as well as unheated troponin[6] antigens were used for the production of hyper-immune sera to identify cooked meats. Of all the antigens reported, adrenal gland antigens were promising but the studies with IgG antigens were inconclusive. Myoglobin and troponin antigens were reported to detect meats heated to 70°C only. In the present study, therefore, heated adrenal gland antigens (BE) and heated IgG antigens of cattle and sheep were selected for a comparative study to raise antibodies in rabbits and in cattle and goats as the case may be, for detection of species specific antigens in cooked meats.

Materials and Methods

Antigens: Cattle, buffalo, sheep and goat adrenal glands procured from slaughter houses and stored at – 20°C were used to isolate respective antigens as per the method of Milgrom *et al*[4]. Twenty grams of adrenal glands were homogenized (Scientronic – homogenizer, Model STH-430, Scientronic Instruments, New Delhi) in 60 ml of 0.14 M sodium chloride solution, sonicated at 150 Watts in an untrasonic disintegrator (Model Braun – Sonic 1510, Van waters and Rogers. San Franscisco, California), heated in boiling water for 1 hr, rehomogenized and centrifuged (20,000 r.p.m.) at refrigerated temperature. The supernatant was autoclaved for half-an-hour at 120°C and centrifuged. This supernatant was subjected to alcohol (71 per cent precipitation and the procedure was repeated thrice. The metal grey precipitate obtained was air-dried at room temperature and ground to fine powder which was called BE (boiling resistant, ethanol precipitable) antigen.

Crude immunoglobulin G (IgG) salt-free suspension from cattle, buffalo, sheep and goat sera was obtained by ammonium sulphate precipitation[7]. The percipitate was heated in a boiling water bath for one hour and homogenized to a fine suspension to be used as IgG (H) antigen.

Raw and cooked antigens: Raw (native) antigens were prepared from muscle, liver, kidney and spleen of catle, buffalo, sheep, goat, pig and chicken. The samples were homogenized with normal saline to get 20 per cent suspension and centrifuged at 5000 rpm, for half-an-hour. The resultant supernatant was

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preserved with thiomersal (1 in 10,000) and stored at −20°C until used.

Heated antigens were also prepared from the aforementioned tissues by cooking them for half-an-hour at 120°C and 15 lb pressure, prior to preparing 20 per cent homogenized suspension as in the case of fresh antigens. Rest of the procedure was same as above.

Mixed antigens: For the purpose of detection of adulteration, cattle meat was mixed with buffalo or goat or sheep meat and sheep meat with buffalo or cattle or goat meat at 1 to 20 per cent levels. The meat extracts were prepared before and after cooking as described for saline extracts of native and heated muscle extract antigens.

Production of antisera: Antisera were raised separately against BE adrenal gland antigens, and heated crude IgG antigens of cattle and sheep by hyper-immunisation of rabbits. Antibodies were also raised in buffalo and goat by hyper-immunisation with BE adrenal gland antigens of cattle and sheep respectively.

Immuno-diffusion tests: Agar gel double immuno-diffusion[8] (DIO) and counter immuno electrophoresis (CIE)[9] tests were performed to study the specificity of the raised antisera and for of detection of adulterant cattle and sheep meats in other species of meat. The gel slides were prepared by pouring 15 ml of 0.6 per cent agarose suspension in sodium phosphate buffer (pH 6.8) over the dried glass slides pre-coated with agarose (size 75 mm × 75 mm). The slides charged with antigens and antibody were incubated at 25°C upto 72 hr, dried and stained with 1.0 per cent solution of amido black, if required.

For immuno-electrophoresis test[10], 0.85 per cent agarose suspension in sodium barbitol buffer pH 8.6 was used to prepare the gel slides as above. The electrophoresis was carried out (15 mA continuous current strength per slide) for one and half hour at 7°C and by further incubation at 25°C for 72 hr. The slides were dried and stained as mentioned above.

Results and Discussion

Loss in antigenicity of proteins on heating has been attributed to their complete coagulation which however, does not occur with all proteins.[11] Antigens isolated from adrenal, brain, testicular and pituitary tissues have been reported to retain antigenicity even after heating at 100°C for 30 min[2-5]. In view of this, two antigens namely adrenal and IgG of cattle and sheep were selected to study the retention of antigenicity after heat treatment and their suitability to produce species specific antisera to identify the homologous antigens.

The antisera raised in rabbits against cattle BE adrenal gland antigen (RAC, BE) formed precipitation lines with cooked homologous (cattle) antigens derived from muscle or other organs of the body suggesting that the adrenal BE antigens retained sufficient antigenicity after heat treatment to produce detectable immune response. However, RAC (BE) antisera also cross reacted with heated buffalo, sheep and goat antigens in both DID and IE tests. The heterologous antibodies, however, could be completely eliminated on absorption with cross reacting antigens and the serum could be made species-specific. The absorbed RAC (BE) serum formed one continuous precipitation line with homologous heated adrenal and heated muscle antigens in DID test. In IE test also, a single precipitation band was formed with the homologous antigens but none with the heterologous antigens from buffalo, sheep and goat.

Like RAC (BE) adrenal serum, RAS (BE) adrenal serum could be made species-specific by absorption. The absorbed serum formed a single precipitation band in DID and in IE test with homologous antigens derived from different organs/tissues of sheep.

The species-specific reaction of RAC (BE) and RAS (BE) sera was consistant as they formed a continuous precipitation band, with all the heated field samples from homologous species only but none with heterologous species of animals.

The absorbed RAC and RAS (BE) sera were used to detect the adulterant cooked beef and mutton respectively in 15 different samples also containing cooked meats of other species of animals. Both the sera specificically reacted with homologous antigens and could detect adulterant beef or mutton, as the case may be to a minimum of 1.0 per cent level. Milgron *et al.*[12] successfully used RAC (BE) adrenal serum to detect the presence of beef in hamburger by employing compliment fixation test but did not report minimum levels of detection. They had prepared test antigens by boiling and ethanol extraction (BE fractions). In the present studies, BE fractions of adrenal antigens were used only as positive controls while the test antigen (field) were isolated by extracting the antigen from autoclaved (120°C, half-an-hour) muscle antigens in normal saline solution without ethanol precipitation. This autoclaved antigen was found to be equally good, as it reacted specifically with homologous serum indicating that there was no necessity of ethanol precipitation for the isolation of the field antigens. This would make the isolation of test antigens simpler, less time consuming and more economical.

The antisera raised in rabbits against heated cattle IgG (RAC – IgG H) and sheep IgG (RAS – IgG H)

reacted with heated homologous as well as with heterologous IgG and muscle antigen in both DID and the IE tests. All attempts to make them species-specific by absorption with cross-reacting antigens were unsuccessful. Similar observations were also made by Karpas *et al.*[1] Failure to make this serum species-specific might have been either due to the normal difficulties experienced in absorption or due to loss of species-specificity and/or increased cross-reactivity of IgG antigens on heating[13,14].

No efforts were made earlier to use RAC (BE) and RAS (BE) adrenal sera to detect native cattle and sheep antigens respectively. Present studies indicated the possibility of using these sera for detection of native cattle and sheep antigens, as both sera reacted with respective native antigens specifically by forming a single precipitation band. They could also detect respective adulterant meats in their native condition. Absorbed RAC (BE) serum detected native adulterant beef in native meat from other species of animals to a minimum level of 2 per cent and RAS (BE) adrenal serum could detect native mutton to 10 per cent level. The precipitation reaction of either RAC (BE) or RAS (BE) absorbed adrenal sera with the native homologous antigens (cattle and sheep respectively) was, however, weak as compared to the reaction obtained with cooked homologous antigens.

No detectable immune response was produced in buffaloes on injecting adrenal BE antigen of cattle, as no visible precipitation reaction was produced either in DID or IE tests by using RAC (BE) serum and the homologous antigens. As observed earlier, BE fractions of adrenal glands of cattle did retain immunogenecity to produce species-specific antibodies in rabbits. It therefore, appears that, to immun ze phylogenetically related species a dose of 20 mg per buffalo might have been very less. It is thus, necessary to extend the studies to standardize the immunization schedule in buffaloes using cattle BE adrenal antigen with different dose levels before concluding that the cross-immunization of buffaloes with cattle adrenal BE antigens is not possible.

The sheep (BE) adrenal antigen evoked immunogenic response in phylogenetically related animals (goats), as the goat anti sheep BE (GAS BE) serum reacted positively, by forming a weak precipitation reaction specifically with heated and native sheep adrenal/muscle antigens. The immune response evoked was, however, of very weak order as the precipitation band formed was very weak, and the serum lost titre within 15 days of storage at $-20°C$. The GAS (BE) serum, however, also cross-reacted with cattle cooked muscle antigen. Further studies are required to standardize the dose of antigen and the immunization schedule to be followed to obtain higher concentration of antibodies.

In the light of above observations made, use of BE antigents to produce species specific antisera for detection of adulteration cooked meats is recommended.

References

1. Karpas A B Myers W L and Segre D, Serological identification of species of origin of sausage meats, *J Fd Sci*, 1970, **35**, 150.
2. Hayden A R, Immunochemical detection of ovine, porcine and equine flesh in beef products with antisera to species myoglobin, *J Fd Sci*, 1979, **44**, 194.
3. Milgrom F Tuggac M and Witebsky E, Immunological studies on adrenal glands. III. Interspecies relation of thermostable adrenal specific antigens, *Immunology*, 1963, **6**, 105.
4. Milgrom F and Witebsky E, Immunological studies on adrenal glands. 1. Immunization with adrenals of foreign species, *Immunology*, 1962, **5**, 46.
5. Hayden A R, Use of antisera to heat-stable antigens of adrenals for species identification in thoroughly cooked beef sausages, *J Fd Sci*, 1981, **46**, 1810.
6. Hayden A R, Detection of chicken flesh in beef sausages, *J Fd Sci*, 1977, **42**, 1189.
7. Williams C A and Chase M W, *Methods in Immunology and Immuno-chemistry*, Vol II *Physical and Chemical Methods*, Academic Press, New York 1968.
8. Ochtertony O, *Handbook of Immunodiffusion and Immuno-electrophoresis*, Ann Arbor Science Publishers Inc., Ann Arbor, Michigan, 1968.
9. Edwards E A, Counter immunoelectrophoresis and double immunodiffusion, In *Rapid Diagnosis in Infectious Diseases*, by Rytel M W, (Ed) CRC Press, Inc., Florida, 1979, 19.
10. Graber P and Burtin P, *Immunoelectrophoretic Analysis*, Elsevier Publishing Co, Amsterdam, 1964.
11. Carpenter P L, *Immunology and Serology*, W.B. Saunders Company, London, 1965, 2nd Edn 56.
12. Milgrom F Tuggac M and Witebsky E, Studies on species specificity, *J Immunol*, 1964, **93**, 902.
13. Hirose S L and Osier A G, Interaction of rabbit antihuman H chain sera with denatured human globulin and its sub-units, *J Immunol*, 1967, **98**, 618.
14. Henney C S and Ishizaka K, Antigenic determinants specific for aggregated G-globulins, *J Immunol*, 1968, **100**, 710.

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Effect of Blackgram Fibre (*Phaseolus mungo*) on the Metabolism of Lipoproteins in Rats

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The effect of blackgram fibre on the metabolism of lipoproteins was studied in rats fed cholesterol-free diet for one month. Rats fed neutral detergent fibre (NDF) from blackgram showed lower concentration of cholesterol in VLDL and LDL fractions when compared to those fed isocaloric, fibre-free diet. Both the concentrations of total protein and apo.B in VLDL and LDL showed a small increase in the NDF diet group. Plasma LCAT activity, activity of lipoprotein lipase in the heart and adipose tissue and post heparin lipolytic activity were also more in the rats of the NDF diet group.

Dietary fibre isolated as neutral detergent fibre (NDF) from blackgram (*Phaseolus mungo*) by the procedure of Goering and Van Soest[1] has been found to show significant cholesterol lowering action in rats fed atherogenic diet.

The effect of blackgram NDF on hepatic HMG-CoA reductase activity, incorporation of labelled acetate into hepatic cholesterol and bile acid synthesis in rats was also reported previously.[2] Rats maintained on cholesterol-free diet and fed NDF from blackgram showed higher incorporation of labelled precursors (1,2-^{14}C) acetate and (U-^{14}C glucose) into hepatic cholesterol, increased activity of hepatic HMG-CoA reductase and higher concentration of cholic acid and chenodeoxycholic acid in the liver. One of the machanisms postulated for the cholesterol lowering action of dietary fibre is that it acts as a bile acid sequestrant. The interruption of enterohepatic circulation of bile acids by a bile acid sequestant like cholestyramine, for example, has been reported to lead to changes in the serum lipoproteins. Stimulation of VLDL secretion into the plasma, promotion of conversion of VLDL to LDL, enhanced hepatic uptake of plasma LDL via higher affinity receptors and increase in plasma HDL_2/HDL_3 ratio have been reported to take place following administration of bile acid sequestrant resins.[3-5]

There does not seem to be any similar report on the effect of dietary fibre on lipoprotein metabolism, eventhough the dietary fibre is believed to act as a bile acid sequestrant like cholestyramine. But there are certain points of difference, the chief of which is that microbial digestion of dietary fibre takes place in the intestine, while a resin like cholestyramine is unaffected. The possibility that some of the products of digestion of dietary fibre may be absorbed and may exert a systemic effect in the liver cannot be over-looked

In view of these, the effect of administration of blackgram NDF on the lipid and apoprotein components of serum lipoproteins has been studied in rats. The activities of two enzymes concerned with lipoprotein metabolism viz. lipoprotein lipase (which is involved in the catabolism of triglyceride rich lipoproteins) and lecithin: cholesterol acyl transferase (LCAT) involved in cholesterol esterification have also been studied. The results are reported in this paper.

Materials and Methods

Animals and diets: Male albino rats (Sprague-Dawley strain, weight 80-100g) were divided into 2 groups of 12 rats each in one experiment and fed as follows.

Group I – Fibre-free diet

Group II – Blackgram NDF diet

The rats in each group were housed individually in polypropylene cages with wire mesh floor in rooms maintained at 25°C. Fibre-free diet contained (in g/100g diet) A – dextrin 67, casein (Vit. fat-free) 20 and groundnut oil 8, NDF diet had A – dextrin 42, casein 20, fibre 25 and groundnut oil 8. both contained B salt mixture-4 and vit. mixture 1.

The compositions of the salt mixture and vitamin mixture have been given earlier[1].

13.1 g of A of NDF diet supply the same calories as 10 g of fibre-free diet. The caloric intake of the two groups was kept the same by adjusting the intake of A. The intake of salt mixture and vitamin mixture was

kept the same in the two groups by giving the same amount of B (0.75g/rat) mixed with the required weighed quantity of A. NDF was taken to contribute very little towards calorie value. Water was provided *ad libitum*. The duration of the experiment was one month. At the end of this period, the rats were deprived of food overnight and blood was removed by cardiac puncture. The animals were killed by decapitation and adipose tissue and heart were removed to ice cold containers for estimation of lipoprotein lipase activity.

Preparation of NDF from blackgram was carried out as described earlier[2]. Total cholesterol was estimated in the whole serum by the method of Abell[6] and total protein by the method of Lowry *et al.*[7] Tetramethyl urea (TMU) soluble protein was determined according to the procedure of Kane *et al.*[8] and the difference between the total protein and TMU soluble protein gives total serum apo-B, VLDL was precipitated from the serum by heparin and magnesium chloride (final concentration, heparin 0.25 per cent and $MgCl_2$ 0.1M) according to the procedure of Burstein *et al.*[9] The supernatant which contains LDL + HDL was analysed for total cholesterol and apo-B. This apo-B gives a measure of LDL apo-B. Difference between the total serum apo-B and LDL apo-B gives VLDL apo-B. VLDL cholesterol was obtained from the difference between serum total cholesterol and the cholesterol in the heparin-magnesium chloride supernatant. Another aliquot of the serum was subjected by heparin-manganese chloride precipitation according to the procedure of Warnick and Albers.[10] The supernatant which contains HDL was analysed for cholesterol to obtain HDL cholesterol. The difference between total and HDL cholesterol gives LDL + VLDL cholesterol. LDL cholesterol is obtained by subtracting VLDL cholesterol from LDL + VLDL cholesterol.

Assay of plasma lecithin: Cholesterol-acyl-transferase (LCAT EC 2.3.1.4.3) : Blood was collected in heparinised tubes maintained at O°C and immediately centrifuged at 0°C to separate the plasma. An aliquot was immediately extracted with acetone:ethanol (1:1) to extract the lipids. Another aliquot of the plasma was incubated at 37°C for 3 hr, at the end of which it was extracted with acetone:ethanol (1:1). Ester cholesterol and unesterified cholesterol were estimated in the lipid extract by the procedure of Schoenheimer-Sperry[11,12]. The increase in the ester cholesterol/unesterified cholesterol ratio is taken as a measure of LCAT activity. Lipoprotein lipase (EC 3.1.1.3) activity of heart and adipose tissue was estimated according to the procedure of Krauss *et al.*[13] Protamine inhibited activity is taken as a measure of lipoprotein lipase activity. The enzyme activity is expressed as micro mole of glycerol liberated per hr per g protein.

Post heparin lipolytic activity: Heparin (100 units/kg body weight) was administered intraperitoneally to rats. After 30 minutes, blood was removed from the heart in heparanised tubes. Plasma was separated by centrifuging at 4°C at 600 **g** for 10 minutes. Total lipolytic activity was determined as above in the absence of protamine sulphate.

Protein was estimated after trichloroacetic acid precipitation by the method of Lowry *et al.*[7]

Differences between the two groups were evaluated for significance by student's 't' test.[14]

Results and Discussion

The gain in body weights (59 ± 2.5g) and the liver weights (5.02 ± 0.32g) of the two groups were comparable.

Concentration of total cholesterol in the lipoprotein fractions: There was significant decrease in the concentration of cholesterol in VLDL, and LDL fractions in the NDF diet group when compared to the fibre-free diet group (Table 1). Maximum decrease was observed in the LDL fraction. HDL cholesterol showed no significant alteration. In this connection, there are some reports that HDL cholesterol is either not affected or increased by dietary fibre[15]. Probably fibres from different sources act differently.

Concentration of total protein and apo-B in VLDL and LDL fractions: Both the concentrations of total protein and apo-B in VLDL and LDL showed an increase in the NDF diet group when compared to the fibre-free diet group (Table 2). The ratio of apo-B/total cholesterol was increased in both VLDL and LDL in the NDF diet group.

Plasma lecithin : cholesterol - acyl-transferase activity: The increase in the ratio of ester cholesterol to free cholesterol after 3 hr was much more in the rats of the NDF diet group than in the fibre-free diet group indicating increased LCAT activity (Table 3).

Lipoprotein lipase activity of heart and adipose tissue and post heparin plasma lipolytic activity (Table 4): The lipoprotein lipase activity in the heart and adipose tissue was significantly higher in the rats of the NDF diet group

TABLE 1. CONCENTRATION (mg/100 ml serum) OF TOTAL CHOLESTEROL IN LIPOPROTEIN FRACTIONS

Group	VLDL	LDL	HDL
Fibre-free diet	5.41 ± 0.14	9.78 ± 0.35	51.31 ± 1.85
Blackgram NDF diet	3.73 ± 0.15	5.27 ± 0.25[a]	45.66 ± 1.30

Values are mean ± SEM of 6 rats.
a = $p < 0.01$

TABLE 2. CONCENTRATION OF TOTAL PROTEIN AND APO-B IN VLDL AND LDL FRACTIONS

Group	Total protein (mg/100 ml Serum)		Apo-B (mg/100 ml serum)		Apo-B	
	VLDL	LDL	VLDL	LDL	VLDL	Cholesterol LDL
Fibre-free diet	10.5 ± 0.33	7.2 ± 0.15	6.2 ±0.12	7.1 ± 0.14	1.14 ±-0.03	0.72 ±0.02
Blackgram NDF diet	11.03 ± 0.34	7.9 ±0.18[b]	6.70 ± 0.15[b]	8.87 ± 0.17	1.79 ± 0.04[a]	1.49 ± 0.03[a]

Values are mean ± SEM of 6 rats., a = $p < 0.01$; b = $p < 0.05$

TABLE 3. ACTIVITY OF LECITHIN CHOLESTEROL ACYL TRANSFERASE

Group	Free cholesterol (mg/100 ml plasma)		Ester cholesterol (mg/100 ml plasma)		Ester cholesterol	
	0 hr	3 hr	0 hr	3 hr	Free	Cholesterol
Fibre-free diet	18.77 ± 0.54	15.2 ± 0.38	43.78 ± 1.14	47.35 ± 0.99	2.33 ± 0.07	3.12 ± 0.09
Blackgram NDF diet	13.6 ± 0.39	7.79 ± 0.23	38.36 ± 1.04	44.17 ± 0.97	2.82 ± 0.08[a]	5.67 ± 0.18[a]

Values are mean ± SEM of 6 rats., a = $p < 0.01$.

TABLE 4. LIPOPROTEIN LIPASE ACTIVITY* AND POST HEPARIN PLASMA LIPOLYTIC ACTIVITY

Group	Lipoprotein lipase activity		Post heparin plasma lipolytic activity
	Adipose tissue	Heart	
Fibre-free diet	142.12 ± 3.84	31.48 ± 1.38	53.12 ± 1.38
Blackgram NDF diet	197.84 ± 6.33[a]	53.38 ± 1.92[a]	76.37 ± 2.37[a]

Values are mean ± SEM of 6 rats., a = $p < 0.01$.
* μ mol glycerol/hr/gm protein.

than in those on the fibre-free diet Post heparin plasma lipolytic activity was also more in the rats of the NDF diet group.

Rat is an animal with an especially efficient mechanism for clearance of chylomicrons and VLDL remnants from the liver via specific receptors.[16-21] This is believed to be responsible for the low LDL levels in rats.[22] Cholesterol ester exchange protein(s), which transfer LCAT-derived cholesterol esters from HDL to LDL is also believed to be absent in the rat which may cause accumulation of cholesterol ester in HDL in these animals.[22] The effect of blackgram NDF on lipoprotein metabolism has to be examined from this point of view.

The increase in apo-B and decrease in cholesterol in VLDL and LDL in the NDF diet group indicates an alteration in the composition of these lipoproteins. Hepatic synthesis of cholesterol is stimulated by NDF as reported earlier.[2] The newly synthesised cholesterol in the liver may be used for VLDL secretion and synthesis of bile acids. One possibility is that more of the newly synthesised cholesterol is channelled into the synthesis of bile acids, which gets support from the observation reported earlier,[1] that the bile acids showed sign ficant increase in the liver in rats fed NDF. If this is the case, then the VLDL secreted will have lower cholesterol content and it would then be converted to similarly depleted LDL. The other possibility is that the mechanism suggested by Sniderman *et al.*[23] whereby cholesterol is removed selectively from the lipoprotein particles and is enhanced in rats fed NDF.

Lipoprotein lipase is involved in the catabolism of

chylomicrons and VLDL and the increase in the activity of these enzymes now observed in rats fed NDF indicates increased catabolism of these lipoproteins. HDL and LCAT operate in conjunction to promote removal of cholesterol from the tissues. Cholesterol esterification is thought to occur on HDL with the transfer of the product ester via. cholesterol ester transfer protein to acceptor LDL or VLDL.[24] Since rat is believed to be deficient in the cholesterol ester transfer protein, cholesterol ester may accumulate in HDL. The HDL may deliver this cholesterol to the liver and some of this cholesterol is converted to bile acids.

The increase in post heparin lipolytic activity in the NDF fed rats which is due to release of lipoprotein lipase and triglyceride lipase on heparin administration, also indicate increased catabolism of VLDL. Both these enzymes act on triglyceride rich lipoproteins (VLDL and chylomicrons). There are no definite reports on the effect of bile acid equestrant resins on lipoprotein lipase and post heparin plasma lipolytic activity. No reports are also available on the effect of resin treatment on LCAT activity and on serum apo-B concentration.

Thus, NDF from blackgram caused decrease in the concentration of cholesterol in serum LDL and VLDL and increase in the concentration of apo B. It also caused increase in plasma LCAT activity and in the activity of lipoprotein lipase in the heart and adipose tissue and post heparin lipolytic activity indicating increased catabolism of lipoproteins.

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References

1. Jayakumari N and Kurup P A Dietary fibre and cholesterol metabolism in rats fed a high cholesterol diet. *Atherosclerosis.* 1973, **33**, 41.
2. Molly thomas, Leelamma S and Kurup P A, Effect of Blackgram fibre (*Phaseolus mungo*) on hepatic hydroxymethyl glutaryl-CoA reductase activity, cholesterogenesis and cholesterol degradation in rats, *J Nutr,* 1983, **113,** 1104.
3. Packard C J and Shepherd T, The hepato biliary axis and lipoprotein metabolism, effects of bile acid sequestrants and ileal bypass surgery, *J Lipid Res,* 1982, **23,** 1081.
4. Witstum J L, Schonteld G and Weidman S W, The effects of cholesterol on the metabolism of very low density lipoproteins in man, *J Lab Clin Med,* 1976, **88,** 1008.
5. Slater M R, Packard C J, Bicker S and Shepherd J, Effects of cholestryamine on receptor mediated plasma clearance and tissue uptake of human low density lipoproteins in the rabbit, *J biol chem,* 1980, **255**, 10210.
6. Abell L L, Levy B B, Brodie B B and Kendall F E, A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J biol Chem,* 1952, **195,** 357.
7. Lowry O M, Rosebrough N J, Farr A L and Randal R J, Protein measurement with the Folin phenol reagent, *J biol chem,* 1951, **193,** 265.
8. Kane J P, Teizosata Hamilton R L, and Richard J, Harvel R J, Apoprotein composition of very low density lipoproteins of human serum, *J Clin Invest,* 1975, **56,** 1622.
9. Bursten M and Scholwick H R, Lipoprotein polyanion. Metal interactions, *Adv Lipid Res,* 1973, **11**, 67.
10. Warwick R C and Albers J M, A Comprehensive evaluation of the heparin-maganese prepitation procedure for estimating high density lipoprotein cholesterol, *J Lipid Res,* 1978, **19,** 65.
11. Schoenheimer R and Sperry N M, A micromethod for the determination of free and combined cholesterol, *J biol Chem,* 1934, **106,** 745.
12. Sperry W M and Webb M, A revision of the Schoenheimer Sperry method for cholesterol determination, *J biol Chem,* 1950, **187,** 97.
13. Krauss R W, Windmuller M G, Levy R I and Frederickson D S, Selective measurement of two different triglyceride lipase activities in rat post heparin plasma, *J Lipid Res,* 1973, **14,** 286.
14. Bennet C A and Franklin N L, *Statistical Analysis in Chemistry and the Chemical Industry,* John Wiley and Sons, Inc., New York 1967.
15. Usha V, Vijayamma P L and Kurup P A, Effect of dietary fibre from banana (*Musa Paradisiaca*) on cholesterol metabolism, *Indian J Exp Biol.* 1984, **22,** 550.
16. Redgrave T G, Formation of cholesterol ester rich Particulate lipid during metabolism of chylomicrons *J Clin Invest,* 1970 **49,** 465.
17. Eisenberg S and Rachmilewitz D, Metabolism of rat plasma very low density lipoprotein, *Biochem Biophys Acta,* 1973, **326,** 378.
18. Eisenberg S and Rachmilewitz D, Metabolism of rat plasma very low density Lipoprotein II. Fate in circulation of apoprotein subunits, *Biochim Biophys Acta,* 1973, **326,** 391.
19. Faergeman O, Sata T, Kane J P and Havel R J, Metabolism of Apoprotein B of Plasma very low density lipoproteins in the rat, *J Clin Invest.* 1975, **56,** 1396.
20. Windler E, Chao Y S and Havel R J, Determinants of hepatic uptake of triglyceride rich lipoprotein and their remnants in the rat. *J Bio Chem,* 1980, **255,** 5475.
21. Stein O, Rachmilewitz D, Sanger L, Eisenberg S and Stein Y, Metabolism of iodinated very low density lipoprotein in the rat, Autoradiographic localization in the liver, *Biochem Biophys Acta,* 1974, **360,** 205.
22. Oschry Y O and Risenberg S, Rat Plasma lipoproteins, re-evaluation of a lipoprotein system in an animal of a lipoprotein system in an animal devoid of cholesteryl ester transfer activity, *J Lipid Res,* 1982, **23,** 1099.
23. Sniderman A, Thomas D, Manpole D and Teng, Low density lipoprotein. A metabolic pathway for the return of cholesterol to the splanchnic bed, *J Clin Invest,* 1978, **61,** 867.
24. Tall A R and Small D M, Body cholesterol removal. Role of plasma high density lipoproteins, *Adv Lipid Res,* 1980, **17,** 1.

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EFFECT OF MAGNESIUM AND MANGANESE IONS ON THE GROWTH OF *LACTOBACILLUS ACIDOPHILUS*

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All the tested strains of *Lactobacillus acidophilus* exhibited higher viable counts, greater acid production and shorter generation time when milk was supplemented with 19.72 Mg^{++}/l or 11.39 mg Mn^{++}/l as compared to the control. Supplementation of milk with both magnesium and manganese had no appreciable effect on these attributes compared to that observed for each individually.

Consumption of acidophilus milk products is considered beneficial in combating intestinal disorders[1], normalising intestinal microflora altered due to antibiotic therapy[2] and maintaining body vigour and vitality[3]. *L. acidophilus* has also been reported to possess anti-carcinogenic[4] and anticholesteremic[5] activity. However, *Lactobacillus acidophilus* grows poorly in milk and therefore, acceptability of such products is poor. Attempts made to prepare acceptable acidophilus products by adding certain nutrients to milk[6] or incorporating other lactic organisms with *L acidophilus* have met with limited success. Agrawal *et al*[7]. reported that addition of sucrose to milk enhanced acid production by *L. acidophilus*. However, the increase in acid production was not sufficient to make a satisfactory product. Lactobacilli require both magnesium and manganese ions for their growth. Therefore, the present investigation was conducted to study the effect of magnesium and manganese ions individually and in combination on the growth of *L. acidophilus* strains in milk.

Fresh skim milk obtained from Livestock Research Center of the University was used in this study. *Lactobacillus acidophilus* NRRL-B-629, NRRL-4239 obtained from Northern Regional Research Laboratory, Peoria, Illinois (USA) and *L. acidophilus*-Russian, Hansen, and 111 ML obtained from National Dairy Research Institute, Karnal (India) were used in this study. The concentrations of the minerals used were: magnesium 9.86, 19.72, and 39.45 mg ion/l and manganese 11.39 and 22.78 mg ion/l. The milk after supplementation with the minerals individually and in combination was sterilized at 121°C for 15 min. Two hundred ml of the experimental medium was inoculated with 16-18 hr test culture at the rate of 1 per cent and incubated at 37°C. Viable counts were determined according to APHA[8] procedure using lactic agar[9]

Titratable acidity was determined according to APHA[8] procedure. Eighteen grams of the sample was titrated with 0.1 N NaOH using phenolphthlein as indicator. Generation time was determined according to Stanier *et al.*[10]

The effect of magnesium and manganese ion supplementation individually and in combination to milk on viable count, titratable acidity, pH and generation time by *L. acidophilus* strains is presented in Table 1. All the tested strains of *L. acidophilus* exhibited greater acid production at 19.72 than at 9.86 mg Mg ion/l. At this concentration, the acid production by different strains ranged from 0.54 to 1.69 per cent as compared to 0.40-1.30 per cent in the control at the end of 16 hr. No further increase in acid production was observed upon increasing the magnesium level to 39.45 mg ion/l. Among the cultures tested *L. acidophilus* Russian showed maximum acid production of 1.63 per cent. The data on changes in pH values are in agreement with titratable acidity values. All the cultures tested exhibited higher viable counts in magnesium added milk than in the control. Magnesium addition resulted in about 5 fold increase in the population of NRRL-B-629 strain as compared to 2-4 folds increase in other strains.

Addition of manganese resulted in greater acid production by all the strains of *L. acidophilus* tested. However, acid production was more or less the same at both 11.39 and 22.78 mg Mn/l tested. Among the strains tested, *L. acidophilus*-Russian showed maximum acid production (1.45 per cent) (Table 1). The changes in pH were more or less in conformity with acid production. The population of NRRL-4239, Hansen and 111 ML strains increased by about 4-6 folds whereas other strains showed slight increase upon addition of 11.39 mg Mn/l than in the control (Table 1).

Addition of magnesium at 19.72 mg ion/l or manganese at 11.39 mg ion/l to milk substantially decreased generation time of different strains. The

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TABLE 1. EFFECT OF MAGNESIUM AND MANGANESE IONS ON VIABLE COUNT, TITRATABLE ACIDITY (%TA), pH AND GENERATION TIME OF DIFFERENT *LACTOBACILLUS ACIDOPHILUS* STRAINS

Strains	Milk (control)				Milk + Magnesium				Milk + Manganese			
	Viable count/ml	%TA	pH	Generation time (min)	Viable count/ml	%TA	pH	Generation time (min)	Viable count/ml	%TA	pH	Generation time (min)
4239	2.4×10^8	0.62	5.20	158.4	1.0×10^9	0.84	4.60	111.6	1.6×10^9	0.92	4.55	97.8
629	2.1×10^8	0.93	4.60	172.7	1.1×10^9	1.21	4.20	95.5	3.0×10^8	0.99	4.40	151.1
Russian	1.3×10^9	1.30	3.95	153.0	2.8×10^9	1.69	3.65	111.5	1.7×10^9	1.45	3.80	124.0
Hansen	1.7×10^8	0.62	5.05	181.8	5.8×10^8	0.68	4.75	116.4	7.6×10^8	0.66	4.60	107.4
111 ML	3.2×10^6	0.40	5.45	186.7	1.1×10^7	0.54	5.00	144.2	2.1×10^7	0.56	4.95	133.3

Average of two determinations
The initial % TA and pH values for milk were 0.24-0.25 and 6.15-6.20, respectively
Minerals added to milk were: Magnesium 19.72 mg ion/l and manganese 11.39 mg ion/l.
Values determined at the end of 16 hr.

ranges of generation time for different strains were 95.5 - 144.2 min in magnesium added milk and 97.8 - 151.1 min in manganese added milk as compared to 153.0 - 186.7 min in the control.

Supplementation of milk with combination of magnesium (19.72 mg ion/l) and manganese (11.3 mg ion/l) showed no appreciable effect on different growth parameters of the strains tested.

The requirements of magnesium and manganese by *L. acidophilus* are well established[11]. Using synthetic medium, Keogh[12] found that *L. acidophilus* required minimum of 20 mg Mg/l for its growth while milk provided 1.2 mg Mg/l[13] . Thus, total availability of magnesium upon supplementation of milk with 19.72 mg ion/l milk works out to be approximately same as observed by Keogh[12] for maximum activity of *L. acidophilus*.

Grebus *et al.*[14] reported optimum requirement of manganese ion at 14.5 mg/l level for the growth of *L. acidophilus*. Milk provides 0.02-0.03 mg Mn/l[13] which is much less than the optimum level required. In the present investigation addition of Mn^{++} at 11.39 mg/l to milk was found optimum for activity of *L. acidophilus*. Further increasing the level of manganese to 22.7 mg/l was without any effect. Thus, the total available manganese upon addition of 11.39 mg ion/l is approximately the same as observed by Grebus *et al.*[14] for maximum activity of *L. acidophilus*.

The results of this investigation showed that supplementation of magnesium or manganese ions to milk enhanced acid production, increased population, and appreciably reduced generation time and pH by *L. acidophilus* strains tested. Thus, addition of either of these minerals could help in preparation of an acceptable acidophilus milk product.

References

1. Shahani K M and Chandan R C, Nutritional and healthful aspects of cultured and culture containing dairy products, *J Dairy Sci*, 1979, **62**, 1685.
2. Laxminarayana H and Shankar P A, Fermented milk in human nutrition, *Indian Dairym*, 1980, **32**, 121.
3. Speck M L, Preparations of lactobacilli for dietary uses, *J Fd Protect*, 1980, **42**, 65.
4. Goldin B R and Gorbach S L, The effect of milk and *Lactobacillus acidophilus* feeding on human intestinal bacterial enzyme activity, *Am J clin Nutr*, 1984, **39**, 756.
5. Gilliland S E, Nelson C R and Maxwell C, Assimilation of cholesterol by *Lactobacillus acidophilus*, *Appl environ Microbiol*. 1985, **49**, 377.
6. Vedamuthu E R, Cultures for butter milk, sour cream and yoghurt with special comments on acidophilus yoghurt, *Cult Dairy Prod J*. 1974, **9**, 16.
7. Agrawal V. Usha M S and Mital B K. Preparation and evaluation of acidophilus milk, *Asian J Dairy Res*, 1986, **5**, 33.
8. *Standard Methods for the Examination of Dairy Products*, American Health Association, New York, 1972, 13th Edn.
9. Elliker P R, Anderson A W and Hannesson G, An agar culture medium for lactic acid streptococci and lactobacilli, *J Dairy Sci*, 1956, **39**, 1611.
10. Stanier R Y, Ingraham A W, Wheelis M L and Painter P R, *General Microbiology*, Mac Millan Education Ltd, London, 1987, 5*th edn*, 183.
11. Sabine D B and Vaselekos J, Trace element requirement of *Lactobacillus acidophilus*, *Nature*, 1967, **214**, 520.
12. Keogh B P, Nutritional deficiencies of co-precipitate for the growth of a strain of *Lactobacillus acidophilus*, *J Dairy Res*, 1970, **37**, 461.
13. Webb B H, Johnson A and Alford J A, *Fundamentals of Dairy Chemistry*, The AVI Publ Comp, Westport, Connecticut, 1974, p.22.
14. Grebus C H, Mas J C and Cremieax A, Effect of various nutritive factors on the growth of *Lactobacillus acidophilus*, *Bull Assoc Dipl Microbiol, Nancy*, 1967, **(105)** 23, (Dairy Sci Abst, 1967, **29**, 2758).

THIN LAYER CHROMATOGRAPHIC DETECTION OF KOKUM BUTTER IN COCOA-BUTTER

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A fairly simple thin layer chromatographic method has been developed to detect kokum-butter in cocoa-butter. Kokum-butter appeared as bluish green spot under U.V. light at Rf 0.5 - 0.6 both, when present alone or in the mixture with cocoa-butter, while the latter did not show up as a spot. The method permits the detection of kokum-butter upto 5% when mixed with cocoa butter.

Kokum-butter[1], a fat obtained from the seeds of kokum-tree (*Garcinia-indica*) is much similar to cocoa-butter[2] in physico-chemical properties. It is used as a cocoa-butter substitute in the manufacture of chocolates, etc. However, the present Indian Standards[3] for cocoa-butter do not permit the presence of kokum-butter/fat in cocoa-butter. The present work was undertaken to develop a simple method for detecting kokum-butter in cocoa-butter using thin layer chromatographic technique.

Pure samples of cocoa-butter and kokum-fat were procured from the producing centres at Bombay. The desired mixtures of 5,7 and 10 per cent of kokum fat in cocoa-butter were prepared as model mixtures.

A 0.25 mm thick silica-gel-G layer was coated on 10 x 20 cm glass plates and dried at room temperature for 30 min. The plates were then activated at 40°C for half an hour and stored in desicator till use. One gram of pure cocoa-butter, 5,7 and 10 per cent mixtures were dissolved separately in 15 ml chloroform. Twentyfive μl of pure cocoa-butter and the three mixtures were spotted on TLC plates 2 cm above the base and 1 cm apart, and developed with benzene : ethylacetate : acetic acid (96 + 4 +)[4]. The plate was then dried in air and observed under ultra-violet lamp (long wave) of 366 nm.

Under UV lamp, the bluish-green curved spots were observed at Rf 0.5 – 0.6 in case of pure kokum fat and the mixtures containing it, but absent in pure cocoa-butter. It was also observed that the Rf. of the spots varied with the variation in ethyl-acetate-acetic acid percentage. The bluish-green fluroscence was very bright with 7 per cent mixture but slightly faint in 5 per cent mixtures, and the method appears to be quite sensitive. Other fats like mango-kernel, Mahua[5] could not be detected by this method.

Various other solvents were tried for this purpose. However, the present solvent system of benzene : ethyl-acetate : acetic acid in the ratio 96:4:1 proved to be more effective.

References

1. The Wealth of India-Raw Materials, Council of Scientific and Industrial Research, New Delhi, 1956, Vol. IV, 102.
2. The Wealth of India – Raw Materials, Council of Scientific and Industrial Research, New-Delhi, 1976, Vol. X, 217.
3. *Prevention of Food Adulteration Rules* 1984, Govt. of India, Ministry of Health Appendix B, Item – A. 10.05, 89.
4. Stahl E, *Thin Layer Chromatography*, George Allen and Lenwin Ltd., London, 1969, 311.
5. The Wealth of India – Raw Materials, Council of Scientific and Industrial Research, New-Delhi, 1962, Vol. VI, 211 and P. 281 – 282.

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SOME ENZYMES AND ENZYME INHIBITORS FROM CHAROLI AND CASHEW NUT

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Charoli (*Buchanania lanzan*) and cashew nut (*Anacardium occidentale*) were analysed for enzymes and enzyme inhibitors. Both the seeds showed low amylase and urease activities. Charoli possessed active lipase while cashewnut exhibited low profile for both lipase and lipoxygenase. Both these seeds showed very low levels of amylase- and trypsin inhibitors. Roasting of the kernels reduced the enzyme activities drastically, however, the effect on inhibitors was not as severe as on enzymes.

Charoli (*Buchanania lanzan*) and cashew nut (*Anacardium-occidentale*) belong to the family *Anacardiaceae*. The kernels are rich in fat and proteins and hence considered to be highly nutritious[1]. They exhibit pleasant nutty taste and are consumed as such or used in milk based beverages and confections. Though much is known about cashew nut and charoli fat, very little information is available on proteins and enzymes present in these seeds. The object of present study was to survey the enzymes that may influence storage stability and acceptability characteristics and enzyme inhibitors likely to affect nutritive value of the kernels.

The kernels of charoli and cashew nut, purchased from local market, were defatted by Soxhlet extraction using petroleum ether (40°-60°C). Residue left behind after extraction was made free of solvent by drying under vacuum at 25 mm Hg and 30°C. It was then ground to an average particle size of 100 microns.

For enzyme extraction, 30 g of defatted flour was extracted twice with 100 ml of distilled water at 20°C for 60 min each. Final volume was made to 250 ml. This was then centrifuged at 5,000 r.p.m. to get clear enzyme extract. Two ml aliquot of this extract was used every time as an enzyme source to determine activities of amylase[2], urease[3] and lipase[4]; using starch, urea and olive oil respectively as substrates. All activities were expressed in terms of μ moles of respective products liberated per minute.

Lipoxygenase was assayed using the procedure of Nicholas, *et.al.*[5] and activity was determined using 0.1 ml of enzyme extract (20 g defatted flour extracted with 100 ml 0.1 M Tris buffer, pH 7.2 at 20°C for 6 hr) and linoleic acid as substrate. Activity was expressed as a unit of activity increasing the absorbance by 0.01 per min at 234 nm.

For trypsin inhibitor, 10 g defatted flour was extracted using water or dilute alkali (pH 7.6) or 0.05 N-hydrochloric acid for 3 hr. Final volume was made to 100 ml. Trypsin inhibitor activity was determined as per Kakade, *et al.*[6] using 1 ml of each extract and benzoyl-arginine-p-nitroanilide (BAPNA) as substrate.

Amylase inhibitor activity of the aqueous extracts of defatted flour was determined using Fungamyl (NOVO Inc, Denmark) as a standard enzyme and starch as substrate. Activity was expressed as decrease in absorbance by 0.01 units at 540 nm. Effect of pH and temperature of incubation on amylase inhibitor activity was observed.

Activities of different enzymes in charoli and cashew-kernels are listed in Table 1. Charoli exhibited higher enzyme activities than cashew nut. The step of roasting used for cracking of shell in commercial decortication of cashew nuts may cause inactivation of many enzymes and enyme inhibitors present in cashew kernels. This is clearly observed from decreased enzyme activities for roasted kernels. (Table 1).

Charoli exhibited higher amylase activity than cashew-kernels. Both the seeds exhibited low urease activity as compared to soyabean[7]. Charoli showed significant lipase and lipoxygenase activity than cashew nut. Lower levels of these two enzymes in cashew nut are reflected in its comparatively longer shelf life.

Table 2 gives trypsin and amylase inhibitor activities of these seed kernels. Highest trypsin inhibitor activity was obtained in acid extract indicating maximum extraction of trypsin inhibitor under acidic conditions. As compared to other oil seeds, like soyabean[8], the trypsin inhibitor activity of these seeds is quite low. Inactivation of trypsin inhibitor due to heating is reflected in lower trypsin inhibitor activity in roasted cashew nut.

Maximum amylase inhibitor activity was obtained at pH 4.9 and 50°C. Variation in amylase activity with

TABLE 1. ACTIVITIES (UNITS PER Mg PROTEINS) OF SOME ENZYMES IN CHAROLI AND CASHEW

Enzyme	Charoli	Cashew kernels	
		Unroasted	Roasted
Amylose	0.064	0.018	0.018
Urease	0.019	0.017	0.002
Lipase	0.033	0.009	trace
Lipoxygenase	10.900	1.130	0.007

Results are expressed as mean of three experiments.

TABLE 2. TRYPSIN - AND AMYLASE - INHIBITOR ACTIVITY (UNITS PER Mg PROTEIN) OF CHAROLI AND CASHEW

Extractant Incubation conditions	Charoli	Cashew kernels	
		Unroasted	Roasted
Trypsin Inhibitor			
Dilute alkali (pH 7.6)	1.20	1.49	0.70
0.05 N HCl	1.48	1.91	0.90
Water	1.18	1.45	0.64
Amylase Inhibitor			
pH 6.0, 37°C	0.075	0.154	0.022
pH 9.0, 50°C	0.219	0.278	0.105

Results shown are mean of three experiments.

change in pH and temperature of incubation and total loss of this inhibitor activity on autoclaving at 15 psi for 15 min suggested proteinaceous nature of amylase inhibitor.

References

1. Gopalar C, Ramasastri R V and Balasubramanian S C, *Nutritive Value of Indian Foods*, National Institute of Nutrition Hyderabad, 1987, 82.
2. Swain R R and Dekker E E, Seed germination studies. I. Purification and properties of α-amylase from the cotyledons of germinating seeds, *Biochem Biophys Acta*, 1966, **172,** 75.
3. Hoffman E, Urease, *In Methods of Enzymatic Analysis*, by Bergmeyer, H U (Ed), Academic Press Inc., New York, 1963, 913.
4. Marchis - Mouron G, Sarda L and Densuelle P, Purification of hog pancreatic lipase, *Arch Biochem Biophys*, 1959, **83,** 309.
5. Nicholas J, Autram M and Drapron R, Purification and some properties of wheat germ lipoxygenase, *J Sci Fd Agric*, 1982, **33,** 365.
6. Kakade M L, Simons L and Liener I E, Evaluation of natural versus synthetic substrates for measuring the antitryptic activity of soyabean samples, *Cereal Chem*, 1969, **46,** 518.
7. Damodaran M and Sivaramakrishnan P M, New sources of urea, *Biochem J*, 1937, **31,** 1041.
8. Kakade M L, Simons N R, Liener I E and Lambert J W, Biochemical and nutritional assessment of different varieties of soyabeans, *J agric Fd Chem*, 1972, **20,** 87.

GREEN COLOUR IN PROCESSED OILS OF BLACK SOYBEAN

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Alkali-neutralized oils from black soybean develop a deep green to bright green colouration on contact with metals like nickel, iron, zinc and copper. On bleaching with activated earth and activated carbon, the colour gets decolourised.

A variety of soybean with black seed coat (locally called *Kalitur*) is being grown in Madhya Pradesh, India. Development of green colouration during hydrogenation of alkali-neutralized oils from this variety of soybean was observed during our investigations. As published data on this phenomenon are not available and as black seed-coat soybean is not processed outside India, preliminary work was undertaken to devise methods to remove the green colour in the hydrogenated soybean oil. The technique is of practical importance to oil hydrogenation factories processing oils from black seed-coat soybean.

The green colour was distinct, bright and persistent. Taking clue from the presence of metallic nickel in the catalytic hydrogenation, trials were conducted with certain other metals such as iron, zinc and copper and it was observed that they also gave rise to the same green colouration. The development of green colour in the oil was slow at the ambient temperature and rapid at elevated temperature. Alkali-neutralized oil from black soybean gave a bright green colour with a speck of metal on heating. However, the corresponding raw soybean oil did not respond to the above treatment. It has been checked that either raw oils or alkali neutralised oils derived from conventional yellow soybean (other than black soybean) do not show this colour phenomenon. The green colour disappears on bleaching with activated earth and activated carbon.

The green colour might be due to the interaction of porphyrin ring system present in the oil and the heavy metals like iron, nickel, copper and zinc resulting in the formation of colour complex. Coloured complexes are broken down on adsorption of metal moiety during earth-carbon treatment. A typical experiment is as follows: 10 ml of soap-free (washed) refined and bleached soybean oil, yellow in colour from black soybean was taken in a test tube, a speck of the metal was added to the oil, shaken well and heated to 180°C. Green colour resulted. The oil was cooled and calculated amount of activated earth (2 per cent) and active carbon (0.2 per cent) was added to the oil, shaken and rapidly heated to 120°C. The green colour in the oil dissappeared and the original yellow colour resulted. The same procedure applies to the final hydrogenated oil.

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STEROL PROFILE OF THE SEED OILS OF A FEW MEMBERS OF COMPOSITAE FAMILY

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Sterol fractions isolated from the seed oils of niger (*Guizotia abyssynica*), safflower (*Carthamus tinctorius*) and sunflower (*Helianthus annus*) belonging to the compositae family have been characterised. These are qualitatively similar but show quantitative differences. This is interesting since a chemical profile corresponds to morphological taxonomy. The sterol profile can be used for distinguishing the oils and detection of mutual adulteration, as for example niger seed oil in mustard oil. Stigmasterol is present in niger seed oil but only traces in mustard oil; brassicasterol is absent in niger seed oil but present in the other. Storage did not alter the sterol profile unlike that of the fatty acids.

Oils require discrete identity for detection of adulteration in them. The physico-chemical characteristics and different qualitative identity tests serve the purpose to the extent possible the sterol profile. The present communication describes examination of a family (with three members) for the sterol profile both at the levels of the family and the individual.

A few samples of the seeds of each variety were collected: Niger seed, Variety-I, from Sargana Dist, Nasik, Maharastra and Variety-II from Dabhadi, Gujarat; Safflower seed, Variety-'Manjira', from Agriculture Research Institute, Hyderabad and Variety-'Tara,' from Agriculture Research Institute, Jalgaon, Sunflower seed, Variety-'EC 68414', from National Seed Corporation, Calcutta.

Two hundred grams of each seed were extracted with 500 ml n-hexane twice and the unsaponifiable matter was prepared by the method of Sengupta *et al.*[1] and separated into four fractions by TLC[2] in order of decreasing polarity, (a) sterols, (b) 4-methyl sterols, (c) triterpene alcohol and (d) less polar compounds. The zones corresponding to the above 4 sterols were identified by running standards. The compounds from the 4 zones scraped quantitatively were eluted with ether. The residues after evaporation of ether under vacuum were stored in a desiccator for GLC analysis. The gas liquid chromatograph (model: Pyeunicam GCV) with column containing 3 per cent OV-17 on Gaschrom-80-100 mesh and a flame ionisation detector was used. Nitrogen was the carrier gas with a flow rate of 40 ml per min. The temperatures of the column, detector and injection port were 240°C, 280°C and 260°C, respectively. The peaks obtained were identified by comparing retention time with those of standard sterols[3,4].

The four fractions of the unsaponfiable component have been shown in Table 1 and percentage sterols in oils in Table 2. Oils are identified by fatty acids, members of unsaponifiable fractions and in certain cases by specific colour reactions. The oils studied here except mustard oil do not lend themselves to easy identification, particularly detection of adulteration. Adulteration by niger seed oil is a problem since it is a cheap oil and not preferred as an edible oil. Modified solstein test[5] and TLC[6] have been used for detection of niger seed oil in sesame oil with varying success. It appears that the sterol profile can be used for identification and detection of adulteration by, these oils to certain extents.

Campesterol, stigmasterol, β-sitosterol, Δ^7-stigmasterol and an unidentified minor sterol are qualitatively comparable (though varying quantitatively) in niger, safflower and sunflower oils. This is interesting since these members of the same family (based on morphological Taxonomy) have comparable profile of a class of chemical constituents, the sterols. However, mustard oil has some difference by having brassicasterol and near absence of stigmasterol. Hence, adulteration of mustard oil with these oils particularly niger oil will bring in stigmasterol, and depress brassicasterol is the flagmark of mustard oil if mixed with others. It is possible that the quantity of the respective sterols as in Table 2 may in some cases be useful to supplement or confirm findings of admixture by other tests. The present findings on sterols are comparable, though not exactly similar, with those of Itoh *et al.*[4,7] Sterols from a sample of nigerseed oil kept in the refrigerator for one year were gas-chromatographed; the peaks were almost the same as those of the fresh oil done earlier.

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TABLE 1. UNSAPONIFIABLE MATTER IN SEED OILS

Content	Niger seed oil		Safflower seed oil		Sunflower seed oil
	Var. I	Var. II	Manjira	Tara	
Unsaponifiable matter (% of oil)	1.09	0.78	1.10	0.85	1.11
Fractions (% of unsaponifiable)					
Fraction-I	16.29	16.96	20.05	22.37	17.46
Fraction-II	7.36	8.16	7.62	11.64	12.48
Fraction-III	4.05	6.25	8.17	5.05	15.29
Fraction-IV	69.90	63.90	52.36	56.00	54.75

TABLE 2. STEROL FRACTIONS IN DIFFERENT SEED OILS

Seed Oils	Brassicasterol	Campesterol	Stigmasterol	β – Sitosterol	Δ^7-Stigmasterol	Un-identified
Niger seed oil (variety-I)	–	17.94	20.51	48.69	9.46	3.38
Niger seed oil (variety-II)	–	15.78	20.52	52.82	9.90	0.95
Safflower seed oil (Manjira)	–	9.47	9.47	40.21	36.49	4.35
Safflower seed oil (Tara)	–	10.05	10.05	40.81	28.08	3.99
Sunflower seed oil	–	12.75	16.40	57.02	13.80	–
Mustard oil	10.68	45.09	–	44.21	–	Trace

References

1. Sengupta P, Sil S and Roy B R, Sterols in tobacco seed oil, *J Oil Technol Ass India*, 1982, **14**, 59.
2. Itoh T, Tamura T and Mutsumoto T, Sterols, methylsterols and triterpene alcohols in three theaccae and some other vegetable oils, *Lipids*, 1974, **9**, 173.
3. Paquot C, *Identification and determination of sterols by Gas-Liquid chromatography*. I.U.P.A.C., Pergamon Press, Oxford, 6th Edn, 129.
4. Itoh T, Tamura T and Mutsumoto T, Sterol composition of 19 vegetable oils, *J Am Oil chem Soc*, 1973, **50**, 122.
5. Arnold W, *Analyst*, 1914, **39**, 86.
6. Pantula PC, Ramamurty R, Bhimsen Rao, Thin layer chromatographic methods for the detection of nigerseed oil in sesame oil. *J Oil Technol Ass India*, 1974, **6**, 23.
7. Itoh T, Tamura T and Mutsumoto T, Methyl sterol composition of 19 vegetable oils, *J Am Oil chem soc*, 1973, **50**, 300.

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PREDICTIVE ANALYSIS OF THE PROTEIN QUALITY OF *PLEUROTUS CITRINOPILEATUS*

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Mushroom, like other proteinaceous foods, bears a non-essential fraction of protein which does not come into use in biological system. *Pleurotus citrinopileatus*, was analysed for amino acid composition to determine its protein quality as compared to whole egg and FAO reference protein. An assessment of protein quality standards (EAA- Index, Amino Acid Score, Biological Value and Nutritional Index) indicated good nutritional significance of the test mushroom. Also, the protein quality standard was compared with *P. sajor-caju* and other food stuffs and its position was assigned in the chronological order of nutritional status.

In the absence of properly designed biological method of feeding trial, there has been considerable difficulty to evaluate the standard of a mushroom protein. As the legible alternative, several biochemical methods have been advanced to make a predictive analysis based on the essential amino acid composition[1,2]. It is well documented that the standard of a proteinaceous food depends on its amino acid composition in relation to the protein content and digestibility. Amino acid composition may also serve as a good relative measure to compare mushroom with other foodstuffs of established nutritive value. Based on this principle, Oser[3,4] proposed Essential Amino Acid Index to rate the dietary protein in terms of highly nutritive reference protein. Bano and co-workers[5] calculated the protein quality and nutritive value of several species of *Pleurotus*. A comprehensive study on the nutritive value of Saudi Arabian truffles was conducted[6] and their in vitro digestibility was found to run between 82.8 - 86.7 per cent. With a view to determine the nutritive value in terms of protein quality of *P. citrinopileatus*[7], quantitative data of amino acid composition were analysed and compared with those of traditional oyster mushroom, *P. sajor-caju*.

Fruit bodies of the mushroom fungi were collected immediately before spore maturity, dried (105°C) to constant weight and ground through 60 mesh sieve, before employing for chemical analysis. Amino acid contents of the mushroom were analysed using Technicon Sequential multipurpose Amino Acid Analyser following the modified method[8] of Moore and Stein[9].

Essential Amino Acid Index is the ratio of essential amino acids contained in a food to the essential amino acid content in reference protein. In the present investigation, EAA calculations were done following the method as advanced by Oser[3,4]. Amino Acid Score, also called Chemical Score, is considered as second alternative to the animal feeding studies for the determination of nutritional value. It is based on the amount of limiting amino acid present in the test protein in relation to its presence in reference protein. Amino Acid Score was calculated using the following formula[2]:

$$\text{Amino Acid Score (AAS)} = \frac{\text{mg amino acid/gm test protein}}{\text{mg amino acid/gm reference protein}} \times 100$$

Biological Value is the calculated measure which helps to predict the nitrogen retention by the feeding organisms and thereby indicates the utilizable fraction of a protein food. Based on the values of EAA-Index, Biological Values were calculated following the regression equation used by Oser[4].

$$\text{Biological Value (BV)} = 1.09\ (\text{EAA} - \text{Index}) - 11.7$$

Qualitative as well as quantitative variations of protein values in different mushroom species tendered difficulty to compare their nutritional status. To overcome this problem, Crisan and Sands[10] advanced an equation considering all the factors with equal importance.

$$\text{Nutritional Index (NI)} = \frac{\text{EAA-Index} \times \%\ \text{protein}}{100}$$

Quantitative data of amino acid profiles of the mushrooms in crude protein nitrogen are presented in Table 1. Essential amino acid contents in *P. sajor-caju* were marginally exceeded over *P. citrinopileatus* except phenylalanine and threonine. While phenylalanine content (460 mg/g) in the latter species was remarkably higher than the former (363 mg/g), threonine contents were almost equal.

Amino Acid Score and sequences of limiting amino acids (Table 2) indicated sulphur containing amino acids to be the most limiting amino acids in both the fungi when whole egg was reference protein. Amino Acid Score in *P. citrinopileatus* and *P. sajor-caju* were 46 and 53, respectively with the sequence of limiting amino acids as Sc (Sulphur containing amino acids), Ileu (Isoleucine) and Val (Valine) in the former species, while, Sc, Ileu and Leu (Leucine) was the sequence in

TABLE 1 AMINO ACID COMPOSITION OF *P. CITRINOPILEATUS* AND *P. SAJOR-CAJU*

Amino acids	Concn of amino acids (mg/g corrected crude protein N)	
	P. citrinopileatus	*P. sajor-caju*
Isoleucine	184	204
Leucine	334	349
Lysine	319	335
Methionine	100	119
Cystine	65	71
Phenylalanine	460	363
Tyrosine	178	208
Threonine	286	282
Tryptophan	nd*	nd
Valine	239	278
Arginine	300	303
Histidine	126	120
Alanine	390	366
Aspartic acid	456	617
Glutamic acid	1113	1231
Glycine	275	268
Proline	249	299
Serine	267	310
Nitrogen content (D.W.)	3.85%	4.05%

*nd not determined.

latter species. But, based on FAO reference pattern, Ileu and Leu became the limiting amino acids in the two species with Amino Acid Score of 74 and 79, respectively. The sequence of limiting amino acids also changed to Ileu, Sc and Val in *P. citrinopileatus*, Leu, Ileu and Sc in *P. sajor-caju*.

EAA-Index of *P. citrinopileatus* based on whole egg and FAO reference pattern were 68.41 and 92.55, respectively compared to the slightly higher values of 72.04 and 97.50 in *P. sajor-caju*. From the calculated values, it seemed pertinent to mention similar dietary standard of protein in both the species of mushrooms. Biological Value (BV) and Nutritional Index (NI) were tabulated (Table 3) along with the values of EAA- Index and Amino Acid Score to evaluate and compare the nutritive value of the mushroom species. Predicted values of all the parameters in the two species of mushroom varied close to each other indicating a good correlation from nutritional point of view.

Protein is the most critical component contributing to the nutritional value of a protein rich food item like mushroom. The determination of its true nutritive value in relation to protein is a complex task because it contains a significant amount of non-protein nitrogen in the chitinous cell wall which interferes during the

TABLE 2. AMINO ACID SCORE AND SEQUENCE OF LIMITING AMINO ACIDS IN *P. CITRINOPILEATUS* AND *P. SAJOR-CAJU*

Amino acids	Amino Acid Score			
	P. citrinopileatus		*P. sajor-caju*	
	Egg	FAO	Egg	FAO
Isoleucine	54.12	73.60	60.00	81.60
Leucine	61.85	75.91	64.63	79.32
Lysine	72.50	93.82	76.14	98.53
Methionine + Cystine	45.83	75.00	52.78	86.36
Phenylalanine + Tyrosine	100.00	167.89	98.45	150.26
Threonine	98.62	114.40	97.24	112.80
Tryptophan	–	–	–	–
Valine	58.29	77.10	67.80	89.68
Limiting amino acids sequence	Sc, Ileu, Val	Ileu, Sc, Leu	Sc, Ileu, Leu	Leu, Ileu, Sc

TABLE 3. ESTIMATED NUTRITIVE VALUE OF *P. CITRINOPILEATUS* AND *P. SAJOR-CAJU*

Mushroom	Essential amino acid (EAA)-index		Biological value (BV)		Amino acid score (AAS)		Nutritional index (NI)	
	EAA Index Egg	EAA Index FAO	BV Egg	BV FAO	AAS Egg	AAS FAO	NI Egg	NI FAO
P. citrinopileatus	68.41	92.55	62.87	89.18	46	74	11.53	15.60
P. sajor-caju	72.04	97.50	66.82	94.57	53	79	12.78	17.30

TABLE 4. STATUS OF OYSTER MUSHROOMS AMONG OTHER FOOD STUFFS

Essential Amino Acid Index	Amino Acid Score	Nutritional index
100 Pork, Chicken Beef	100 Pork	59 Chicken
99 Milk	98 Chicken, Beef	43 Beef
98 *P. sajor-caju*	91 Milk	35 Pork
91 Potato, Kidney bean	79 *P. sajor-caju*	31 Soybean
89 *P. citrinopileatus*	74 *p. citrinopileatus*	26 Spinach
88 Corn	63 Cabbage	25 Milk
86 Cucumber	59 Potato	21 Kidney bean
79 Peanut	53 Peanut	20 Peanut
76 Spinach	50 Corn	17 *P. sajor-caju*
69 Turnip	46 Kidney bean	17 Cabbage
44 Tomato	42 Cucumber	16 *P. citrinopileatus*
	33 Turnip	14 Cucumber
	31 Carrot	11 Corn
	28 Spinach	10 Turnip
	23 Soybean	9 Potato
	18 Tomato	6 Carrot

estimation of protein. In the absence of sufficiently critical mechanical device or biological feeding studies, most reliable measure presently available for determining the nutritive value of mushroom protein appears to be their amino acid content, especially the essential amino acid composition. Analytical data show that, all the essential amino acids required for human nutrition are present in adequate quantities in *P. citrinopileatus* and thus ensure its standard of higher protein quality.

Nutritional standards (EAA-Index, AAS, BV and NI) were always on the higher side, indicating good nutritional significance of the test mushrooms. They were also compared well with the calculated nutritional standards of other foodstuffs against FAO reference pattern[10] and its position was assigned in the chronological order of nutritional status (Table 4). Present study clearly revealed that the sporophores of *P. citrinopileatus* though marginally inferior to *P. sajor-caju*, formed a good source of protein having proportionately good amino acid composition to make it a nutritionally rich food.

References

1. *Amino acid contents of Food and Biological Data on Proteins*, Nutr. Stud. No.24, Food Policy and Food Sci. Serv. Nutr. Div., Food Agric. Organ, UN, Rome, 1970.
2. *Energy and Protein Requirements*, Report of a Joint FAO/WHO ad hoc Expert Committee, Food Nutr. Meet. Rep., Ser. No.52, Food agric. Organ, UN, Rome, 1973.
3. Oser B L, Method for integrating essential amino acid content in the nutritional evaluation of protein, *J Am diet Ass*, 1951, **27**, 396.
4. Oser B L, An integrated essential amino acid index for predicting the biological value of proteins, in *Protein and Amino Acid Nutrition* by Albanese AA, (Ed) Academic Press, New York, 1959, 281.
5. Bano Z, Bhagya S and Srinivasan K S, Essential amino acid composition and proximate analysis of the mushroom *Pleurotuseous* and Pleurotus florida, Mushroom Newsletter Trop, 1981, **1**, 6.
6. Sawaya W N, Al-Shalhat A, Al-Sogair A and Al-Mohammed M, Chemical composition and nutritive value of truffles of Saudi Arabia, *J Fd Sci*, 1985, **50**, 450.
7. Ghosh N and Chakravarty D K, Introduction of a new Indian edible mushroom (*Pleurotus citrinopileatus*) for cultivation, *Indian Agric*, 1986, **30**, 125.
8. Gopinathan M C, Babu C R, Chatterjee S R and Abrol Y P, Nutritional potential of *Vigna minima* (Roxb) Ohwi and Ohashi, I. Seed protein content and amino acid composition. *Qualitus Plant : Pl Fd Human Nutr*, 1986, (in press).
9. Moore S and Stein W H, chromatographic determination of amino acids by automatic recording equipment, in *Methods in Enzymology*, 1963, **6**, 819.
10. Crisan E V and Sands A., In *Nutritional Value, in the Biology and Cutivation of Edible Mushrooms*, by Chang S T and Hayes W A, (Eds) Academic Press, New York, 1978, 137.

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ENZYMIC BROWNING IN RIPENING PLANTAIN PULP (*MUSA PARADISIACA*) AS RELATED TO *ENDOGENOUS FACTORS*

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Ascorbic acid, total phenolic content and polyphenol-oxidase (PPO) activity were determined in the pulp of a Nigerian cultivar of plantain, at five stages of ripening and correlated with browning tendency. The dark green (unripe) pulp had relatively low PPO activity and concentration, low total phenolic content but high ascorbic acid level and exhibited the least browning potential. The increased browning potential observed with ripening has been related with relatively high PPO activity and level, low ascorbic acid content and high total phenolics.

Plantain is one of Nigeria's staples from which a wide range of menu is prepared. It is widely cultivated because of its nutritional values and economic importance. Different cultivars of plantain are found in different parts of the world. In Nigeria, three main cultivars are found[1], namely; (i) "Ogbutu" or true horn plantain; (ii) "Bini" or false horn plantain; (iii) "Unereike" or French plantain.

Plantain is consumed in both the unripe (green) and ripened stages in Nigeria. The fruit has a short shelf-life under the prevailing temperature and humidity conditions in this country. Its proper and prolonged storage in the unripe and ripe stages for direct consumption and conversion into other food products is, therefore, of paramount interest and importance. Controlled atmospheric storage[2] and irradiation[3] have been used to extend the shelf-life of plantain but these methods are very expensive in a developing country like Nigeria. Slices of the fruit soaked in a 1 per cent aqueous sodium chloride solution has also been used to prevent browning[4], while rubbing of the unpeeled fruit with a paste of ash from different types of wood, is used locally to accelerate ripening of the unripe fruit. Plantain, like other fruits is susceptible to browning during prolonged storage. Browning, in the pulp ("Ogbutu") a Nigerian cultivar of plantain, (*Musa paradisiaca*) is investigated as a storage problem. Jayaraman *et al.*[5] have reported seasonal variations in polyphenol oxidase (PPO) activity and level, ascorbic acid content, total phenolic content and pH in the ripe pulp of five banana cultivars and correlated these parameters with enzymic browning. These authors[5] observed sharp increases in PPO activity and level during winter months. The variations of these endogenous factors were also examined as a function of ripening in three different cultivars of banana pulp by Jayaraman and Ramanuja[6]. Weaver and Charley[7] have also studied the enzymic browning of ripening bananas. There is no information available on such endogenous factors influencing browning in plantain cultivars, obtainable in Nigeria. We report here, the variations of ascorbic acid, total polyphenol content, polyphenoloxidase activity and level in the pulp of "Ogbutu" a Nigerian cultivar of plantain as a function of ripening and examine to what extent, these factors influence browning. We also compare our results with those previously reported for banana cultivars by Jayaraman and Ramanuja[6].

Variations in endogenous factors have been determined at five stages of ripening and browning potential correlated with the variations in these endogenous factors in order to identify which stage of ripening, on account of least browning tendency, may have a relatively longer shelf-life.

A bunch of matured, green plantain freshly harvested from a local farm was used for these experiments. Samples were taken from the same "hand" of the bunch, and divided into five portions. Each pulp was sliced longitudinally into four approximately equal portions. Fifty grams of each of these slices were used for the assay. Unpeeled plantains were stored in a wooden box and covered with a fibre bag and allowed to ripen under ambient conditions.

Ascorbic acid was extracted and assayed according to the description of the National Canners Association Research Laboratories[8] and values are expressed as mg per gram pulp. PPO was by the method of Walter and Purcell[9]. The sample was homogenised, centrifuged and filtered. The absorbance of the clean extract at 425 nm was taken initially and after 180 min at room temperature in a Bausch and Lomb Spectronic 20 photometer. The change in absorbance (Δ A) within the time interval was used as measure of browning potential. Total phenolics was by the method of Guadagni *et al*[10] and the results were expressed as mg caffeic acid per 100g pulp.

The physico-chemical characteristics of "Ogbutu" plantain pulp at five stages of ripening are presented in Table 1. Total phenolic content was low in the green (Day-1) stage and increased with ripening. The value for the (Day-13), ripe pulp was about seven times higher than the green (Day 1) pulp. Our results differ from

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF PLANTAIN DURING RIPENING

Ripeness stages	Ascorbic acid (mg/g)	Total phenolics (mg/100g)	Change in absorbance (Δ A) after 180 min.	PPO* activity (units/ml)	PPO level (units/g pulp)
Day-1 (green pulp)	0.19 ± 0.02	12.75 ± 0.09	0.44	9.07 ± 0.44	18.15 ± 0.87
Day-4 Intermediate	0.18 ± 0.01	15.24 ± 0.10	0.46	16.25 ± 0.66	32.50 ± 1.32
Day-7 Stages of ripeness	0.14 ± 0.01	27.55 ± 0.12	0.73	21.25 ± 0.68	42.50 ± 1.36
Day-10	0.09 ± 0.01	62.67 ± 0.16	1.49	37.71 ± 0.72	75.42 ± 1.43
Day-13 (Fully ripe)	0.08 ± 0.00	89.68 ± 0.14	1.75	40.75 ± 0.74	81.50 ± 1.48

All values are averages of five determinations (Mean ± S.D.); *50g pulp homogenised with 100 cm^3 of 0.1M sodium phosphate buffer solution at pH 6.5.

those of Jayaraman and Ramanuja[6] who reported significantly high values for the green pulp and low values for the ripe pulp in banana varieties – "Poojabale" and "Rasabale". "Pachabale", a cultivar of banana, however, gave values that were low in the green pulp and remained almost the same during ripening. They concluded therefore, that total polyphenol content was not a factor influencing browning. Our results show otherwise. Ndubizu[1] reported that plantain is more starchy than banana. The general increase in polyphenol content in plantain during ripening may be partly due to such compositional differences in the same or different cultivars[6]. Ripe plantain (Day-13), the most susceptible to browning, had relatively high PPO level and activity and low ascorbic acid content, while the green pulp (Day-1), the least susceptible to browning, had low activity and high ascorbic acid content. These trends were also observed in cultivars of banana[5,6]. The degree of browning in peaches[11], avocado[12] and apples[13] was also found to correlate with PPO activity.

Susceptibility to browning by visual observation of the sliced pulp, showed a gradual increase during ripening. The change in absorbance, (Δ A) of the clear extract also correlated with increased browning tendency, in agreement with the visual observation.

Ascorbic acid, an inhibitor of enzymic browning, was reported to gradually decrease in content during ripening[14,15]. Our results also show the same trend. The gradual decrease in ascorbic acid level during ripening may also account for the gradual increase in total phenolics as ripening progressed.

Quinones inactivate enzyme reaction and this leads to a decrease in PPO activity[16]. This study shows a general increase in PPO activity during ripening. It has also been reported[14] that ascorbic acid shows a non-competitive type of inhibition on plantain PPO.

Some endogenous factors have been shown to vary seasonally in five cultivars of banana[5]. Since low PPO activity, low total phenolics and high ascorbic acid levels are associated with least browning potential, it is probable that, the green pulp (Day-1) of "Ogbutu" plantain, has the least browning potential and longer shelf-life considering browning as a storage problem. Ambient temperatures and humidity conditions vary with the seasons of the year. A choice of storage temperature similar to the ambient temperature of the season in which least browning is observed and in combination with the stage of ripening having low PPO activity, low total phenolics and high ascorbic acid content will provide longer shelf-life on account of least browning. This investigation shows that "Ogbutu" plantain may be stored or distributed in the dark green stage.

References

1. Ndubizu T O C, The Nigerian bananas, *The Nigerian Field*, 1981, **46**, 118.
2. Olorunda A O, Effect of ethylene absorbent on the storage life of plantain packed in polythene bags, *Nigerian J. Sci*, 1976, **10**, 19.
3. Olorunda AO and Aboaba F O, *Food preservation by irradiation; Vienna*, International Atomic Energy Agency, Vol. 1, 1978, 53.
4. Giami S Y, Effects of pretreatments on the sensory properties of plantain and yam on frozen storage, *Intern J Fd Sci Technol*, 1989, **24**, 195.
5. Jayaraman K S, Ramanuja M N and Vijayaraghavan P K, Enzymatic browning in some banana varieties as related to polyphenoloxidase activity and other endogenous factors, *J Fd Sci Technol*, 1982, **19**, 181.
6. Jayaraman K S and Ramanuja M N, Changes in polyphenol-oxidase and other endogenous factors during ripening in some banana varieties, *J Fd Sci Technol*, 1987, **24**, 67.
7. Weaver C and Charley H, Enzymic browning of ripening bananas, *J Fd Sci*, 1974, **39**, 1200.
8. *Laboratory Manual for Food Canners and Processors*, Vol. 2; Analysis, sanitation and statistics; National Canners Association Research Laboratories, U.S.A., AVI Publishing Co; Inc. Connecticut, 1968, 199.
9. Walter W M, (Jr.) and Purcell A E, Effects of substrate levels and po yphenol oxidase activity on darkening in sweet potato cultivars, *J agric Chem*, 1980, **28**, 941.
10. Guadagni D G Sober D G and Wieber J S, Enzymatic oxidation of phenolic compounds in peaches, *Fd Technol Champaign*, 1949, **3**, 359.

11. Jenn J J and Kahler K R, Characterisation of PPO in peaches grown in the South East, *Hort Sci*, 1974, **9**, 590.
12. Kahn V, Latency properties of polyphenoloxidase in two avocado cultivars differing in their rate of browning, *J Sci Fd Agric*, 1977, **28**, 233.
13. Vamo-Vigyazo L, Gajzayo I, Naduvari-Markos V and Mihalyi K, Studies into the enzymic browning and the polyphenol-polyphenoloxidase complex of apple cultivars, *Confructa* 1976, **21(3)**, 24 (*FSTA*, 1977, **9** 5J684)
14. Omuaru O T, Izonfuo W-A L Braide S A and Obomanu, F G, Polyphenoloxidase activity and ascorbic acid levels in plantain during ripening, *Nig J Natural Sci*, 1988, (in press).
15. Omuaru V O T and Izonfuo W-A L, Effect of ripening on the chemical composition of plantain peels and pulps (*Musa paradisiaca*), *J Sci Fd Agric*, 1988, **45**, 333.
16. Ponting J D, The control of enzymic browning of fruits, *Food Enzymes*, Schultz, H W. (Edn). AVI Publishing Co. Inc. Connecticut, 1960, 105.

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INNOVATION OF TECHNOLOGY FOR PREPARATION OF *RASOGOLLA* ANALOGUE FROM SOY MILK

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Soy-rasogolla was successfully prepared from soy-chhana using 2% calcium lactate as coagulant at 85°C for coagulation which resembled the market *Karapak* rasogolla made from milk. Use of rose flavour could considerably overcome the beany flavour of soybean.

Rasogolla is the most popular indigenous milk product prepared from *chhana*. A method for preparation of *rasogolla* from cow's milk has been standardized but not fully from buffalo milk[1-4].

Soy-milk is a low cost and excellent source of good quality protein and fat. Coagulated products like *paneer* and *chhana* were prepared from soybean[5-7]. No effort has been made to utilize soy-milk for preparation of *rasogolla*. Hence, the present study was undertaken to utilize soy-milk for preparation of *rasogolla* analogue.

For preparation of soy-milk, soybean, were soaked in water containing one per cent sodium hydroxide over-night at room temperature in the ratio of 1:4 (W/V). After soaking, the husks were removed by rubbing with hand. For extraction of soy-milk, the clean soybean (200g) were ground in mixer with lukewarm water (1000 ml). The resulting suspension was then filtered through a double layer muslin cloth.

For preparation of soy-*chhana*, soy-milk was heated to coagulation temperature (80°, 85° and 90°C) in a beaker and two per cent calcium lactate solution was added with gentle and continuous stirring till coagulation completed. The contents were left undisturbed at room temperature for 20 min. Whey was removed by filtering through muslin cloth and hanged for 30 min or till dripping of free whey was completely ceased. The soy-*chhana* was manually kneaded into a smooth dough. Round balls of uniform size of about 8 g each were prepared by hand and cooked for 15 min in boiling clarified 50-55 per cent sugar syrup. Concentration of sugar in the syrup was maintained by adding hot water at frequent intervals. In separate trials, a sugar ball is inserted at the centre of the *chhana* ball before cooking. The cooked soy-*rasogolla* were placed in hot 40 per cent sugar syrup and were allowed to cool to room temperature. The soy-*rasogollas* were stored at room temperature in the same sugar sugar syrup overnight and were used for sensory evaluation and chemical analysis. In some trials, rose and vanilla flavour (James Pokpins & Co.) were added to sugar syrup at 1 ml per litre.

The soy-*rasogollas* were chemically analysed for fat, protein, moisture and sucrose (Lane-Eynon method) content following the method of IS : 4079[8].

Sensory evaluation was carried out using 9 - point Hedonic scale by a panel of 6 judges.

Soy-*rasogolla* prepared using calcium lactate coagulant was found to have good elasticity and sponginess The sponginess of soy-*rasogolla* increased with insertion of sugar ball at the centre of soy-*rasogolla*.

Soy-*rasogolla* prepared using soy-*chhana* coagulated at 90°C was comparatively harder than those prepared at either 80° or 85°. The chemical composition of soy-*rasogolla* is shown in Table 1. The results reveal that the composition of soy-*rasogolla* differ with coagulation temperature of soy-*chhana*. The moisture content of soy-*rasogolla* ranged between 46.12 and 48.07 per cent while coagulation temperature ranged from 80° to 90°C. The fat, protein and sucrose contents ranged between 1.83 and 2.39 per cent; 9.86 and 12.68 per cent and 38.27 and 39.58 per cent, respectively. An increase in moisture and sucrose and decrease in fat and protein of soy-*rasogolla* were observed with increase of temperature of coagulation of soy-milk. The reason for such changes is not well understood. The average moisture, fat, protein and sucrose contents of market samples of milk *rasogolla* have been reported to be 37.99, 5.66, 5.43 and 48.36 per cent, respectively[9] and 30.9, 5.7, 8.0 and 55.1 per cent respectively[3]. The large variation observed in the composition of market samples of *rasogolla* might be due to the compositional variation of milk and concentration of sugar solution used for manufacture of *rasogolla* - as no standard scientific method for preparation of *rasogolla* is followed by the trader. A significant difference could be noted from the above data that soy-*rasogolla* though contained less fat but was very rich in protein content compared to milk *rasogolla*. The soy-*rasogolla* also contained less sucrose than the milk *rasogolla*.

The sensory score of soy-*rasogolla* is reported in Table 2. Irrespective of the coagulation temperature of soy-*chhana*, the soy-*rasogolla* had beany flavour. It was observed that addition of rose flavour in sugar solution and dipping the cooked soy-*rasogolla* in that solution had significant effect on the reduction of beany flavour. However, addition of vanilla flavour had no significant

TABLE 1. EFFECT OF COAGULATION TEMPERATURE ON THE COMPOSITION OF SOY-*RASOGOLLA*

Coagulation temp (°C)	Moisture (%)	Fat (%)	Protein (%)	Sucrose (%)
80	46.12 ± 0.046	2.39 ± 0.018	12.68 ± 0.063	38.27 ± 0.086
85	47.72 ± 0.049	1.95 ± 0.04	10.43 ± 0.023	39.26 ± 0.040
90	48.07 ± 0.063	1.83 ± 0.029	9.86 ± 0.033	39.58 ± 0.054

Data are mean of three determinations ± SE.

TABLE 2. SENSORY EVALUATION OF SOY-RASOGOLLA

Coagulation temp (°C)	Colour	Appearance	Body and texture	Flavour
80	8.3 ± 0.163	7.2 ± 0.124	7.1 ± 0.169	7.3 ± 0.125
85	8.1 ± 0.169	7.8 ± 0.339	8.6 ± 0.081	7.3 ± 0.124
90	8.0 ± 0.235	7.6 ± 0.169	8.3 ± 0.124	7.4 ± 0.309

Data are the mean of three determinations ± SE.

effect. The colour of soy-*rasogolla* was slightly yellow (creamy), which was similar to that of *Karapak* milk *rasogolla*. The body and texture of soy-*rasogolla* were smooth and spongy. The best quality soy-*rasogolla* was obtained from soy-*chhana* coagulated at 85°C.

References

1. Date W B Lewis Y S Johar O S and Bhatia D S, Studies on the preparation and preservation of rasogolla, *Fd Sci, Mysore*, 1958, 7. 217.
2. Jagtiani J L Iyengar J R and Kapur N S, Optimum conditions for rasogolla making and storage, *Fd Sci, Mysore*, 1960, 9, 46.
3. Soni K Bandopadhyay A K and Ganguli N C, Manufacture of rasogolla from buffalo milk, *Indian J Dairy Sci*, 1980, **33**, 357.
4. Vaghela M N Vyas S H Upadhyay K G Patel M T and Patel G C, Evaluation of the effects of the superheating of condensed milk and additives on the quality of rasogolla from buffalo milk, *Indian Dairym*, 1984, **36**, 382.
5. Chander P, *Utilization of soy-milk in formulating some of the dairy products*, 1972, M.Sc. Thesis, *Pune Uni*.
6. Vijay Lakshmi K, *Nutritional Quality of Paneer from Soybeans, Sunflower Seeds and Skim Milk combinations Sensory Evaluation of the paneer and Its Products*, 1980, M.Sc. thesis, *Univ Agric Sci*.
7. Mohd Nasim Mital B K and Tyagin S M, Development of a process for preparation of soy-paneer, *J Fd Sci Technol*, 1986, **23**, 69.
8. *Specification for Canned Rasogolla*, IS:4079-1967, Indian Standards Institution, New Delhi.
9. Singh G P and Ray T K, Effect of milk coagulant on the quality of rasogolla and sandesh, *J Fd Sci Technol*, 1977, **14**, 149.

J. Fd. Sci. Technol., 1990, Vol. 27, No. 4, 244 – 246

EFFECT OF PROCESSING ON MEAT QUALITY OF CLAMS (*MERETRIX CASTA*)

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Meat quality during different stages of processing *viz.* depuration, shucking, blanching and freezing was evaluated in the clam (*Meretrix casta*). Glycogen content varied significantly while total lipid did not vary during all the stages of processing. Water soluble protein (WSP) and salt soluble protein (SSP) contents of the meat decreased significantly during shucking and blanching respectively. Peroxide value (PV) and alpha-amino nitrogen (AAN) during shucking and total volatile base nitrogen (TVBN) during blanching increased significantly.

Until recently, clam meat was used only on a very limited scale for human consumption. However, an awareness has been created regarding the importance of clam meat as a protein-rich food and its potential for processing into different products which could command domestic as well as export markets. Particular care must be given on handling and processing techniques. During the processing of clams either by freezing or canning, various operations such as depuration, shucking, blanching, etc. are carried out. The present paper describes the effect of processing on the meat quality of clams, *Meretrix casta*.

Clams (*Meretrix casta*) were harvested during September from Talapady estuary, Mangalore. Clams ranging from 22 mm (L) × 19 mm (B) × 14 mm (W) to 36 × 23 × 28 mm with the mean dimensions of 29 × 21 × 16 mm and mean wt 10.32 g were used in the study. They were washed in running water to remove crust, mud and sand.

Depuration was carried out by keeping clams in a tub under running water for 24 hr followed by 5 p.p.m. chlorinated water for 2 hr. The depurated clams were immersed in water at 60°C for 2 min to facilitate shucking. The meat was picked up manually. A portion of shucked meat was blanched in 2 per cent brine solution for 5 min after which the liquid was drained completely and the blanched meat air-dried for 3-5 min.

Shucked meats (250 g each) were packed in galvanized iron freezing trays lined with polythene sheets and frozen in a coil freezer at – 28°C for 48 hr. Fresh, depurated, shucked, blanched and frozen clam meats were analysed for various biochemical parameters. Moisture and total ash[1], glycogen[2] and total lipids[3] were determined by standard methods. Total nitrogen (TN) was according to Srikar and Chandru[4] while WSP and SSP contents were by the method of Gornall *et al.*[5] 2-thio barbituric acid (TBA) reactive substances, Peroxide value (PV), AAN and TVBN were determined by the methods of Taraldgis *et al.*[6], Jacobs[7], Pope and Stevens[8] and Beatty and Gibbons[9] respectively. The data from chemical analyses were subjected to analysis of variance and Duncan's new multiple range test[10] was applied to determine differences between different processing stages. In all instances, where $P < 0.05$, mean values were considered different.

The average meat yield used in the study varied from 7.7 to 8.5 per cent with a mean value of 8.1 per cent. The proximate composition of clam meat during different processing stages is given in Table 1. Moisture did not vary significantly during depuration ($P > 0.05$). A significant decrease in moisture content was observed during shucking, blanching and freezing ($P < 0.05$). These findings are in accordance with Saralaya and Nagaraj[11] who reported, a similar decrease in shellfishes during steaming, boiling and blanching. Besides, a reduction observed after freezing may be attributed to the loss occurring during thawing of frozen meat. Total nitrogen content showed reduction during different processing stages of clam meat. However, a significant reduction was observed in blanched meat ($P > 0.05$). This decrease may be attributed to the loss of nutrients due to leaching during blanching in hot water[12]. No significant change in total lipid was observed during different processing stages ($P > 0.05$). A slight increase observed during shucking could be due to loss of moisture. Shucking, blanching and freezing do not seem to affect the total lipid content. The sum of moisture and lipid contents works out to 79.5 per cent. During depuration, the total ash content decreased significantly from 1.21 to 0.79 per cent ($P < 0.05$) whereas no significant change was observed due to other processes. These results are similar to the observations made by earlier workers.[11,13]

The glycogen content of the clam meat decreased significantly during different processing stages ($P < 0.05$). During depuration, the decrease is mainly due to the utilisation of glycogen reserve as a source

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TABLE 1. BIOCHEMICAL COMPOSITION OF MEATS OF *MERETRIX CASTA* DURING PROCESSING STAGES

Type of meat	Moisture % mean ± S.D.	Total nitrogen % mean ± S.D.	Total lipid % mean ± S.D.	Total ash % mean ± S.D.	Glycogen % mean ± S.D.
Fresh	78.64 ± 0.34(5)	2.06 ± 0.13(5)	1.86 ± 0.01(3)	1.21^{a} ± 0.06(5)	7.49 ± 0.34(5)
Depurated	78.62^{a} ± 0.22(5)	2.05 ± 0.11(5)	1.87 ± 0.04(3)	0.79^{a} ± 0.01(5)	6.79^{ab} ± 0.72(5)
Shucked	77.95^{abc} ± 0.12(5)	1.93^{a} ± 0.04(5)	1.98 ± 0.03(3)	0.79 ± 0.02(5)	6.34^{bcd} ± 0.50(5)
Blanched	75.26^{b} ± 0.28(4)	1.80^{a} ± 0.03(5)	1.98 ± 0.03(3)	0.83 ± 0.06(5)	5.08^{c} ± 0.33(5)
Frozen	77.26^{c} ± 0.02(5)	1.82 ± 0.06(3)	1.97 ± 0.02(3)	0.83 ± 0.06(5)	6.23^{d} ± 0.21(5)

Values in each column with different superscripts differ significantly ($P < 0.05$)
Figures in parenthesis indicate number of samples analysed.

of energy for metabolic processes. A comparison of the carbohydrate, protein and lipid nutrients in fresh and depurated clams points to the preferential utilization of glycogen by *M. casta* rather than lipid or protein source. The loss of glycogen during shucking and blanching may be due to their loss in hot water due to leaching[12]. The decrease in glycogen content during freezing may be due to the breakdown of glycogen in post-mortem muscle and partly due to the loss along with thaw drip, the drip loss accounting to the extent of 7.79 per cent. Similar observation has been made by Slabyj and Carpenter[1] in *Mytilus edulis*.

The effect of processing on water soluble proteins (WSP), salt soluble proteins (SSP) and chemical indicators of freshness is shown in Table 2. WSP decreased during different processes. However, a significant decrease was observed due to shucking ($P < 0.05$), the loss being due to leaching. SSP content of the clam meat did not show any significant change during depuration, shucking and freezing. However, a significant reduction was observed during blanching ($P < 0.05$) as clam meat was cooked in salt solution.

Clam meat contained a relatively high quantity of alpha-amino nitrogen (AAN). Due to different processes, the AAN content increased but a significant increase during shucking ($P < 0.05$), due to the release of amino nitrogen by the breakdown of proteinaceons matter was observed. The TVBN content increased during different processing stages, but a significant increase observed during blanching ($P < 0.05$) may be due to the protein degradation during cooking of the meat. Among the lipid freshness parameters, TBA number did not show any significant change ($P < 0.05$) while peroxide formed during shucking and blanching was found to be significant ($P < 0.05$).

The results of the present investigation indicate that during different processing stages of clam meat, quality was found to be altered as reflected by a decrease in glycogen and protein contents as well as by freshness parameters.

References

1. *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, D.C., 1975, 12th Edn.
2. Seifter S, Dayton S, Novic B and Muntwyler E, The estimation of glycogen with the anthrone reagent, *Arch. Biochem*, 1950, **25**, 191.
3. Bligh E G and Dyer W J, A rapid method of total lipid extraction and purification. *Can J Biochem Physiol*, 1959, **37**, 911.
4. Srikar L N and Chandru R, Determination of organic nitrogen by Kjeldahl digestion using hydrogen peroxide, *J Fd Technol*, 1983, **18**, 129.
5. Gornall A G, Bardawlll C J and David M M, Determination of

TABLE 2. WATER SOLUBLE PROTEIN (WSP), SALT SOLUBLE PROTEIN (SSP), PEROXIDE VALUE (PV), THIOBARBITURIC ACID (TBA), TOTAL VOLATILE BASE NITROGEN (TVBN) AND ALPHA AMINO NITROGEN (AAN)

Type of meat	WSP % mean ± S.D.	SSP % mean ± S.D.	PV mean ± S.D.	TBA number mean ± S.D.	TVBN (mg %) mean ± S.D.	AAN (mg %) mean ± S.D.
Fresh	5.35 ± 0.15(4)	6.96 ± 0.21(4)	Nil	0.10 ± 0.02(6)	4.09 ± 0.96 (5)	43.84 ± 0.00(3)
Depurated	5.24^{a} ± 0.26(3)	6.99 ± 0.18(3)	Nil^{a}	0.13 ± 0.02(6)	4.34 ± 1.29(5)	42.69^{a} ± 1.92(3)
Shucked	4.71^{b} ± 0.14(4)	6.62^{a} ± 0.14(4)	0.79^{b} ± 0.32(4)	0.10 ± 0.02(6)	5.26^{a} ± 1.16(5)	52.96^{b} ± 1.71(3)
Blanched	4.36 ± 0.24(4)	6.13^{b} ± 0.16(4)	0.81 ± 0.28(4)	0.12 ± 0.02(6)	6.75^{b} ± 1.49(5)	53.93 ± 2.20(3)
Frozen	4.37 (3)	6.52 ± 0.24(3)	0.89 ± 0.24(4)	0.11 ± 0.01(6)	5.62 ± 0.85(5)	52.39 ± 0.97(3)

Figures in parenthesis indicate number of samples analysed
Values in the same column with different superscripts differ significantly ($P < 0.05$)

serum proteins by means of the Biuret reaction, *J Biol Chem,* 1949, **177,** 751.

6. Taraldgis B G Wattos B M and Youndthan M T, A distillation method for the quantitative determination of malonaldehyde in rancid foods, *J Am Oil Chem Soc,* 1960, **37,** 44.
7. Jacobs M B, *The Chemical Analysis of Foods and Food Products.* Kreiger Publishing Co. Inc., New York, 1958, 393.
8. Pope C G and Stevens M F, The determination of amino nitrogen using a copper method, *Biochem J,* 1939, **33,** 1070.
9. Beatty S A and Gibbons N E, The measurement of spoilage in fish, *J Biol Bd. Can,* 1937, **3,** 77.
10. Duncan D B, New multiple range and multiple F tests, *Biometrics,* 1955, **11,** 1.
11. Saralaya K V and Nagaraj A S, Studies on the utilisation of shell fishes of Karnataka coast by canning, *Mysore J Agric Sci,* 1978, **12,** 484.
12. Lee F A, The blanching process, *Adv Food Res,* 1958. **8,** 63.
13. Balachandran K K and Surendran P K, Purification of live clams for processing, *Seafood Export J,* 1985, **17,** 5.
14. Slabyj B M and Carpenter P N, Processing effect on proximate composition and mineral content of meats of blue mussels (*Mytilus edulis*), *J Fd Sci,* 1977, **42,** 1153.

BOOK REVIEWS

Focus on Artificial Sweeteners, Vol.5: Ed. by M.G.E. Wolters and Y. Botma, TNO. CIVO-Institutes, attn. Marion Vander Vorn, 3700 AJ ZEIST, The Netherlands, Price: Dfl. 200; Dfl. 219 (abroad); 1988.

The acceptance of artificial sweeteners has been subjected to numerous scientific challenges during the past 20 years. Low calorie sweeteners are widely applied in diet beverages. Artificial sweeteners are employed in the preparation of chewing gum, table top sachets, ice cream, powdered drink mixes, cereals, dry mixes of gelatins, desserts and toppings, and pre-sweetened coffees and teas. The review lists 43 interesting scientific papers on artificial sweeteners collected from 38 international journals.

Newly approved U.S sweetener acesulfame is used in a variety of products worldwide. Following changes in regulation, more and more soft drinks are using aspartame. Figures for the first half of 1988 show that the sales of regular carbonated soft drinks in the U.K. grew by 5% in litres, but that low calorie products grew by 310%.

Although there is no sweetener on the market which exactly matches the taste of sugar, much work is done on the use of blends of sweeteners which act synergistically to fool the mouth as to the level of sweeteners. The review mentions that acesulfame, alitame and sucralose are the sweeteners now under review by FDA. It is said that these three hold the best potential for the near future because they are the only ones to reach the petition stage.

The quarterly contains many interesting articles titled 1) Two more Natural Sweeteners - Steria and Sucanat, 2) Sugarless Confectionary using sorbitol, mannitol and lycasin, 3) a review on sweeteners, 4) newly approved U.S. sweetener used in a variety of products worldwide, 5) low calorie alternatives, 6) sweet taste of success, 7) exciting future for non-calorie sweeteners, 8) FDA clears Hoechst's non-calorie sweeteners for use in dry foods, 9) sweeter than sugar, and 10) xylitol in sugar-free- confectionery.

The Booklet describes Stervia and Sucanat as two new natural sweeteners for trials. Both fit into the definition of natural sterioside as they are extracted from the dried leaves *Stevio rebaudiana*. These have been most regularly used in Japan, Brazil may clear it for use it in soft drinks. Stevia is a low calorie, heat stable, intensely sweet, 300 times sucrose at 0.4% level. Sucanat contains all the vitamins and trace minerals lost during refining of sugar cane,

The Booklet contains the reviews dealing with the available information on non-nutritive sweeteners and their uses in the production of diet foods. The sensorial, chemical, physical and toxicological properties of saccharin, cyclamate, aspartame, acesulfame-K, thaumation, stevisoide, monellin, glycyrrhizin, phyllodulein, hernandulcin, miroculin, alitan, sucralose and dihydrochalcone.

The Booklet is quite informative and interesting, worth possessing by those interested in the study of artificial sweeteners.

J.R. RANGASWAMY,
CFTRI, MYSORE

Food Additives: Ed. by A. Larry Branen, P. Michael Davidson and Seppo Salminen, (Food Science and Technology Series/35) Marcel Dekker, Inc. 270, Madison Avenue, New York, NY 10016, 1990; Pp: 752; Price: $180.00

Food additives are meant to enhance the overall acceptability of the food. Often serious doubts are raised about the safety of such additives. There is a preference for additives from natural sources, on the assumption that they are safe. However, it is known that all that is 'natural' need not necessarily be 'safe'. Therefore, there is a need to have an integrated view of food additives such as their safety, use in processed foods, acceptable daily intake, regulatory status, etc. In this direction, this book provides the most up-to-date information available on the subject in seventeen chapters written by eminent people in the field. It is stated in the preface of the book that 'Authors and the editors have been selected from throughout the world, so as to bring an international perspective to this work' Further it is stated that 'this is important since current regulations, additive use, and approaches to toxicological evaluation differ worldwide'. However, I find an exception for these statements as 'India does not figure in the book.

The first two chapters provide an introduction to the food additives with discussion of each of the major food additive categories and information on estimation of food additive intakes. The latest figures indicate that the per capita consumption of additives in USA exceeds 4-5 kg and tops the list. Acceptable daily intake (ADI) values are shown to be useful for food safety evaluation. The estimated intake and ADI are compared to assess the safety of that food additive. Chapters 3 to 14 deal with specific food additive categories namely,

nutritional additives, antimicrobials, antioxidants, flavourings, sweeteners, colouring agents, emulsifiers, polysaccharides, enzymes, acidulants and miscellaneous additives. Each chapter gives a general introduction and discussion of chemistry, functions, mechanism of action, analytical methods and regulatory aspects, toxicological concerns of additives and references.

Under nutritional additives (Ch.3), vitamins are covered in detail; however, very little is said on essential fatty acids, although quite an amount of information and specific indepth studies are available on the essential fatty acid intake. The chapter on antimicrobial agents (Ch.4) deals in addition to common preservatives, the biologically derived anti-microbials like nisin and natamycin. The 5th chapter on antioxidants starts with the chemistry of food fats and oils and their deteriorative changes. Applications on phenolic antioxidants, their mechanism and use in vegetable oils and fried foods, cereal products, etc., and toxicity studies are discussed. The chapter on Flavouring Agents (Ch.6) emphasises the distinction between spices and essential oils and simulated flavours. Chapter 7 gives a good reading on flavour enhancers, namely glutamates and 5' nucleotides. Sweeteners are dealt in detail in Chapter 8. The continued use of saccharin in USA as a result of United States Senate Moratorium on the proposed ban of saccharin by the FDA is an example of balancing risks and benefits of additives. We are living and continue to live in a society in which food additives are 'a way of life'. The other chapters namely colouring agents, emulsifiers, polysaccharides, enzymes, acidulants and miscellaneous additives are also well covered and highly informative. Safety evaluation methods, hypersensitivity and risks and benefits of foods and food additives are discussed in the final three chapters.

The book is useful as a source book for the selection of additives for use in foods, for understanding the benefits and risks of additives and for the regulatory agencies. The book is invaluable to those interested in Food Science and Technology, consumer organisations, research scholars and scientists working in this area.

M.N. KRISHNAMURTHY
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Evaluation of Certain Food Additives and Contaminants: 33rd Report of the Joint FAO/WHO Expert Committee on Food Additives, Technical Report Series 776, WHO, Geneva, 1989; pp: 64; Price: Sw fr.8.

The Report deals broadly on three aspects namely; 1) General considerations 2) Comments on specific food additives and contaminants and 3) Revision of certain specifications followed by setting priorities for the safety review of food flavouring ingredients, future work and recommendations. The report is also supplemented with five annexures about 1) Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives 2) Acceptable daily intakes, other toxicological information, and information on specifications. 3) Further toxicological studies and other information required or desired, 4) A method for setting priorities for the safety review of food flavoring ingredients, and 5) Matters arising from the reports of sessions of the Codex Committee on Food Additives (and contaminants).

Limits formerly given as provisional maximum tolerable daily intakes for many metal contaminants were changed to provisional tolerable weekly intakes (PTWIS). The rationale for establishing a weekly intake was that such contaminants are able to accumulate within the body at a rate and to an extent determined by the level of intake and by the chemical form of the heavy metal present in food.

Since the common antioxidant Butylated hydroxy-anisole (BHT) was found to cause oesophageal hyperplasia in pigs and monkeys, a temporary ADI of 0 to 0.3 mg per kg of body weight was established with a recommendation that it would be desirable to carry out additional studies to determine the mechanism of action of BHT on the fore stomach. Flavouring agent trans-anethole at a temporary ADI of 0-2.5 mg per kg body weight was found to show incidence of hepatomas in female rats, a recommendation has been made for conducting epidimiological study of consumers of food and beverages containing high levels of trans-anethole. The committee is of the opinion that the bromate should not be present in foods as consumed. It was found by studies that the treatment of flour upto 62.5 mg potassium bromate per kg of flour resulted in the absence of bromate residues in the resultant bread. So, an ADI of 0 60 mg of potassium bromate per kg of flour is proposed.

Likewise the committee has done a commendable job of going through in detail about all specific food additives and their recommendations are presented in the 33rd report.

The Report provides flood of information on these specifications for those who are engaged in the line.

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Fish Smoking and Drying: Ed by J.R. Burt, Elsevier Applied Science, London and New York; 1988; pp:166, Price: £34.

The book consists of contributions to the subject by members of a working group set up under the joint aegis of the International Union of Food Science and Technology and the International Union of Nutritional Sciences. The objective of the book is enunciated in the extended title, "The effect of smoking and drying on the nutritional properties of fish". Since such traditional methods are practised primarily for avoiding wastage, the main theme appears to be to weigh the extent of such saving, which is often effected through relatively harsh techniques, against possible losses in terms of nutritional value of the products. There is also a concern expressed regarding the effect by the consumption that such products might have on the gut pathology of people.

The introductory chapter briefly covers such aspects like water activity, temperature and pH and their influence on spoilage and nutritional elements. The next three chapters have been devoted to specific areas viz., proteins, lipids and vitamins as affected by smoking and drying. Chapter 5 and 6 are concerned with toxins, particularly carcinogens from smoke. This is followed in Chapter 7 by the description of a general procedure, drawn from regional practices, for the processing and storage of these products. There is a review fully devoted to Japanese experiences in Chapter 8. The next and last chapter provides in tabular form, details of products, species used, processes in brief, composition and properties, all arranged according to the country of origin.

Traditional dried fish products may be broadly divided into (1) dried, (2) salt-cured and dried, and (3) salted, smoked and dried. The degree of salting and extent of drying and smoking may vary for each type of product. Mostly, sun-drying is being practised due to consideration of cost in developing countries like India. Barring advantages gained in time-temperature and humidity control, mechanical dryers introduced in this area serve merely as an extension of the traditional sun-drying process. Salt-curing is a unique step by itself, contributing largely to the flavour profile of the finished product. The practice of smoking is generally followed in Europe and the Far East. Smoke deposited on the product imparts some preservative effect, attractive colour and a characteristic flavour.

Guided apparently by their own perceptions, the authors have been tempted to ignore the specific role of salt-curing, and slightly over-play the importance of smoking in relation to quality and nutritional effects. Oddly, the numerous publications from India on this subject are also conspicuous by their absence in the literature cited. The presence of polycyclic aromatic hydrocarbons, the major health hazard in smoked products, could only be assumed in smoked fish through analogy from data on similarly processed meat products, due to lack of published information. The dangers from nitrosamine in fish are remote because nitrite is rarely used in fish curing. There appears to be a degree of imbalance among the various chapters with regard to scope and amplitude. The chapter on processing for instance, could have been little more elaborate; the review on Japanese studies, in contrast, is expansive with too many experimental details. Incidentally, statements suggesting that biological value is a measure of digestibility of protein should have been avoided.

Despite these minor shortcomings, the book is a valuable contribution mainly because it is probably the only one of its kind, dealing specifically with nutritional and toxicological aspects of traditional fish products. The insight provided in the discussions on the cause-effect relations will be immensely useful for those concerned with quality and safety of these products.

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CFTRI., MYSORE.

AFST(I) News

Annual General Body Meeting 1989

The Annual General Body Meeting of the Association for the year 1989 was held on 11th May 1990 at CFTRI, Mysore. The following have been elected as office bearers for the year 1990-91.

President	: Dr. V. Sreenivasa Murthy
President-designate	: Dr. P.J. Dubash
Vice-President HQ	: Dr. M. Mahadeviah
Vice-Presidents	
Chapters	: Dr. M.M. Krishna
	Dr. P. G. Adsule
	Dr. L.P. Rajput
	Dr. S.V. Krishnaswamy
Hon. Exec. Secretary	: Dr. K. Vidyasagar
Hon. Jt. Secretary	: Dr. M.S. Prasad
Hon. Treasurer	: Dr. N.S. Mahendrakar

The membership (including all categories) of the Association at the end of the year 1989 was 2039.

AFST(I) Activities for the year 1989-90

A National Convention on 'Recent Developments and Future Trends in Milling and Baking Technology' was held during 10-12th May 1990. Shri K.H. Ranganath, Hon'ble Minister for Education and Youth Services, Government of Karnataka inaugurated the convention, Dr. B.L. Amla, Director, CFTRI, Mysore delivered the Presidential address, Shri. Kantilal D. Singhee, President, R.F.M.F.I., reviewed the status of Milling Industry in India and Shri. S.K. Alagh, Managing Director, Britannia Industried Ltd., reviewed the status of Baking Industry in India. The convention was well attended by over 400 delegates. A 25 year cumulative index of the Journal of Food Science and Technology and a bibliography on Bakery Additives were released during the convention.

AFST(I) awards for the year 1989

Prof. V. Subrahmanyan Industrial Achievement Award was given to Shri. H.C. Bhatnagar, a well known Food Consultant.

Laljee Godhoo Smarak Nidhi Award was jointly awarded to Dr. J.R. Rangaswamy, Scientist, CFTRI, Mysore and Dr. J.S. Sidhu, Professor and Head, Department of Food Science, Punjab Agricultural University, Ludhiana.

The recipient of Young Scientist Award was Shri. M.C. Nandeesha, Assistant Professor, Fisheries College, Mangalore.

Gardener's Award was jointly given away to Dr. A.S. Bawa, Dr. W.R. Usborne and Dr. H.L. Orr for their best research paper entitled 'Effect of levels of fillers and extenders on the functionality of a meat system' published in the Journal of Food Science and Technology, Volume 25, No.2, 1988.

Best Student Award was received by Shri. Pradeep Kumar Sharma, Student, M.Sc. (Food Tech.), CFTRI, Mysore.

AFST(I) Fellowship

The Fellowship of the Association was bestowed on the following distinguished personalities:

1. Dr. A. Sreenivasan, Food Consultant, Bangalore.
2. Dr. K.K.G. Menon, Consultant, NDDB, Bombay.
3. Mr. N.A. Pandit, Food Consultant and Former President, AFST(I).
4. Dr. A.M. Nanjundaswamy, Sr. Scientist, CFTRI, Mysore.

Indian Food Industry Journal

The Journal has entered the 9th year of publication. From 1990 onwards, the journal has become bimonthly and the first issue was released in Delhi during the Symposium on 'Production, Processing and Marketing and Export of Untapped Indigenous Fruits and Vegetables' on 7th April 1990.

AFST(I) Education and Publication Trust

The Trust, this year has decided to award scholarships to four students pursuing post-graduate studies in Food Science and Technology. The scholarship is for two years at the rate of Rs.500/- per month and was awarded to the following persons;

1. Shri. Ganapathy Subramanian, IFTTC, CFTRI, Mysore,
2. Shri. M. Elias Baigh, G.B.P.U.A., & T., Pantnagar,
3. Kum. B. Gowri, Tamil Nadu Agricultural University, Madurai,
4. Shri. Dilip Sharma, UDCT, Bombay.

The Trust has also sanctioned Rs.20,000 for publishing a book on 'Rice' by the V. Subrahmanyan Memorial Trust, CFTRI campus, Mysore.

Bombay Chapter

A seminar on 'Biotechnology in food processing industry' was organised on 16th September 1989, A National symposium on 'Food irradiation – perspectives and prospects' was held during January 3-5, 1990 and a seminar on 'Clean Industries' was also held on 4th March 1990.

Poona Chapter

A half day special seminar and an Exhibition on 'Food Adulteration' were organised on 21st September 1990.

Delhi Chapter

A one day National seminar on 'Production, processing, marketing and export of untapped indigenous fruits and vegetables' was organised on April 7, 1990.

Hissar Chapter

A day's seminar on 'Recent trends in food science and technology' was organised on 16th September 1990 and many eminent food scientists were also felicitated during the symposium.

Bangalore Chapter

A Seminar on 'Dietetic processed products' and industry oriented training programme on 'Quality for food processing industry' was held on March 16-April 4th, 1990, and also organised about 10 special lectures on various topics.

Madras Chapter

A technical get-together of food colour manufacturers was organised on 6th September 1989 and conducted a seminar-cum-workshop on 'Traditional Convenient Foods' on 2nd November 1989. A training cum workshop on marketing of fresh and processed food products was also conducted from 12th March 1990 to 16th March 1990.

Calcutta Chapter

A Half day seminar on 'Margarine Industry – its prospects' was organised on 26th August 1989 and conducted a National seminar on 'Problems and prospects of food processing industry' on 31st December 89 to 1 January 1990.

Jabalpur Chapter

A one day seminar on 'Health and Nutrition' was organised on 25th October 1989.

Karnal Chapter

A Symposium on 'Food Security through High Technology: Option before us' was organised.

Ludhiana Chapter

A one day Symposium on 'Development of Food processing industry in Punjab: Constraints and opportunities' was held.

All the chapters celebrated the Founder's Day in memory of the Founder-President Dr. V. Subrahmanyan.

Complaints regarding the non-receipt of the journal should reach within 6 months form the publication of the respective issue of the journal.

Subcribers are requested to contact AFST(I) Headquarters for subscribing to the journal. Journal will not be distributed through agents in India.

Errata

27 (1), Page 40, Table 1 last row: printed as 0.900 ±, read as 0.090 ±

27 (3), Page 176, line 1 from top: printed as 0.8 kg.Sq. m; read as about 9 kg/sq. m.

INDIAN FOOD INDUSTRY JOURNAL

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

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

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

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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.

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

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