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CONTENTS

Research Papers

Research Notes

Direct Assessment of the Toxicity of Malathion and Fenitrothion Residues in Flours with the Adult Flour Beetle, *Tribolium castaneum* (Herbst)

SYED S. H. QADRI AND ZAREENA BANU
Department of Zoology, Post Graduate Centre, Warangal, A.P.

Manuscript Received: 19 *December* 1975

The toxicity of organophosphorus insecticides is assessed by feeding insecticide treated flour to flour beetles in a glass walled small cubical cell. A glass plate with holes provided access to the head of the beetle to feed on insecticide treated flour *ad libitum*, and avoided unnecessary contact of other parts of the body during feeding. Another similar glass plate provided free access to air and avoided a build up of insecticidal vapour, if any, during the operation.
The toxicity of malathion and fenitrothion deposits was assessed in terms of mortality rate, which has shown potentiation of toxicity of malathion and fenitrothion to flour beetles in the presence of rice, jowar, barley, *maida* and bajra flours.

Use of pesticides is necessary for the conservation of foods. But the order of toxicity after combining with active biological agents present in foods over short or long term periods is unknown. Most of the toxicological assessments have been based on original active compounds. Yet what is consumed may be an altered form of the parent compound and the altered products either may be quite toxic or may bring a change in physico-chemical properties as a result of transformation of the parent compound. Moreover, factors like synergism, inhibitory and masking effects, detoxification and other chemical agents significantly modify the total quantum of toxicity[1]. Therefore, treated food, as such, and compound corresponding to all the constituents of the actual terminal residues in the food have to be toxicologically evaluated[2,3].

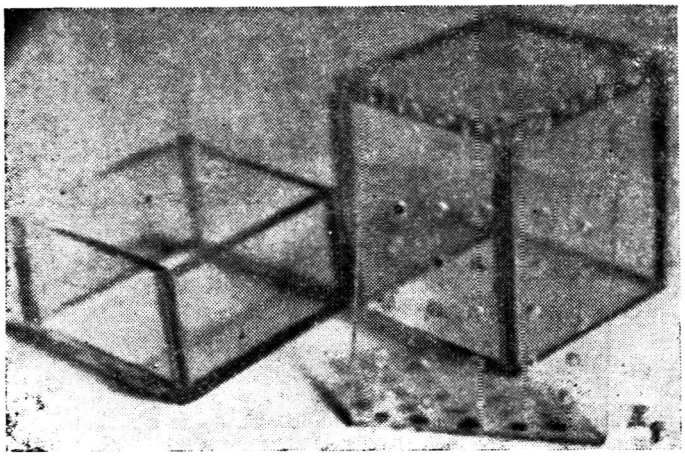
Existing physical and chemical methods to detect and determine the residues of pesticides can only indicate the presence of active chemical moiety in an experimental sample irrespective of their overall toxicity potential. These methods cannot give information on the total toxicity of the pesticidal compound as modified by the constituents of the substrate[4-6]. It is therefore, considered that no other method will be more useful and reliable than the bioassay technique where in the toxicity of the chemical is measured on the basis of response of the organism as a total function of the active ingredient as influenced by the substrate composition. In the present study therefore, attempt has been made to develop a bioassay technique to estimate the total chemical effect (of pesticides, malathion and fenitrothion) present in association with the modifying factors in foods, the substrate.

Redeteff *et al*[7] recommended malathion for the control of stored grain pests. No chronic toxicity hazard was found with malathion unless it was administered in high dosages[8]. Similarly, no health hazards were observed with malathion even if treated food grains were consumed immediately after highest treatment dosage of 50 ppm[9]. Further, the acceptance of malathion as a grain protectant for direct admixture with wheat upto a tolerance limit of 8 ppm has opened up a vast possibility of admixture with other commodities. Studies carried out by CFTRI, Mysore, on the imported wheat, by PAU, Ludhiana and NCL, Poona on the FCI wheat revealed the presence of malathion at 3.56–6 ppm level[10].

Fenitrothion combines low mammalian toxicity[11] with a high degree of effectiveness against stored products insects[12]. Further, experiments carried out by Lemon[12] with dust formulations on wheat showed that the mortalities produced by 1.0 ppm of fenitrothion and 4.0 ppm, malathion are roughly in the ratio of the acute oral toxicities of the compounds to rats[13], so that the dangers of hazard for any of these treatments may be approximately the same. However, there is little information available on the use of fenitrothion as grain protectant probably because of its recent arrival in the country and the overwhelming acceptance of malathion. Therefore, in the present study fenitrothion has been included along with malathion.

Materials and Methods

Oral feeding of insecticide-flour-bait ad libitum *by flour beetles:*[2] Methods have been described to evaluate efficacy of pesticides against stored grain pests.[14,15] Techniques described in the literature suffer from certain disadvantages. One of these is that insects not only



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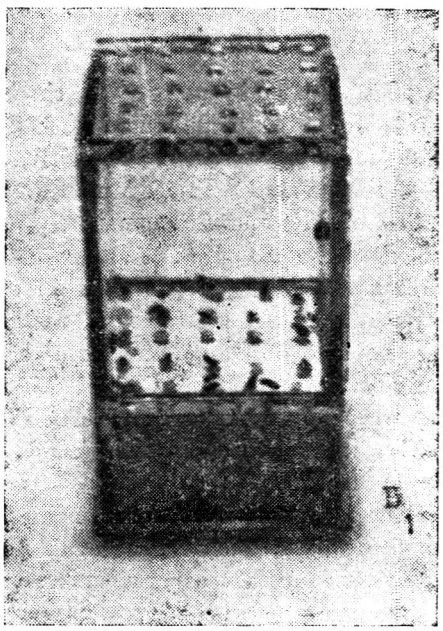

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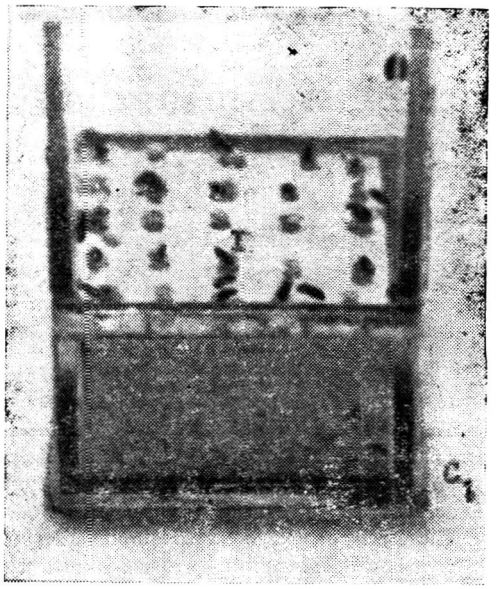

C_1

Fig. 1. The apparatus to administer insecticide flour bait orally to flour beetles.

A_1: Different parts of the apparatus B_1: Apparatus ready for operation
C_1: Internal view of the apparatus to show flour beetle (I) feeding on insecticide-flour bait (H).

feed on the food-poison-bait, but also remain in contact with the poison bait. Therefore, mortality recorded is the total effect of contact and oral toxicities, although the sensitivity of the oral assay is somewhat less than of the contact assay as reported by Fawaley *et al*[16]. and Dewey[17]. Therefore, the adoption of such a combination of trials will definitely cause discrepancy in the results, and the ultimate picture obtained will not be due strictly to oral toxicity.

In the present study a technique was designed to make oral administration with negligible contact of the insects with the flour-insecticide-bait during the operation. The technique (Fig. 1) consists of two square shaped four walled glass cells: (1) A square shaped four small-walled glass cell (A) measuring $4.4 \times 4.4 \times 2.2$ cm in size with a removable square glass plate (C) of 4.5×4.5 cm in size used as a bottom plate, another removable square glass plate (D) of the same size bearing holes (F) of 1 mm diameter 1 cm apart, used as a removable top. The holes provided in the plate allowed beetles (I) to feed on flour-insecticide-bait (H) already filled in the lower glass cell without contact of the body with the bait.

(2) A glass cube (B) made of glass plates measuring $4.4 \times 4.4 \times 4.4$ cm in size, with holes in the glass plate(E) similar to D used as a removeable top. These holes maintained free access to atmospheric air in the testing chamber, Thus the toxicity, if any, caused by insecticidal fumes was negated. All the square glass plates used were 3 mm in thickness.

Operation of the apparatus: The assembled cell (B_1) contained the flour-insecticide-bait (H). Twenty-five beetles starved for 5-6 days were released in the four walled glass (B) by sliding the hole bearing top glass plate (E). The beetles, thus released were allowed only to feed on flour baits for 24 hr *ad libitum*. Mortality data were recorded by observing the beetles through the top glass plate.

This method had the following advantages: (*i*) sufficient space was provided to accommodate 25 beetles to feed at a time without crowding and (*ii*) vapour toxicity caused by fumes was negated to a large extent.

LD_{50} of Malathion and Fenitrothion with wheat and other flours: The susceptible strain of flour beetles (*T. castaneum* Hbst.) was raised at a temperature $28 \pm 2°C$

TABLE 1. LD_{50} VALUES OBTAINED BY THE ORAL FEEDING OF MALATHION AND FENITROTHION IN WHEAT FLOUR TO FLOUR BEETLE FOR 24 HOURS

Insecticide	Regression equation	LD_{50}* g/g	S.E. of Log LD_{50}	Relative toxicity
Malathion	$y = 1.320 + 2.080x$	112.20	3.040 ± 0.251	41.25
Fenitrothion	$y = 2.160 + 1.950x$	2.72	1.460 ± 0.064	1.00

*Read from regression line.

and R.H. of 65 to 75 per cent. The experiments were carried out under the same conditions. One-to four-week old flour beetles were selected, belonging to the same progeny in a set of experiments. Known quantities of malathion (97 per cent premium grade, supplied by Cynamide Industries Ltd., Bombay, India) and fenitrothion (technical grade, supplied by Tata Fison Industries Ltd., Bombay, India) in acetone were mixed in enough ether to allow application to all exposed surfaces of the flour in a crystallizing dish while the contents were stirred with a glass rod for thorough application. Malathion and fenitrothion were thus tested in the range 0.5 — 1.80 μg/flour and 0.1 —0.5μg /g flour respectively. Then a stream of air was blown gently to evaporate and remove the ether. The last traces of the ether were removed by exposing the flour at room temperature. Beetles were grouped 5-6 days before they were subjected to insecticide-baits, each group consisting of 25 individuals. There were four replicates for each treatment, and the mean mortality was calculated at the end of 24 hr of feeding *ad libitum*. Data obtained from a plot of the log-dosage and probit kill were subjected to probit analysis[18], and the LD_{50} values calculated are shown in (Table 1).

Generally the rate of consumption of flour-insecticide-bait for 25 beetles was about 8 mg in 24 hr. This was determined by taking the initial weight of flour-insecticide-bait holding square shaped four walled cell (A) with bottom and top glass plates (D & F) and subtracting the final weight of flour-insecticide-bait holding square shaped four walled cell with bottom and top glass plates after 24 hr feeding of the beetles on insecticide bait. However, a reduction in the consumption rates from 3 to 5 mg was observed when beetles were provided lethal dosages. Parallel control trials were also run with the respective flour treated only with acetone by following the same procedure.

To study the effect of different substrates on the toxicity of malathion and fenitrothion residues further experiments were carried out. The selected flours were thoroughly treated with insecticide-acetone solution in ether as mentioned earlier and the LD_{50} values were estimated. Parallel control trials were run for comparison as describedr. Mortality data were recorded after 24 hr of feeding on insecticide-flour-bait and each dosage was calculated on the basis of flour-insecticide-bait consumed by the beetles. Data thus collected were subjected to probit analysis.

Computation of toxicity unit: LD_{50} values presented in (Table 1) were calculated from their respective regression lines. The relative toxicity units for different substrates were calculated by taking LD_{50} value of malathion-wheat flour-bait and fenitrothion-wheat flour-bait as 1.00 in the respective trials. On the basis of these values per cent increase or decrease in the toxicity of malathion and fenitrothion was calculated (Table 2) respectively.

Results and Discussion

To determine whether the results could be duplicated with the apparatus, tests were conducted with flour beetles at 24 hr exposure period. Each test was carried out by providing wheat flour-insecticide-bait to beetles. Data obtained are shown in (Table 1). From LD_{50} values it is clear that fenitrothion was more toxic to beetles than malathion.

The study of the influence of food substrates on the toxicity of malathion and fenitrothion residues was carried out by feeding the known doses of the mix of malathion-acetone and flour as mentioned earlier. The data obtained for the LD_{50} are shown in Table 2.

In these experiments bajra, jowar, rice, barley and maize flours have shown the potentiation of toxicity of both malathion and fenitrothion to flour beetles as compared to wheat flour. The results of these experiments have shown that toxicity unit (T) could be used as a measure of the comparative toxicities of malathion and fenitrothion as influenced by the substrate factors. Thus these data have indicated that the toxicity of a particular insecticide is modified profoundly by the substrate composition.

There is no doubt that man and animal ingest pesticide residues along with the food. The ingestion is not limited to a single chemical. They are ingested mostly in combination with a large number of chemicals and food factors. Therefore, the manifestation of toxicity to man and animal is governed by the interactions of the pesticidal compounds and food factors

TABLE 2. REPRESENTATIVE DATA COLLECTED AFTER ALLOWING 1 TO 4 WEEK OLD FLOUR BEETLES TO FEED DIRECTLY ON MALATHION AND FENITROTHION TREATED FLOUR FOR 24 HOURS

Insecticide	Foodstuff (as flour)	LD_{50}* g/g	% increase/ decrease in toxicity
Malathion	Barley	50.12	+55.32
	Rice	45.71	+59.26
	Jowar	22.39	+80.04
	Bajra	60.26	+37.29
	Maize	17.18	+93.60
	Wheat	112.20	**
Fenitrothion	Barley	0.295	+92.83
	Rice	0.115	+99.74
	Jowar	0.209	+95.91
	Bajra	0.155	+97.91
	Maize	0.251	+94.40
	Wheat	2.723	**

*Read from regression line.
**indicates decrease in toxicity; (+) indicates increase in toxicity.

and not as function of a single pure pesticide or a toxicant[19].

Although, the tolerances of pesticidal chemicals on foods are established on the basis of elaborate studies and using various safety factors in computation of these, there appears to be a great need for investigation of the present day pesticidal chemicals with reference to the influence on modification of the toxicities by their substrate factors. This concept of toxicity unit opens up vast possibilities for assessing comparatively the potential toxicological hazards of residues of the pesticides or the combination of pesticides together with their interactions of the substrate factors.

Further, studies carried out on grain treatment by Zutshi and Lallan Rai[20] showed that whole wheat flour made from malathion treated grains at 24 ppm level contained less than 6 ppm malathion residues. Similarly Parkin[21] indicated that in wheat freshly treated with 5 ppm fenitrothion and subjected to milling 3.9 ppm of the applied dose was recovered from offal and flour fractions. Generally, after the admixture, the treated grains are stored. Malathion residues dropped to low levels with time, whereas fenitrothion residues remained effective for prolonged period of 10 months[22]. In practice, these grains are released to market. As the pesticides are basically toxic to man, the contamination of these lead to consumer hazards. Hence, the food materials have to be assessed for contamination before they are released in the markets for human consumption. The present method furnishes an accurate assay of the joint or total effect of the combination of insecticides and other biologically active agents present in grain flours. Hence, a knowledge of the hazards under our climatic, dietary and nutritional conditions for assessing the maximum tolerable concentrations and long range chronic toxicity of fenitrothion to mammals are desirable.

The method described is sensitive at as low as 17 ppm of malathion and 0.12 ppm of fenitrothion residues in grain flours. It is also possible to estimate the residues of organophosphates from the treated grain flours by running parallel control trials with known amounts of pesticides without involving any extraction and clean-up steps.

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Development of Foams in Fish Slurries

B. P. NIGAM
Central Food Technological Research Institute, Mysore-570013

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Stable foams have been developed out of slurries of precooked trash fish like croaker (*Otolithus* sp.) under experimental conditions of maintaining carbon dioxide flow rate of 1500–2000 ml/min for 30 min at pH 7.0 and consistencies corresponding to 0.16 s/w [solids (exclusive of fat or additive] to water ratio) with 0.5% (w/w) GMS (Glyceryl mono-stearate) and system pressure of 2–3 cm of Hg at 8-10°C while agitating at 60 rpm and as well as at consistencies corresponding to 0.13 s/w with 0.5-1% (w/w) GMS and system pressure of 2-12 cm of Hg at temperatures of 6.5° 10°C.

The development of foam has been concluded by observing its physical porous structure, increase in volume and lowering of density whereas the stability was judged by its sufficient resistance to collapse, on handling it for subsequent operations of the process. Preliminary attempts have been made to dry the foamed product and find out its physical as well as organoleptic characteristics.

Foam-mat drying technique is a recent commercial breakthrough[1] developed to provide an economical method for the production of a wide variety of high quality instantly soluble foods from concentrates. The technique has been applied to fruit juices, potato slurries and baby foods[2]. Literature however does not show any mention of its application to fish slurries.

The object of the present investigation was to develop foam in fish slurries with a view to obtaining a dried product with properties of good reconstitution and dispersability.

Materials and Methods

Pretreatment of the raw material: Fresh croaker (*Otolithus* sp.), purchased from the local market was washed, dressed by beheading, eviscerating, finning and scaling and cooked in open steam for 30 min. The exuded water during precooking was discarded and the cooked fish stored at —1°C approximately, until required (after thawing) for analysis and other experimental purposes. The minced and analysed fish was weighed and blended for 30 min in a waring Blender with water (a pre-calculated amount) and GMS emulsion to give a desired s/w ratio and GMS per cent in the slurry.

Preparation of GMS emulsion: The GMS emulsion was prepared by the method of Morgan *et al*[3].

Foam development: The apparatus for foam development comprised of a three-necked flask equipped with a glycerine-sealed motor-driven stirrer designed for low pressures, a thermometer and the gas inlet and outlet lines as well as the desired ice bath. The gas line consisted of a single stage regulator, a rotameter, a manometer, a compound pressure gauge, a gas bubbler and suitable stopcocks. The gas discharge line included suitable empty and moisture absorption traps, with necessary stopcocks for connection to vacuum pump.

Procedure: The system was evacuated initially after the contents of the flask attained the desired temperature and the vacuum broken by introduction of carbon dioxide gas. Next, the system pressure was adjusted, followed by setting in motion the stirring device. This, as well, marked the commencement of the whipping and gas injection time periods. The final evacuation was also resorted to in some of the earlier experiments. The foam developed after a definite time period was allowed to attain the room temperature and jerked manually, followed by transferring to another container to determine the extent of its collapse. Its physical characteristics were observed visually and the density determined.

In an attempt to determine and compare the properties of dried samples, i.e., the foamed product (F), non-foamed but slurried with same S/W ratio as that of F (NF) and the non-slurried cum non-foamed product, without any additional water (SL) with their precalculated weights, were spread uniformly on petridishes so as to manitain the same solid percentages per unit area in all the three cases. The drying was carried out in a laboratory air oven at temperatures between 55° and 65°C with the entering air varying in relative humidities from 42 to 80%. The samples were covered, removed and weighed and care was taken to maintain identical conditions during drying.

ISI methods were used for analysing the oil and moisture content of the fish used.

Results and Discussion

Table 1 gives the results of a series of experiments

TABLE 1. RESULTS OF FOAM DEVELOPMENT IN FISH SLURRIES

pH	7.0
Initial evacuation (final evacuation in Expt. 1 & 2)	–19 to –21 psig
CO_2 flow rate	1500–2000 ml/min.
Stirring rpm	200 approx
In expt. 5	260 approx
In expt. 6	60
Stirring and gas injection duration	30 min
Densities at 27°C	
(*a*) Foamed (F)	0.615 g/cm^3
(*b*) Non-foamed but slurried (NF)	0.940 g/cm^3
(*c*) Non-foamed cum non-slurried (SL)	1.002 g/cm^3

Expt No.	S/W	Slurry feed volume (ml)	GMS (%) (w/w)	Pressure (cm Hg)	System temp (°C)	Foaming characteristics
1.	0.13	200	1.0	10–12	15	Transient
2.	0.13	200	1.0	10–12	10	Stable
3.	0.13	200	0.75	10–12	10	"
4.	0.13	225	0.50	10–12	10	"
5.	0.13	200	0.50	2.3	7	"
6.	0.16	200	0.50	2.3	9	"
7.	0.16	175–200	0.25–0.45	2.3	8–14	No foam

conducted for development of a stable foamed structure out of fish slurries and conclusions drawn relate strictly to the experimental conditions mentioned in this table.

Results of experiments 1 and 2 reflect probably the favourable change of consistency of fish slurry or exhibit an improved property of GMS by decrease of temperature to 5°C. Such an effect of temperature lowering appears to persist till an optimum minimum value of 0.5 per cent of GMS is reached (Experiment 2, 3 and 4). Under the conditions studied, it is evident that the maximum temperature level of the system should not be increased beyond 10°C for stable foam generation. Another interesting finding of the investigation, highlighting the importance of consistency, is from the latter experiment that higher S/W values (0.16) can offset a much lower CO_2 flow rate with subsequent pressure drop over the system to generate the stable foam and also that an increase in S/W by 0.03 can be accompanied by a decrease in stirring rpm by 70 per cent for the same (Experiment No. 6).

Since these studies were carried out for evolving a new process for stable foam generation, only when once stable foam could be obtained under certain set of conditions, the foam density has been determined in some cases. The density obtained was very slightly higher (i.e. by 0.015 g/cm^3 at 27°C) as compared to the value given by Copley and Van Arsdel[4]. This can be attributed to this unexplored system with entirely a different composition under the given set of conditions. Since GMS has been reported by Morgan *et al*[3] as the more widely used as well as more versatile, and hence promising, foaming and stabilising agent, it had been arbitrarily chosen for the present system also. With the limited studies made here, the foaming times could not be reduced to find out if foams with lower densities could be obtained. Also CO_2 was preferred to other inert gases on account of its low cost, easy availability and its ability to displace adsorbed oxygen[1].

Notwithstanding the observations made by Hart *et al*[5], who reported that foams formed using CO_2 were unstable once they were removed from the carbon-dioxide atmosphere, it was possible to obtain stable foams in the present case of fish slurries.

TABLE 2. PHYSICAL CHARACTERISTICS OF FOAM MAT DRIED SAMPLES

sample type	Physical characteristics
SL	Yellowish; rough and almost continuous mat; most fragile; least shrunken.
NF	Whitish, longitudinal perforated ridges in the non-continuous mat; harder to break as compared to SL more shrunken than SL.
F	Whitish with obvious porous spots on a continuous mat; hardest to break and more shrunken than SL.

The apparent physical characteristics as observed in the dried mats are compiled in Table 2. It is to be assumed that during drying at 55°–65°C foams stability would be affected and hence the product quality would be lowered. In spite of this limitation, the product was quite acceptable and reconstituted well.

The results of preliminary attempt to evaluate the hydration characteristics of the dried products and their texture by organoleptic evaluation seem to indicate that the foam mat dried product had better properties in this regard. This is however subject to confirmation by further tests.

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Distribution of Lysine in Different Legumes and Some Species of Amaranthus Seeds*

HAMIDA ABDI AND MAHARAJ K. SAHIB
Division of Biochemistry, Central Drug Research Institute, Lucknow-226001

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Protein and lysine content of seven commonly occurring leguminous seeds have been determined. Protein of horse gram seed has highest lysine content. Black gram, Pea and red gram proteins are also rich in lysine. Seeds of different species of Amaranthus have been analysed for their protein and lysine contents. *A. hypochondrium* was found to have the highest lysine content. Leguminous seed coats contain non-protein nitrogen and some bound lysine. The seed coat of horse gram accounts for about 15% of the total lysine present in the seed. Horse gram kernel and Amaranthus seeds have been fractionated into four different proteins. Albumin constituted about 8% of the horse gram kernel and the major protein fraction in Amaranthus seeds. Most of the lysine of the seeds is present in albumin fractions and a small portion in the globulin and glutelin fractions. Prolamin contained very little lysine.

The amino acid composition of some common Indian pulses has been studied by several workers[1-3]. Bagchi *et al*[4] determined the essential amino acid content of some pulses by quantitative paper chromatography. Chemical methods for amino acid analysis have been used for sulphur containing amino acid by De and Desikachar[5].

The present paper reports values for lysine in seven legumes and its distribution in different parts of the seed. The lysine content of some species of Amaranthus seeds has also been determined.

Materials and Methods

Material: Pea, Bengal gram, black gram, red gram, green gram lentil and horse gram purchased from the local market, were soaked overnight in water at 4°C; seed coats and kernels were separated and dried to constant weight at 60-80°C. Dried samples were finely powdered. Samples of Amaranthus seeds obtained from National Botanical Gardens, Lucknow were dried and finely powdered.

Protein content: The protein content of the samples was determined by the microkjeldahl method.

Acid hydrolysis of the samples: The samples (0.1 g) were taken in glass ampoules and hydrolysed with 3 ml of 6N HCl for 24 hr at 105°C according to the method of Holmes[6]. After hydrolysis excess HCl was removed by repeated evaporation in a vacuum desiccator. The volume was made to 2 ml with borate buffer (pH 9.2) and used for analysis.

Communication No. 2164.

Lysine estimation: The method according to Salim and Ahmed[7] is based upon the formation of ε—DNP—lysine. One ml of freshly prepared copper phosphate suspension[8] was pipetted into 0.6 ml of protein hydrolysate plus borate buffer (pH 9·2) thoroughly mixed at 40°C for 10 min by using a mechanical shaker.

The suspension was centrifuged and to 1 ml of the supernatant 2.5 mg of 1—fluro—2,4—dinitrobenzene in 0.02 ml methanol was added and mixed by shaking at 40°C for one hour in dark. 2N HCl (2 ml) were added and H_2S was then bubbled for 2 min. The precipitated copper sulphide was removed by centrifugation and washed with 1N HCl. Supernatant and washings were mixed and the volume was made to 10 ml with 1N HCl. DNP derivatives of amino acids other than lysine were removed by repeated extraction with ether. The yellow colour of ε—DNP—lysine was measured at 390 mμ using a spectrophotometer.

Standard ε—DNP—lysine was prepared from lysine monohydrochloride and suitable blank of protein hydrolysate was prepared in which 1—fluro—2,4—dinitrobenzene was added just before ether extraction.

Protein fraction:[2] Horse gram kernel and Amaranthus seeds were fractionated into four different protein fractions–albumin, globulin, glutalin and prolamin according to the method of Mitchell[9]. The fractionation scheme is outlined in Fig. 1.

Results

Protein and lysine content of legumes: It is evident from Table 1 that all the commonly used pulses contain high protein content. Lysine is also high in case of legumes, horse gram showing the highest value.

Lysine content of leguminous seed coat: Table 2 represents the nitrogen and lysine contents of leguminous seed coats. The seed coat contained a fair amount of lysine in bound form, though devoid of protein. Non-protein nitrogen was present in almost all the pulses. Horse gram seed coat contained the highest amount of lysine in bound form.

TABLE 1. DISTRIBUTION OF PROTEIN AND LYSINE IN LEGUME

Legumes	Protein %	Lysine % of protein
Black gram (*Phaseolus mungo*)	23.6	7.5
Pea (*Pisum sativum*)	21.2	8.5
Green gram (*Phaseolus radiatus*)	17.3	6.9
Bengal gram (*Cicer arietinum*)	18.6	5.2
Red gram (*Cajanus indica*)	22.0	7.3
Lentil (*Lens esculanta*)	24.6	6.5
Horse gram (*Dolichos biflorus*)	22.6	14.5

Protein was calculated as N×6.25. Both the values are on dry wt basis.

Protein and lysine content of some Amaranthus species: Seeds of seven different species of Amaranthus. *A. hypochondrium. A.caudatus* var. *albiflora*, *A. caudatus* var. *alopecurus*, *A. caudatus* var. *Ag*-5 *A.metazzianus Ag*-16, *A.21BB* and *A.N.G. Lko-A*19—were analysed for their protein and lysine contents. The commonly available species *A.hypochondrium*, was found to be very rich in lysine as shown in Table 3.

TABLE 2. NITROGEN AND LYSINE CONTENT OF LEGUMINOUS SEED COATS

Legumes	Nitrogen % dry wt	Lysine % dry wt
Black gram	0.16	0.13
Pea	0.23	0.03
Green gram	0.18	0.16
Bengal gram	0.37	0.05
Red gram	1.00	0.18
Lentil	0.100	0.06
Horse gram	2.40	0.90

Both were calculated as per cent of dried seed coat.

TABLE 3. PROTEIN AND LYSINE CONTENT OF AMARANTHUS SEEDS

Amaranthus species	Protein %	Lysine % of protein
Amaranthus hypochondrium	15.00	10.0
A. caudatus var. *albiflora*	16.25	8.2
A. caudatus var. *alopecurus*	19.37	7.9
A. caudatus var. *Ag*-5	16.88	7.5
A. metagazzinnus Ag-16.	14.38	9.9
A. 21 *BB*	17.50	7.8
N. G. Lko-A 19	15.62	7.9

Protein was calculated as N×6.25 and reported on dry wt. basis.

TABLE 4. DISTRIBUTION OF LYSINE IN DIFFERENT PROTEIN FRACTIONS OF HORSE GRAM KERNEL

Protein fractions	Recovery % of kernel	Protein % %	Lysine % of protein
Albumin	8.00	85.6	6.80
Globulin	1.21	30.0	2.30
Glutelin	0.67	51.8	0.13
Prolamin	0.45	13.2	0.06

*On dry wt. of the fraction.

Percent recovery was calculated on the dry wt of protein fraction obtained from 100 g of kernel. Percent protein was calculated on dry wt of the protein fraction obtained. The protein fraclions were in crude state and were not of 100% purity.

TABLE 5. PROTEIN FRACTIONATION OF AMARANTHUS HYPOCHONDRIUM AND DISTRIBUTION OF LYSINE IN THE FRACTIONS

Protein fractions	Recovery % of seed	Protein* % %	lysine % of protein
Albumin	4.40	48.9	3.80
Globulin	1.00	13.7	1.20
Glutelin	0.56	8.5	0.60
Prolamin	0.76	7.2	0.18

*On dry wt. of the fraction

Percent recovery was calculated on the dry wt. of protein fraction obtained from 100 g of seed. Percent protein was calculated on dry wt. of the protein fraction obtained. The protein fractions were crude and were not of 100% purity.

Distribution of lysine in different protein fractions of horse gram kernel and Amaranthus seeds: Albumin, globulin, glutelin and prolamines were isolated from horse gram kernel and *A. hypochondiurm* seeds. The isolated proteins were reprecipitated by ammonium sulphate; dialysed against water and lyophylised. The protein and lysine content of powdered protein fractions were determined (Table 4 and 5).

The albumin fraction of horse gram kernel constituted 8 per cent of the whole seed (on dry wt basis). Albumin contained 80-90 per cent of protein and nearly all the lysine present in the seed kernel. About 2.5 per cent lysine was found in globulin; glutelin contained some lysine and very little or no lysine was present in prolamine (Table 4).

In Amaranthus seeds also the major content of lysine rich protein was albumin, constituting about 4.4 per cent of dry seed and containing about 50 per cent protein (Table 5).

Discussion

In India 70-80 per cent of the diet is made up of cereals which are deficient in lysine. Lysine deficiency not only affects the overall growth of the animals but also the development of lymphoid system making the animals more susceptible to infections[11]. When wheat or corn is supplemented with lysine or lysine rich protein, amino acids supplied are adequate Among the plant proteins pulses are a rich source of lysine. Lysine content of legumes is about 6 to 8 per cent of the protein.

It is evident that horse gram, black gram, pea, red gram and Bengal gram contain a very good amount of lysine; horse gram showing the maximum content of lysine (Table 1). Horse gram also contains an appreciable amount of methionine while the other legumes are deficient in methionine. Hence its nutritional value is higher than that of other pulses[4]. It would be an ideal material for supplementation of rice and wheat diets.

No chemical studies about the structure and amino acid content of leguminous seed coat have been reported. The present results suggest that the leguminous seed coat contains amino acids which also occur in the whole seedor kernel. Of these lysine is of special significance. The nature of the linkage of lysine or for that matter the non-protein nitrogen in the seed coat remains to be elucidated.

Another lysine rich source of plant origin is the protein of Amaranthus seeds having 8-10 per cent protein (Table 3). Nutritive values of legumes[12] and Amaranthus[13] have been investigated by many workers but not much work has been reported on their chemical analyses, protein fractionation or their amino acid content. *Amaranthus hypochondrium* has been found to be one of the richest sources of lysine of the commonly available species of Amaranthaceae.

During this investigation it was also found that nearly all the lysine content of horse gram kernel or Amaranthus seeds was present in the albumin fraction of the protein (Tables 4 and 5). Albumin constituted about 80-90 per cent of protein in the horse gram kernel but only 45-50 per cent in Amarnathus seeds.

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Factors Affecting Proteolysis in Milk by *Streptococcus cremoris*

B. M. KRISHNA AND S. M. DUTTA
National Dairy Research Institute, Karnal, Haryana, India

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Factors affecting proteolysis in milk by *Streptococcus cremoris* were studied. No significant difference in proteolytic activity of milk from cow, buffalo or goat was observed. Proteolytic activity increased with the intensity of heat treatment. A progressive increase in proteolysis in milk by *Str. cremoris* was also observed, when incubation temperature was raised from 15° to 37°C. Addition of metal salts to the milk did not increase proteolytic activity.

Lactic acid bacteria play an important role in the development of characteristic flavour and aroma of various milk products specially cheese[1-3]. The breakdown products of milk proteins viz. peptides, amino acids etc. contribute either by directly providing background to the cheese flavour or indirectly by conversion into compounds which contribute to the characteristic cheese flavour[4].

The present communication reports on the factors affecting proteolysis in milk by *Str. cremoris*.

Materials and Methods

The culture of *Str. cremoris* (C—1) for the present study was obtained from the culture collection of the Institute. It was propagated in sterlized reconstituted non-fat milk at 30°C for 24 hr and stored in the refrigerator until use.

Kind of milk: Non-fat dry cow milk obtained from experimental dairy of National Dairy Research Institute and reconstituted at 10 per cent level in distilled water in a homogenizer was used in addition to cow, buffalo and goat skim milk. It was sterilized at 121°C for 20 min.

Heat processing: Cow skim milk was subjected to different heat treatments–pasteurization (63°C/30 min), steaming (30 min) and sterilization (121°C/20 min).

Effect of metal ions: Metal salts viz. $MgSo_4$, $CuSO_4$ $CoCl_2$, $ZnSO_4$, $NiSO_4$, $MnCl_2$, $CaCl_2$, $LiSO_4$, $Fe_2(SO_4)_3$, and Ferric-citrate at 0.1 per cent level were added to cow skim milk and the milk was sterilized at 121°C/20 min. Milk was cooled to room temperature (30°C) and inoculated with *Str. cremoris* at 1 per cent level. It was incubated at 30°C and examined periodically for proteolytic activity by Hull's method[5].

Incubation temperature: Sterilized cow skim milk inoculated with *Str.cremoris* was incubated at 15, 22, 30 and 37°C and examined for proteolytic activity periodically.

Results and Discussion

The growth and activity of starter cultures vary with the brand of milk powder used in the preparation of reconstituted milk[6] as well as the kind of milk[7]. These variations may either be due to initial quality of milk, the intensity of heat treatment during processing milk to milk powder or due to variations in composition of milk from different species. Proteolysis by *Str. cremoris* was lower in goat and reconstituted skim milk as compared to skim milk from cow and buffalo (Table 1). The activity in cow and buffalo skim milk was however of the same order. This is important since in our country both cow and buffalo milk are used for the preparation of fermented milk products like butter and cheese.

Heat treatment which is associated with preparation of various milk products, brings about denaturation of milk proteins[8], the extent of which is dependent on the intensity of heat treatment[9]. An increase in proteolytic activity with the increase in the intensity of heat treatment (Fig. 1) may, therefore, be due to the denaturation of milk protein substrate by the intracellular protease of the organisms. Higher proteolytic activity observed with pasteurization as compared to steaming is interesting and need further investigation.

Mineral requirement for growth and activity of lactic acid bacteria is well established[10-13]. No increase in proteolytic activity could be recorded by any of the salts (Table 2). This may be due to the fact that the organism *Str. cremoris* used in the present study has no mineral requirement over and above the level present in milk.

Incubation temperature is known to be an important factor affecting growth and activity of starter cultures[14]. A progressive increase in proteolytic activity with increase in temperature of incubation (Fig. 2) may be due to enhanced rate of multiplication of the organisms.

TABLE 1. EFFECT OF DIFFERENT TYPES OF MILK ON PROTEOLYSIS BY *STR. CREMORIS*

Type of skim milk	Proteolytic activity (mg tyrosine liberated/g of curd) at different periods (hr) of incubation						
	4	6	9	12	15	24	30
Reconstituted	0.072	0.128	0.184	0.200	0.220	0.260	0.280
Cow	0.152	0.160	0.200	0.248	0.260	0.280	0.304
Buffalo	0.120	0.152	0.184	0.240	0.264	0.283	0.380
Goat	0.052	0.104	0.144	0.200	0.224	0.248	0.272

TABLE 2. EFFECT OF METAL IONS ON PROTEOLYSIS IN MILK BY *STR. CREMORIS*

Metal salts added	Proteolytic activity (mg tyrosine liberated/g of curd) at different periods (hr) of incubation					
	6	12	15	24	36	48
Control	0.180	0.218	0.234	0.272	0.306	0.318
$Mg\ So_4$	0.068	0.148	0.168	0.204	0.224	0.224
$Li\ SO_4$	0.044	0.110	0.130	0.180	0.210	0.224
$Cu\ SO_4$	0.112	0.172	0.200	0.240	0.268	0.280
$Co\ Cl_2$	0.076	0.148	0.172	0.212	0.238	0.280
$Fe_2(SO_4)_3$	0.092	0.160	0.180	0.220	0.260	0.286
$Zn\ SO_4$	0.068	0.152	0.178	0.224	0.248	0.248
$Ni\ SO_4$	0.064	0.172	0.188	0.212	0.226	0.226
$Mn\ Cl_2$	0.048	0.168	0.186	0.208	0.216	0.222
Fe-citrate	0.072	0.156	0.172	0.238	0.264	0.280
$Ca\ Cl_2$	0.172	0.204	0.222	0.250	0.292	0.308

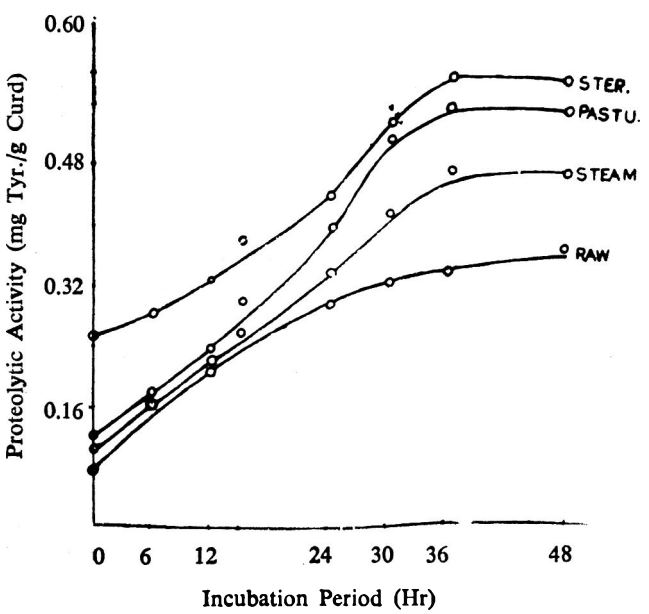

Fig. 1. Effect of heat treatment on proteolysis in milk by *S. cremoris*.

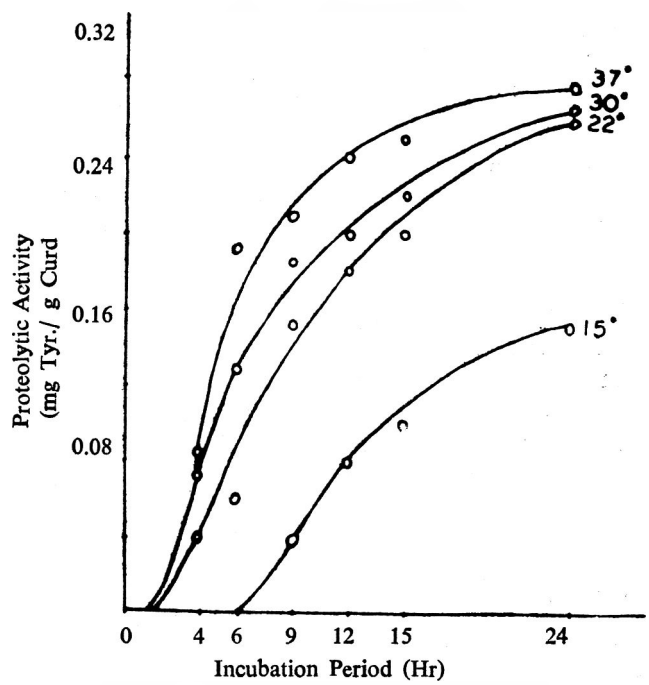

Fig. 2. Effect of incubation temperature on proteolysis in milk by *S. cremoris*.

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Estimates of Multiple Correlation Coefficients between Carcass Traits and Indicators of Meatiness of Lambs

J. S. SHARMA* AND C. F. PARKER

Department of Animal Science, Ohio Agricultural Research and Development Centre, Wooster, Ohio, U.S.A.

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Estimates of the multiple correlation of the 12 carcass traits (chilled carcass weight, average loin eye area, fat thickness over ¾ *L. dorsi* muscle, fat thickness over 5/6 shoulder, fat thickness at thickest point over 12th rib, fat thickness over 4-5 lumbar region, kidney and pelvic fat as percentage of chilled carcass weight, trimmed legs as percentage of chilled carcass weight, final grade, conformation grade, maturity grade and quality grade), and age at slaughter with 6 indicators of meatiness (estimated edible portion as percentage of slaughter weight or shipping weight, total fat as percentage of chilled carcass weight, U.S.D.A. yield grade, total edible portion of right leg and right shoulder, edible portion right legs×2 and total fat trim), are presented and discussed.

Chilled carcass weight was found to be the best predictor of meatiness as it was fitted first (both in male and female) for four out of the six indices of meatiness. Fat thickness at 5/6 shoulder was fitted first for both male and female with R values 0.430 and 0.567 respectively, for total fat as percentage of chilled carcass weight. This can be physiologically explained as more fat deposition is found in females as compared to males due to higher level of estrogen. For U.S.D.A. yield grade, fat thickness ¾ *L. dorsi* was fitted first in both males and females with R values of .955 and .928 respectively. Thus the variability in U. S. D. A. yield grade, explained by this single trait was found to be 91.2% and 86.1% in males and females respectively. Average loin eye area was found to be relatively less important in predicting indicators of meatiness. Grades were also not found to be important in predicting indicators of meatiness.

Lamb provides the major portion of the sheep producer's income. The producer continually faces problem of satisfying consumers demand for his product while producing it as cheaply as possible.

The human population is increasing while acreage available for live-stock production is decreasing. More over rising prices and the introduction of new products (synthetic fibres) have led to need for a change in sheep business. These events accentuate the need for efficiently produced lamb carcass that meet the nutritional and palatability requirements of the consumer. A recent trend in consumer preference is for meatier lamb cuts with less excess fat. These factors have stimulated research to produce lambs with improved carcass merit.

This study was undertaken to estimate the multiple correlation coefficients between carcass traits and indicators of meatiness of lamb.

Materials and Methods

The sheep (Targhee, Columbis and their crosses), maintained at Ohio Agricultural Research and Development Centre, Wooster, Under NC-50 project were used for this study.

**Present addresss:* Department of Animal Breeding, College of Animal Sciences, Haryana Agricultural University, Hissar, India.

In lamb raising, one major variable influencing early growth from birth to weaning from an environmental stand point was judged to be the quality and quantity of supplemental feed offered to the lambs during the nursing period. To eliminate supplemental diet as a major variable among nursing lambs and to permit more precise measurements and comparisons of growth rates, a complete ration diet, processed into 3/16″ pellets, was formulated. The pellets were fed free-choice to all lambs[1].

The lambs for slaughter were fed to a weight of 95 to 105 lb or to 180 days, whichever was earlier. The carcass traits and indicators of meatiness were recorded as described by Sharma and Parker[2].

Stepwise regression techniques were used for both the sexes separately to determine if several carcass traits would be any more useful in predicting indices of meatiness and also their relative importance in such predictions. Age at slaughter was fitted as a variable just to get rid of the variability which might have been in the data due to the different age at slaughter of the lambs. A total of 443 male and 182 female lambs were used in this study.

Results and Discussion

Overall averages of the carcass traits of experimental (males and females) separately are tabulated in Table 1. Multiple correlation coefficients between carcass traits and indicators of meatiness are tabulated in Tables 2 and 3.

Carcass weight: Chilled carcass weight was the variable fitted first for estimated edible portion slaughter weight (per cent), total edible portion, right leg and shoulder (lb) edible portion, right leg × 2 (lb) and total fat trim (lb) in both the sexes indicating its importance in predicting the indices of meatiness. It was not found to be of any importance in predicting U.S.D.A. yield grade. Thus chilled carcass weight was the variable most highly correlated with indicators of meatiness except when the carcass traits were the parts of the variable with which they were being correlated. Viz, correlation of fat thickness over $\frac{3}{4}$ *L. dorsi* muscle and kidney and pelvic fat with U.S.D.A. yield grade.

Studies concerning influence of carcass weight upon cutability have generally shown a reduction in percentage of consumer cuts with an increase in carcass weight. Barton and Kirton[3] found that the amount of separable fat in the lamb carcass was highly correlated with carcass weight of mature sheep, but some what lower in the association when considering light weight lamb carcass. This was in general agreement with the reports of several workers[4-11].

It has also been pointed out by various workers that as carcass weight increases, the rate of increase in *L. dorsi* area is reduced and fat deposition increases. Esplin *et al.*[12] reported that the error in adjusting the loin-eye area as a direct ratio to the chilled carcass weight resulted from basing adjustments on a ratio at a given weight and projecting this as a constant ratio; the ratio actually changed greatly due to large changes in weight and limited changes in loin-eye area. Similar findings are evident from the data of other investigators[9,13-23].

L. dorsi: Average loin-eye area was found to be relatively less important in predicting indicators of meatiness. Mathews[13] reported that cross sectional measurement of the loin-eye or rib-eye muscle were not as highly correlated as were fat measurements with either percentages of wholescale cuts or separable lean in the rack. Other workers, Botkin *et al*[24] and Stanley[25], reported higher correlations for the cross sectional areas of the leg than for rib-eye area when related to carcass or whole-sale lean content. Carpenter *et al.*[19] suggested that rib eye area was a fairly accurate measure of muscling in carcass of a narrow weight range but

TABLE 1. CARCASS TRAITS OF LAMBS

Trait	Sex	Overall average	Standard deviation
Chiled carcass wt (lb)	M	45.269	±3.145
	F	47.108	±3.278
Average loin-eye area, (in²)	M	1.940	±0.213
	F	1.854	±0.204
Fat thickness over $\frac{3}{4}$ *L. dorsi* (in.)	M	0.153	±0.065
	F	0.218	±0.069
Fat thickness over $\frac{5}{8}$ shoulder (in.)	M	0.348	±0.105
	F	0.485	±0.119
Fat thickness at thickest point over 12th rib (in.)	M	0.389	±0.142
	F	0.588	±0.173
Fat thickness over 4-5 lumber region (in)	M	0.331	±0.103
	F	0.424	±0.119
Kidney and pelvic fat as % of chilled carcass wt	M	2.968	±0.965
	F	3.123	±0.987
Trimmed legs as % of chilled carcass wt	M	22.657	±0.983
	F	22.362	±0.997
Final grade (1-15)	M	11.132	±0.853
	F	11.561	±0.875
Conformation grade	M	11.118	±0.865
	F	11.547	±0.891
Maturity grade	M	2.631	±0.498
	F	2.786	±0.517
Quality grade	M	11.448	±0.852
	F	11.877	±0.883

TABLE 2. MULTIPLE CORRELATION COEFFICIENTS BETWEEN CARCASS TRAITS AND INDICATORS OF MEATINESS

	Chilled carcass wt.		Av. loin eye area		Fat thickness								Kidney & pelvic (%)	
					3/4 L. dorsi		5/6 Shoulder		Thickest pt.		(6) 4-5 lumbar			
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Est. edible portion slaughter wt. (%)	0.620 (1)	0.550 (1)	0.712 (6)	0.735 (7)	0.663 (2)	0.657 (2)	0.698 (4)	0.705 (3)		0.731 (6)	0.740 (8)		0.721 (9)	0.725 (5)
Total fat chilled carcass wt. (%)	0.500 (2)	0.744 (5)	0.592 (4)	0.759 (6)	0.613 (5)	0.666 (2)	0.430 (1)	0.567 (1)		0.768 (8)	0.624 (6)	0.764 (7)	0.635 (7)	0.737 (4)
U.S.D.A. yield grade					0.955 (1)	0.928 (1)			0.999 (5)				0.955 (2)	0.997 (2)
Total edible, r. leg & shoulder (lb)	0.710 (1)	0.680 (1)		0.927 (7)		0.928 (9)	0.920 (5)	0.924 (4)	0.920 (4)	0.929 (10)		0.927 (8)	0.919 (3)	0.925 (5)
Edible portion r leg×2 (lb)	0.702 (1)	0.696 (1)				0.996 (3)	0.998 (5)		0.998 (4)			0.997 (6)		
Total fat trim	0.477 (1)	0.603 (1)	0.661 (4)	0.797 (4)	0.676 (5)	0.779 (3)	0.592 (2)	0.737 (2)		0.813 (7)	0.684 (6)	0.816 (8)	0.692 (7)	0.805 (5)

Numbers in the brackets indicate the consecutive step at which the independent variable was fitted.

that it added little to the predictive value if reliable measures of fatness were available.

Fat thickness: Fat thickness over ¾ *L. dorsi* was fitted Ist for U.S.D.A. yield grade and in the second position for estimated edible portion as slaughter weight (per cent) in both the sexes. Fat thickness 5/6 shoulder was fitted first for total fat chilled carcass weight per cent and in the second position for total fat trim. Fat thickness at thickest point and at 4-5 lumbar region were found to be less important in predicting the indicators of meatiness. Kidney and pelvic fat (per cent) was fitted in second position for the U.S.D.A. yield grade. Callow[4] and Willford and Carrigus[26] recognized early that the amount and distribution of fat was the major variable influencing lamb carcass composition.

TABLE 3 MULTIPLE CORRELATION COEFFICIENTS BETWEEN CARCASS TRAITS AND INDICATORS OF MEATINESS

	Trim'd legs Chilled carcass wt. (%)		Age at slaughter		Final grade		Conformation grade		Maturity grade		Quality grade	
	M	F	M	F	M	F	M	F	M	F	M	F
Est. edible portion slaughter wt (%)	0.716 (7)	0.746 (13)		0.742 (10)	0.687 (3)	0.746 (12)	0.719 (8)	0.714 (4)	0.707 (5)	0.741 (9)		0.745 (11)
Total fat chilled carcass wt (%)		0.772 (10)	0.550 (3)	0.773 (13)		0.773 (11)		0.773 (12)	0.636 (8)	0.771 (9)		0.716 (3)
U.S.D.A. yield grade	0.999 (4)				0.999 (7)		0.999 (3)	0.999 (3)			0.999 (6)	
Total edible, r. Leg & shoulder (lb)	0.916 (2)	0.916 (2)		0.922 (3)	0.920 (6)	0.926 (6)	0.921 (8)				0.921 (7)	
Edible portion r. leg×2 (lb)	0.998 (2)	0.996 (2)	0.998 (7)			0.997 (5)	0.998 (8)		0.998 (6)		0.998 (3)	0.996 (4)
Total fat trim			0.632 (3)			0.810 (6)			0.694 (8)	0.819 (9)		

Number in the brackets indicates the consecutive step at which the independent variable was fitted

Carpenter *et al*[19] found that as carcass weight, kidney fat or fat thickness over the 12th rib increased, there was an increase in fatness which resulted in a decrease in the yield of retail cuts.

Trimmed legs: Trimmed legs as percentage of chilled carcass weight were fitted in second position for the total edible right leg and shoulder (lb) and edible portion right leg $\times$ 2 (lb) in both the sexes.

Age at slaughter: Age at slaughter was fitted in 3rd position for total fat, chilled carcass weight (per cent) and total fat trim (lb) in the case of males and for total edible right leg and shoulder (lb) in the case of females.

Grades: Final grade was fitted in 3rd position for estimated edible portion as slaughter weight (per cent) in the case of males only. Similarly conformation grade, maturity grade and quality grade were also found less important as predictor of indices of meatiness. Today, lamb carcass grades are extensively used in spite of their apparent low relationship to cutability.[11,26,27] In this study also, the correlations among the grades and indicators of meatiness were not high.

Conclusion: (*i*) Chilled carcass weight was found to be the best predictor of meatiness. (*ii*) Amount and distribution of fat was found to be the major variable influencing lamb carcass composition. (*iii*) Average loin-eye area was found to be less important in predicting indicators of meatiness. (*iv*) Final grade was also not found to be much important in predicting indicators of meatiness.

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Effect of Stage of Lactation on the Distribution and Composition of Phospholipids in Milk Products

T. K. KUCHROO* AND K. M. NARAYANAN
National Dairy Research Institute, Karnal

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The distribution of phospholipids in cream and butter was influenced by the stage of lactation and species of milk used. The average proportions of phospholipids transferred from colostrum to cream and from cream to butter were 67 and 66% respectively in the case of buffaloes, and 60 and 64% respectively in case of cows. The proportions of phospholipid transferred from milk to cream and from cream to butter were found to decrease as the lactation progressed. The composition of phospholipids of cream, butter, butter-milk and skim-milk were almost the same as that of milks from which they were prepared.

The distribution of the fat globule in milk is affected by various factors such as stage of lactation, breed, and species,[1–3]. Since the phospholipids exist on the membrane surface of fat globules, the size of fat globule in milk may influence the phospholipid content of cream and butter. Since the average size of fat globules of buffalo and cow milks are known to be different[2,3], the percentage distribution of the phospholipid in cream and butter obtained from these two species should be different. In the present investigation an attempt was made to study the effect of stage of lactation on the distribution and composition of phospholipids in milk products prepared from both buffalo and cow milks.

Materials and Methods

Pooled milk samples from 5 to 7 animals were collected at different stages of lactation from the herd kept at the Institute separately from cows and buffaloes. Colostrum samples used for the study were only the first milkings after the calving. Early lactation milk was collected from animals between 30 and 60 days of lactation period, and middle lactaion milk was obtained from animals between 120 and 160 days of lactation period. Late lactation milk was collected from animals which had completed 280 days of lactation period. Samples in each group were collected at the same time for comparative purposes and three trials in each group were conducted.

The cream and butter were prepared from both cow and buffalo milks using standard manufacturing procedure. The distribution of phospholipids during separation of milk and churning of cream was calculated from the actual yields and phospholipid contents of the products obtained.

Fat percentage of milk and milk products was determined by Rose-Gottlieb method using Mojonnier fat extraction apparatus[4]. Phospholipid content of milk and milk products was estimated according to method of Rama Murthy and Narayanan[5]. Individual phospholipids were separated by thin layer chromatography and determined as described by Kuchroo and Narayanan[6].

Results and Discussion

Distribution of phospholipids in cream and butter: From Table 1 it is evident that the average phospholipid content of cream, skim-milk, butter and butter-milk obtained from buffalo colostrum were 252.0, 16.9, 307.0 and 187.0 mg/100 g respectively. The corresponding values for cow colostrum were 302.0, 22.4, 386.0 and 218.0 mg/100 g respectively. From Table 2 it is seen that the average proportion of phospholipids passed from colostrum to cream was 67 per cent in buffaloes and 60 per cent in cows indicating that the proportion of transfer of phospholipids to cream was slightly greater in buffaloes than in cows. The average proportions of phospholipids passed from buffalo and cow colostral cream to butter were 66 and 64 per cent respectively.

During the preparation of butter from buffalo and cow milks, the distribution of phospholipids in cream, skim-milk, butter and butter-milk varied depending upon the types of the milk used (Table 2). On separation of buffalo milk, the average proportions of phospholipids passed from early, middle and late lactation milks to cream were 60, 56 and 50 per cent respectively,

**Present address:* Haryana Milk Foods, Pehowa, Kurukshetra
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TABLE 1. EFFECT OF STAGE OF LACTATION ON FAT AND PHOSPHOLIPID CONTENTS OF MILK AND MILK PRODUCTS*

Product	Species	Stage of lactation: Colostrum Fat %	Colostrum phospholipid mg%	Early Fat %	Early phospholipid mg%	Middle Fat %	Middle phospholipid mg%	Late Fat %	Late phospholipid mg%
Milk	Buffalo	5.5	45.1	6.5	32.0	8.4	37.0	9.3	48.0
	Cow	4.5	50.4	4.1	30.0	4.8	35.0	5.6	45.0
Skim-milk	Buffalo	0.3	16.9	0.1	13.9	0.1	19.8	0.1	29.0
	Cow	0.4	22.4	0.1	13.2	0.1	18.0	0.1	28.0
Cream	Buffalo	44.0	252.0	46.0	136.0	44.0	111.0	44.0	114.0
	Cow	41.0	302.0	44.0	189.0	43.0	173.0	42.0	161.0
Butter	Buffalo	80.7	307.0	81.8	161.0	82.0	130.0	82.0	119.0
	Cow	81.0	386.0	82.8	225.0	81.2	208.0	81.0	159.0
Butter-milk	Buffalo	0.7	187.0	0.8	104.0	0.6	89.0	0.6	109.0
	Cow	0.8	218.0	0.7	149.0	0.6	139.0	0.8	163.0

*Average of 3 samples

indicating that it decreased as the lactation progressed. The corresponding values for cow milk were 57, 53 and 47 per cent respectively. These showed that average proportions of phospholipids passed from milk to cream were lower in cows than in buffaloes. Similar findings were also observed during preparation of butter. Using early, middle and late lactation buffalo milks, the average proportion of phospholipids passed from cream to butter were 66, 62 and 55 per cent respectively. Similarly the values using early middle and late lactation cow milks were 63, 59, and 50 per cent respectively.

The above results indicate that proportions of phospholipids passed from milk to cream and from cream to butter were influenced by the stage of lactation and species of the milk used. Since much of the phospholipids in milks is in fat globule layer it is probable that those variations in the distribution of total phospholipids between cream and skim-milk in the separation of milk, and between butter and butter-milk in the churning of cream, were due to differences in the sizes of fat globules in milk. It has been reported[7] that the average size of the globule in colostrum was high and as the lactation advanced there was a decrease in the fat globule size of buffalo and cow milks. The greater transfer of phospholipids in cream and butter observed in the present study from milks containing higher average fat globule size may be due to the affinity of the bigger fat globules to go along with the cream and butter, and of the smaller ones to skim-milk and butter-milk during separation and churning respectively.

There are no reports available on the distribution of phospholipids in cream and butter as influenced by stage of lactation. McDowell[8] found that the proportion of phospholipids passing from cow's whole milk to skim-milk varied from 40.6 to 48.0 per cent. Rama Murthy and Narayanan[5] observed that during separation of cow milk about 46-63 per cent of total phospholipids went into cream, against 57-68 per cent in buffalo milk. Asker *et al.*[9] have reported high distribution of phospholipids (about 75 per cent of the total phospholipids) in cream from buffalo milk. Few reports are also available on the distribution of phospholipids in butter. Holm *et al.*[10] reported that using cow milk approximately 30-45 per cent of total phospholipids passed from cream to butter. McDowell[8] reported that average proportion of phospholipids lost was from 57.1 to 61.8 per cent. Rama Murthy

TABLE 2. EFFECT OF STAGE OF LACTATION ON THE DISTRIBUTION OF PHOSPHOLIPIDS DURING SEPARATION OF MILK AND CHURNING OF CREAM*

Stage of lactation	% distribution of phospholipids: Milk separation Cream	Milk separation Skim milk	Cream churning Butter	Cream churning Butter milk
Buffalo				
Colostrum	67.0	33.0	66.0	34.0
Early	60.0	37.0	66.0	34.0
Middle	56.0	44.8	62.0	38.0
Late	50.0	49.0	55.0	45.0
Cow				
Colostrum	60.0	38.0	64.0	36.0
Early	57.0	40.0	63.0	37.0
Middle	53.0	45.0	59.0	41.0
Late	47.0	53.0	50.0	50.0

*Average of 3 samples.

TABLE 3. COMPOSITION (WEIGHT %) OF PHOSPHOLIPIDS OF MILK AND MILK PRODUCTS*

Components	Milk		Skim-milk		Butter-milk		Cream		Butter	
	Buffalo	Cow	Buffalo	Cow	Buffalo	Cow	Buffalo	Cow	Buffalo	Cow
PC	29.5	32.2	28.9	30.0	30.0	28.9	28.7	32.2	30.2	31.3
PE	29.9	38.6	29.8	38.5	30.0	36.1	31.0	37.4	29.8	38.3
PS	3.1	5.5	3.6	6.2	3.7	5.1	3.6	5.0	3.9	5.3
Sph	32.0	19.2	33.3	20.6	31.7	23.5	31.2	20.0	31.5	20.0
PI	4.4	4.0	3.8	4.3	3.9	5.4	4.7	4.8	4.1	4.5
LPC	0.3	0.2	0.2	0.1	0.2	0.3	0.2	0.2	0.2	0.2
LPE	0.4	0.3	0.4	0.3	0.5	0.6	0.5	0.4	0.3	0.4

*Average of 3 samples.

PC = Phosphatidyl choline
PE = Phosphatidyl ethanolamine
PS = Phosphatidyl serine
Sph = Sphingomyelin
PS = Phosphatidyl inositol
LPC = Lyso phosphatidyl choline
LPE = Lyso phosphatidyl ethanolamine

and Narayanan[5] observed that buffalo cream showed more transfer of phospholipids to butter than cow cream. In the present study, the differences noticed in cow and buffalo milks on the percentage distribution of phospholipids in cream and butter are in general agreement with the above findings.

Composition of phospholipids in milk and milk products: Since differences were observed in the percentage distribution of total phospholipids during the preparation of cream and butter from buffalo and cow milks, it was also of interest to study the composition of phospholipids in these products. Pooled samples of buffalo and cow milks were separately used for this investigation and the composition of phospholipids in milk, cream, skim-milk, butter and butter-milk obtained are presented in Table 3.

From these it is seen that although there were slight differences in proportion of individual phospholipids of buffalo and cow milks, the percentage distribution of individual phospholipids in skim-milk, cream, butter and butter-milk was almost the same as that of milks from which they were prepared. Earlier, a few investigators have also studied the composition of phospholipids in dairy products obtained from cow milk. Rhodes and Lea[11] compared the composition of phospholipid fractions present in butter, butter-milk and skim-milk, and found no appreciable differences. Patton *et al*[12] have demonstrated by TLC the same classes of phospholipids with similar intensities of spots in milk, skim-milk, cream and butter-milk. Nakanishi and Kaya[13] studied the phospholipids in cream, and skim-milk, and reported same classes of phospholipids with similar concentrations. The presen study confirms the above findings and establishes that though buffalo milk and cow milk differ in their distribution of total phospholipids in products, the phospholipid composition of these products are more or less the same as that of milk from which they were prepared.

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Suitability of Indian Durum Wheats for Semolina Milling and Vermicelli Preparation

A. Rahim, C. Prabhavathi, P. Haridas Rao and S. R. Shurpalekar
Central Food Technological Research Institute, Mysore

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Among several varieties of Indian durum wheats evaluated, *NI*-5759, *A*-11, *A*-1-8-1, *MPO*-159, *HI*-7597, *HD*-4519, *HD*-4530 and *WL*-1002 gave excellent semolina yields of more than 55% which were comparable to Canadian durums—*Stewart* 63, *Wakooma* and *Hercules* 72 used as control. Twenty-two Indian varieties and Canadian *Hercules* 72 and *Wakooma* were found to have good to excellent vermicelli making quality. The Indian variety, *A*-1-8-1 showed excellent semolina milling quality and vermicelli making quality.

The Farinograph characteristics of semolina showed that *A*-11, *A*-1-8-1, *A*-9-30-1 and *MPO*-159 and Canadian *Wakooma* and *Hercules* 72 had a low dough development time of 3-6 min and very high tolerance index of 90 B.U. or more. Dough development time for durum semolina covered a wide range of 3 to more than 35 min.

Quality and utilization of Indian durum wheats (*Triticum durum*) have attracted very little attention of research workers in the past. Although durums are mainly milled as semolina and used in different types of paste goods, very little information is available on Indian varieties.

Among different paste goods, vermicelli is popularly used in India for savoury as well as sweet preparations. Since Canadian durums are widely used in the manufacture of paste goods, a comparative study of vermicelli making quality of semolina from Indian and Canadian durums can serve as an index of their suitability for different paste goods. Results of studies on semolina milling quality, Farinograph characteristics of the semolina doughs and vermicelli making quality of semolina from 28 varieties of Indian durums as compared to 3 varieties of Canadian durums are presented in this paper.

Materials and Methods

Raw materials: Twenty-eight varieties of durum wheats (listed in Table 2) covered under All India Coordinated Wheat Research Programme and received from different Agricultural Research Stations were used for evaluation of semolina milling and vermicelli making quality. The durum wheats—*Hercules* 72, *Stewart* 63 and *Wakooma* procured from Grain Research Laboratory Winnipeg, Canada were used as control in these studies.

Semolina milling: The semolina yielding quality of various durum wheats was evaluated using Buhler laboratory mill (Model MLU-202) under modified conditions according to Rahim *et al*[1]. Clearances for break roolls B_1 and B_3 were maintained at 0.30 and 0.20 mm respectively.

The semolina fraction was mechanically sieved in Buhler Plansifter to obtain coarse (-28 and +45 mesh) and fine (–45 and +6xx) fractions. The flour fraction consisting of throughs of 6xx sieve was combined with the other flour fraction from break rolls.

Farinograph characteristics: The Farniograph characteristics of fine semolina were studied according to procedures described by Irvine *et al*[2]. However, for dough formation, 17.5 ml of water was added to 50g of semolina on 14 per cent moisture basis. The Farinograph curves were allowed to run until 5 min after reaching maximum consistency. The data regarding the dough development time, maximum consistency and tolerance index were recorded.

Vermicelli making quality: For assessing the vermicelli making quality of fine semolina, a dough having a maximum consistency of 500 B.U. was obtained. The dough was removed from the Farinograph mixer 1 min after reaching the maximum consistency. Vermicelli were then extruded from a hand-press, using a brass disc with perforations of 1.5 mm diameter. Ten cm lengths were cut and dried at 60°C in a oven (TEMPO : Model TI-1268) with forced air circulation. The drying period was adjusted to about 2 hr so as to obtain a product having about 6 per cent moisture.

Cooking quality of vermicelli: For evaluating the cooking quality of vermicelli, 10 g of vermicelli were added to 100 ml of simmering water at 95-97°C. Cooking was continued for 30 min. The cooked vermicelli was then drained over a Buchner funnel. Total solids in

the drained water and the water absorbed by the cooked product were determined according to the procedure adopted by Binnington *et al*[3]. The colour, odour, texture, stickiness and disintegration characteristics of the cooked product were also evaluated.

Results and Discussion

Semolina milling quality: The yields of semolina and other products are given in Table 1. Of the 28 Indian durums evaluated, 8 varieties yielding more than 55 per cent semolina were graded as excellent, while 15 varieties with yields ranging between 50 and 55 per cent were classified as good. Other varieties yielding less than 50 per cent were considered as fair. All the 3 Canadian durums had excellent semolina yields. It was also observed that in all but two varieties, the yields of fine semolina ranged between 37 and 42 per cent. Interestingly enough, 8 varieties had bran yield of 25 to 30 per cent, while 6 varieties yielded 31 to 39 per cent of bran fraction. This may be attributed to the same conditions of milling such as tempering moisture level of 18.5 per cent[4] clearance between break rolls, etc., for different varieties varying in physical characteristics such as size, shape and hardness of the grains. All the 3 Canadian and 11 Indian durums gave flour yields of 20 per cent or more.

Farinograph characteristics: Half of the varieties

TABLE 1. SEMOLINA MILLING QUALITY OF DURUM WHEATS[a]

Variety	Semolina yields				
	Fine[b] (%)	Coarse[c] (%)	Total (%)	Flour[d] (%)	Bran (%)
A-11	39.8	18.2	58.0	17.7	24.3
A-1-8-1	39.7	15.4	55.1	20.5	24.4
HD-4519	41.8	13.3	55.1	21.5	23.4
HD-4530	39.9	15.7	55.6	21.3	24.2
HI-7597	41.2	15.4	56.6	19.2	24.2
MPO-159	41.9	17.2	59.1	17.5	23.4
NI-5759	41.2	15.4	56.6	19.2	24.2
WL-1002	39.4	16.2	55.6	13.2	31.2
A-1-1-2-3	38.9	14.6	53.5	15.3	31.2
A-206	40.2	12.6	52.8	21.0	26.2
A-624	36.9	14.1	51.0	22.8	26.2
A-9-30-1	40.1	14.3	54.4	21.1	24.5
HD-4502	37.6	14.3	51.9	20.6	27.5
HI-7483	40.4	13.8	54.2	22.2	23.6
HI-7747	38.1	15.8	53.9	13.6	32.5
Bansi	40.9	12.8	53.7	16.4	29.9
MPO-157	37.6	14.1	51.7	18.2	30.1
MPO-161	37.8	13.6	51.4	18.9	29.7
N-59	41.2	10.6	51.8	21.7	26.5
NP-404	40.9	11.1	52.0	20.1	27.9
Raj-911	38.0	11.6	49.6	13.4	37.0
Raj-912	41.4	8.4	49.8	16.4	33.8
Bijaga Yellow	39.6	10.6	50.2	16.1	33.7
HI-7720	28.0	17.4	45.4	15.7	38.9
Amrit	37.8	10.0	47.8	16.4	35.8
Local red	37.2	10.5	47.7	16.5	35.8
Bijaga red	35.5	8.6	44.1	20.0	35.9
MPO-142	29.6	11.0	40.6	23.1	36.3
Stewart-63	39.5	16.1	55.6	25.1	19.3
Wakooma	41.6	15.1	56.7	20.5	22.8
Hercules-72	42.4	16.8	59.2	20.9	19.9

[a]–Break roll clearances of 0.30 mm for B_1 and 0.20 mm for B_3 were used.
[b]–Throughs of 45 mesh and overtails of 6xx.
[c]–Throughs of 28 mesh and overtails of 45 mesh.
[d]–Throughs of 6xx sieve,

TABLE 2. FARINOGRAPH CHARACTERISTICS OF DURUM SEMOLINA[a]

Variety	Dough development time (min.)	Maximum consistency (B.U.)	Tolerance index (B.U.)
Local red	21.5	380	20
Bijaga yellow	16.0	380	0
Amrit	15.0	460	20
Bijaga red	22.0	420	0
N-59	8.0	520	60
NI-5759	7.0	400	60
A-206	10.0	600	80
A-624	4.8	450	70
A-1-1-2-3	14.0	660	40
A-11	4.5	500	180
A-1-8-1	4.0	500	160
A-9-30-1	6.0	520	110
MPO-157	6.0	560	40
MPO-142	5.5	450	50
MPO-159	3.0	470	100
MPO-161	4.0	480	50
Raj-911	5.5	660	20
Raj-912[b]	—	—	—
NP-404	10.0	600	60
HI-7483	11.0	760	40
HI-7747	35.0	600	40
HI-7720	5.5	565	40
HI-7597	3.5	400	70
WL-1002	10.0	400	40
HD-4519	12.0	450	50
HD-4530	6.0	440	60
HD-4502	4.5	380	60
Bansi (Commercial)	11.5	440	0
Stewart-63	6.0	420	60
Wakooma	6.0	480	90
Hercules-72	3.5	490	120

[a]At 35% water absorption.
[b]Could not form the dough at 35% water absorption.

TABLE 3. COOKING QUALITY OF VERMICELLI FROM DURUM SEMOLINA[a]

Variety	Creamy colour	Odour	Texture	Stickiness	Water absorption (%)	Solids lost in cooking (%)
Local red	Pale	Mild	V. soft	V. sticky	540	8.0
Bijaga yellow	Bright	Somewhat strong	Soft	Nil	620	7.7
Amrit	Dull	Mild	Soft	Sticky	580	7.8
Bijaga red	,,	Strong	Slightly hard	,,	575	7.8
N-59	Normal	,,	Soft	Nil	665	9.9
NI-5759	,,	,,	,,	,,	663	9.9
A-206	,,	,,	,,	,,	690	7.4
A-624	Bright	Mild	,,	Slight	640	11.5
A-1-1-2-3	Pale	Strong	,,	Nil	680	9.3
A-11	Bright	Mild	,,	,,	550	8.5
A-1-8-1	Pale	,,	,,	,,	634	7.3
A-9-30-1	Bright	,,	,,	,,	675	7.5
MPO-157	,,	,,	,,	Slight	690	7.4
MPO-142	,,	,,	,,	Nil	610	9.3
MPO-159	,,	,,	,,	,,	610	9.6
MPO-161	,,	,,	Slightly hard	,,	560	7.6
Raj-911	,,	,,	Soft	,,	580	11.3
Raj-912	Pale	,,	,,	,,	560	9.5
NP-404	Bright	Strong	,,	,,	650	10.1
HI-7483	,,	,,	,,	,,	675	9.7
HI-7747	,,	,,	,,	Slight	530	6.2
HI-7720	,,	,,	Slightly hard	Nil	520	9.9
HI-7597	,,	,,	Soft	,,	650	10.0
WL-1002	Pale	,,	,,	,,	600	11.2
HD-4519	Bright	Mild	,,	,,	530	11.7
HD-4530	,,	,,	,,	,,	585	10.0
HD-4502	,,	,,	,,	,,	645	10.9
Bansi	,,	,,	,,	Slight	607	9.4
Stewart-63	Pale	,,	,,	,,	565	11.4
Wakooma	Bright	,,	,,	Nil	640	9.6
Hercules-72	,,	,,	,,	,,	625	10.0

[a]No disintegration was observed except in Local red and Amrit varieties which showed slight disintegration.

tested had a dough development time ranging between 3 and 9 min as observed also in Canadian wheats (Table 2). The durums with low development times are generally suitable for macaroni type of dough as it is associated with higher protein content and good grade semolina for macaroni preparation. Nine varieties had a dough development time of 10-16 min while the remaining 3 varieties - *Local red*, *Bijaga red* and *HI*-7747 - had excessively high dough development time of 22 min or more. Maximum dough consistency of 10 varieties exceeded 500 B.U., while values for remaining varieties including Canadian durums ranged between 380 and 500 B. U. Higher the mixing consistency higher is the protein content of the semolina which is a desirable feature for minimising the cooking losses. For macaroni type of products semolina should have less elastic and more extensible gluten in the dough and this is reflected by the higher tolerance index of the dough. Only varieties *A*-11, *A*-1-8-1, *A*-9-30-1, *MPO*-159 had high tolerance index of 90 B.U. or more like those of the Canadian *Wakooma* and *Hercules*-72. As such, they are likely to be suitable for macaroni type products. Eleven varieties had a low tolerance index of 0-50 B.U. while those of the remaining 12 varieties including *Stewart*-63 ranged between 50 and 80 B.U.

Evaluation of cooked vermicelli: For grading of cooked vermicelli, a minimum of 500 per cent water absorption during cooking was considered as one of the main criteria. The other impotrant criterion was loss of less than 8, 10 and 12 per cent solids in cooking water for excellent, good and fair grades respectively. In addition to these criteria colour, odour, texture, stickiness and disintegration also contributed to the

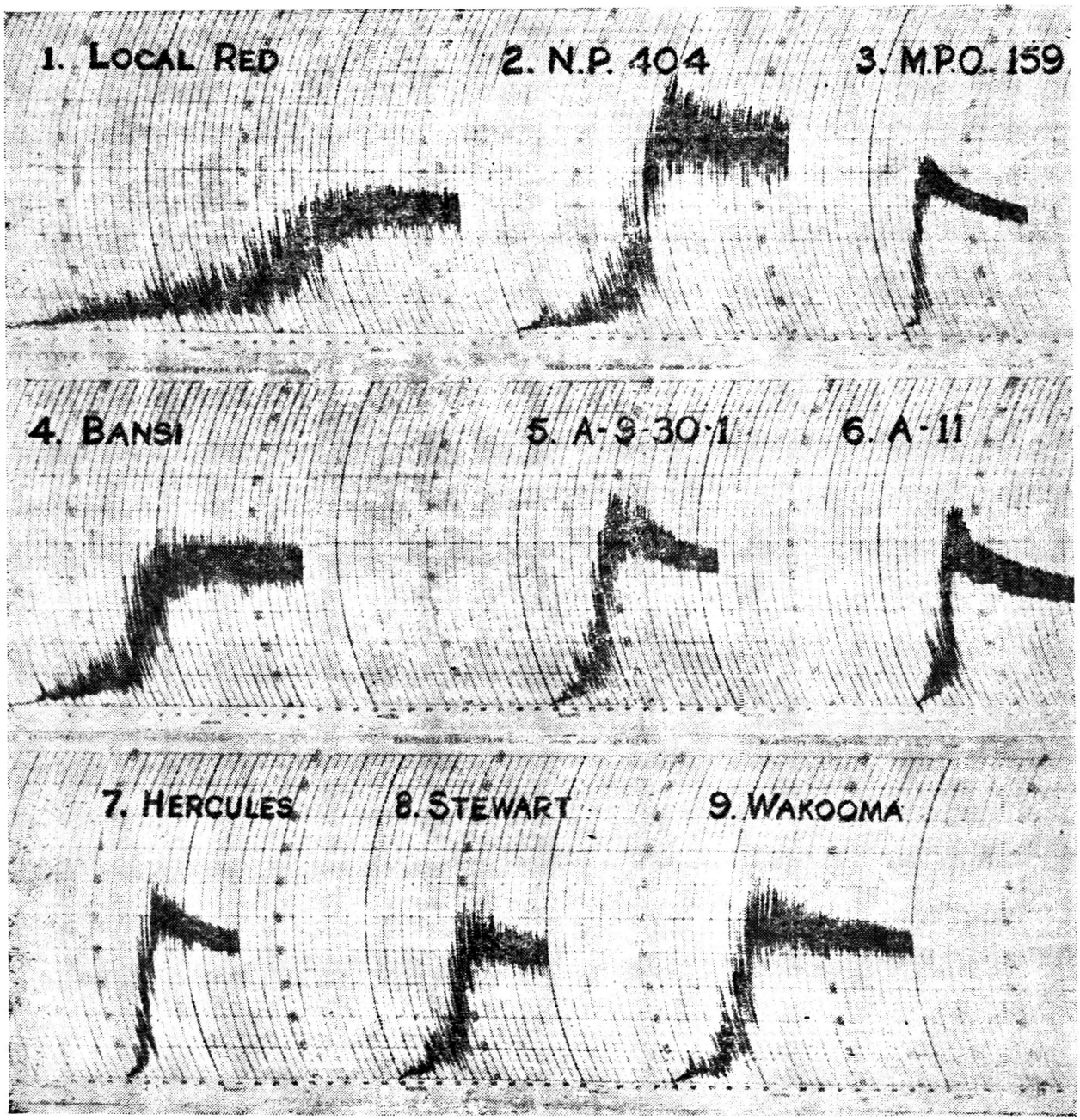

Fig. 1. Typical farinograph curves for semolina from some varieties of durum wheats

Varieties 1, 2 & 3 indicate long, medium and short dough development time respectively. Varieties 4, 5 & 6 show low, medium and high tolerance index respectively. Varieties 7, 8 & 9 are Canadian durums as control.

overall acceptability of the cooked vermicelli. Of the 31 varieties evaluated on this basis, 10 could be classified as excellent and 14 varieties including the Canadian *Wakooma* and *Hercules*-72, as good (Table 3). The remaining varieties were classified as fair. Inspite of having a very soft texture, vermicelli from *Local red* and *Amrit* were sticky and disintegrated slightly. On the contrary, varieties *MPO*-161 and *HI*-7720 had slightly hard texture but were neither sticky nor displayed any disintegration.

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Effect of Germination Temperature on Amylolytic and Proteolytic Activities of Bajra and Barley During Germination

ASHA PAL, D. S. WAGLE AND V. S. SHEORAIN*
Department of Chemistry & Biochemistry, Haryana Agricultural University, Hissar-125004

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Decreasing germination temperatures from 35 to 25°C in Bajra and from 25 to 15°C in barley, a significant increase in total amylolytic activity as well as proteolytic activity of green malts prepared from the two cereals was noted. Increase in total amylolytic activity was mainly due to β-amylase in both the malts, more so in bajra malt. Apparently there was no significant difference among the three varieties of either of the two cereals. Present study suggests that the germination at low temperature leads to better yield as well as quality of malt.

Preliminary work from this laboratory[1] showed that bajra grains possess all the qualities needed for malt preparation when compared with barley. Subsequently Pal *et al*[2]. prepared malt from the two cereals in a laboratory trial and compared them for their various properties and concluded that bajra malt was as good as barley malt. Amongst the many factors affecting the yield as well as quality of malt, germination temperature is one which plays an important role[3–6]. Optimum germination temperature for barley during malting had been found to be 15-17°C, though maximum germination and growth of seedlings was noted at 25°C. Therefore, the present study was aimed at finding a suitable germination temperature at which maximum enzyme activities in green malt could be obtained.

Materials and Methods

Three varieties each of bajra viz., 'HB-1', 'HB-3' and 'HB-4' and of barley viz., 'BG-1', C-138' and'C-164 were procured from the Department of Plant Breeding, Harayana Agricultural University Hissar. Germination studies were carried out as described elsewhere[1] at different temperatures for 3 and 6 days for bajra and barley respectively. Green malts were prepared from these cereals following the method of Pal *et al*[2]. Enzymes were extracted according to the method of Nason[7] with a few modifications. The amylolytic activity (i.e. total, α-and β-amylases) was assayed by the method described by Sheorain and Wagle[8]. Proteolytic activity was estimated by the method reported by Arima *et al*[9]. Protein was estimated according to Lowry

TABLE 1. EFFECT OF GERMINATION TEMPERATURE ON AMYLOLYTIC AND PROTEOLYTIC ACTIVITIES IN BAJRA DURING GERMINATION

Germination temp (°C)	Varieties	Total amylolytic activity	α-amylase activity	β-amylase activity	Proteolytic activity[1]
		mg maltose formed/3 min/mg protein at 37°C			
25	HB-1	46.41	15.51	30.90	55.09
	HB-3	32.19	11.97	20.22	46.43
	HB-4	38.56	13.66	24.90	37.78
30	HB-1	30.48	12.27	18.21	46.43
	HB-3	29.28	10.53	18.75	32.81
	HB-4	27.80	11.82	15.98	36.42
35	HB-1	19.08	11.88	7.20	29.64
	HB-3	14.97	9.21	5.76	26.71
	HB-4	15.83	10.35	5.48	25.18

[1] μg tyrosine formed/20 min/mg protein at 35°C

**Present address:* Deptt. of Biochemistry, Postgraduate Institute of Medical Education & Research, Chandigarh-160011. INDIA.

TABLE 2. EFFECT OF GERMINATION TEMPERATURE ON AMYLOLYTIC AND PROTEOLYTIC ACTIVITIES IN BARLEY DURING GERMINATION

Germination temp (°C)	Varieties	Total amylolytic activity	∝-amylase activity	β-amylase activity	Proteolytic activity*
		mg maltose formed/3 min/mg protein at 37°C			
15	BG-1	99.35	32.75	66.60	32.14
	C-138	80.61	22.62	57.99	31.46
	C-164	71.05	23.34	47.71	35.12
20	BG-1	71.04	26.44	44.40	27.80
	C-138	70.38	16.92	53.46	29.09
	C-164	56.39	22.94	33.45	33.81
25	BG-1	59.66	16.76	42.90	22.27
	C-138	63.90	14.10	49.80	19 45
	C-164	35.85	10.05	25.80	21.05

* μg tyrosine formed/20 min/mg protein at 35°C

et al[10]. Activity of amylases was expressed as mg of maltose formed in 3 min at 37°C per mg protein and proteolytic activity was expressed as micrograms of tyrosine formed in 20 min at 35°C per mg protein.

Results and Discussion

From a perusal of amylolytic activity of bajra malt prepared from seeds germinated at three different temperatures (Table 1), it was found that the total amylolytic activity increased in all the three varieties. This increase was mainly attributed to increase in β-amylase activity rather than ∝-amylase since activity of the former increased significantly with the decrease in germination temperature and ∝-amylase, however, was not much affected. Almost a similar trend was observed in the case of barley malt (Table 2) except that β-amylase from this source was not sensitive enough to the germination temperature i.e., gain in total amylolytic activity was equally contributed by both ∝-amylase as well as β-amylase.

Higher proteolytic activity was noted in the case of bajra malt (Table 1) when germination temperature was lowered from 35 to 25°C unlike the barley malt (Table 2) where activity showed some increase when temperature was lowered from 25 to 20°C but further lowering of temperature to 15°C did not lead to significant increase in the activity. It was interesting to note that there was no varietal difference in any of the two cereals tried for amylolytic or proteolytic activities because all of them followed a similar pattern.

The present study, therefore, revealed that effect of germination temperature on the two enzymes under investigation was more or less same both in bajra and barley malts. This observation has confirmed the earlier reports by Leners[5,6] in case of barley malt whereas it gave new temperature of germination for bajra. On the basis of this study gremination temperature of 15 to 25°C in case of barley and bajra respectively can be recommended on account of higher amylolytic and proteolytic activities which are the attributes to a good quality malt.

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A Simple Methylene Blue Reduction Test to Distinguish Cotton-Tract Buffalo Ghee from Normal Ghee

O. P. SINGHAL, N. C. GANGULI AND NOSHIR N. DASTUR*
National Dairy Research Institute, Karnal

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A simple and quick methylene blue dye reduction test for differentiating cotton-tract buffalo ghee from normal ghee is described. Methylene blue was instantaneously decolourized by cotton-tract buffalo ghee, whereas normal buffalo or cow ghee showed no such decolourizing effect.

The component responsible for such reduction was tentatively identified as the cyclopropene fatty acids present in cotton-tract ghee on the basis of the Halphen test and spectral analysis.

Cottonseed feeding to milch animals alters the composition of milk fat markedly. Achaya and Banerjee[1] examined noraml ghee samples from buffaloes, and ghee samples from the Saurashtra-Gujarat (India) areas where cottonseeds are fed to buffaloes in liberal quantities. They observed that while the Reichert value of normal ghee was 37.4, that of ghee from cottonseed fed animals was 20.7. Similar results were shown by Anantakrishnan *et al*[2] by actually feeding cottonseed oil to milch animals. Such wide variation in the physico-chemical properties of ghee samples poses a grave problem in detecting the adulteration of ghee with foreign fats, particularly animal body fats. In particular it would be most helpful to be able to distinguish cottonseed tract ghee (CTG) both from noraml ghee (NG) and ghee adulterated with animal body fats (GAF).

Singhal *et al*[3] observed that cow ghee from cottonseed fed animals rapidly loses its yellow colour, implying quick discolouration of carotene. To test whether this is an oxidation-reduction phenomenon in CTG, methylene blue was tested as an artificial electron acceptor in the system. As a consequence, a testing procedure to distinguish CTG from NG using the methylene blue reduction test was developed.

Materials and Methods

(*a*) *Normal ghee* (*NG*). These were prepared from cow and buffalo herd milk maintained at the Institute farm by direct cream and *desi* methods essentially as described by Ganguli and Jain[4]

(*b*) *Cottonseed tract ghee* (*CTG*). These were prepared by the *desi* method and procured through the courtesy of the Agricultural Marketing Adviser to the Government of India.

Body fats: Buffalo, goat, pig and sheep body fats were prepared by heating the fat tissues of the animals (obtained from slaughter houses) in a stainless steel vessel below 125°C.

Adulterated ghee samples. Normal cow and buffalo ghee samples were artificially adulterated with different body fats (buffalo, goat, pig and sheep) at 5, 10, 20 and 50 per cent levels.

Cotton seed oil: Cottonseed oil was soxhlet extracted from cottonseeds (obtained from the local market) using hexane, and the oil was alkali-refined (10 per cent NaOH) and bleached using Fuller's earth[5].

Sterculia foetida oil: Sterculia oil was a gift through courtesy of the United States Department of Agriculture, New Orleans, Louisiana, U.S.A.

(*a*) *Assay system with methylene blue added directly to the fat:* The dye solution consisted of a 0.1 per cent methylene blue (BDH laboratory reagent) solution in chloroform: methanol mixture (1:1). The clear fat

TABLE 1. METHYLENE BLUE DYE REDUCTION BEHAVIOUR OF DIFFERENT FATS

Nature of fat	Species	Optical density at 650 nm			
		0 min	10 min	20 min	30 min
Ghee	Cow (I)	0.44	0.44	0.41	0.38
	Buffalo (I)	0.42	0.42	0.40	0.38
	Buffalo (C)	0.06	0.06	0.06	0.07
Body fat	Pig	0.28	0.28	0.27	0.28
	Sheep	0.24	0.24	0.22	0.22
	Goat	0.23	0.23	0.21	0.20
	Buffalo	0.22	0.22	0.20	0.20

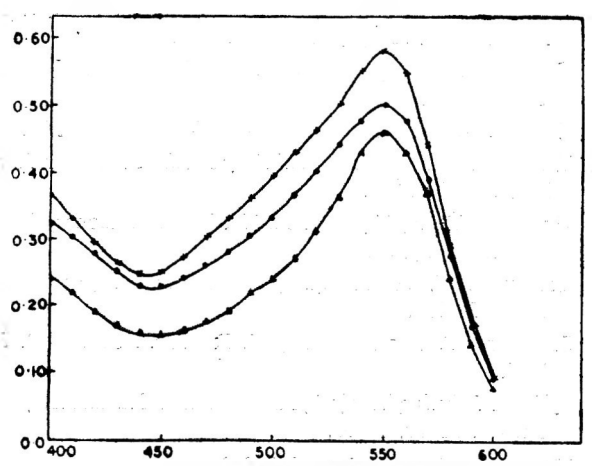
I = Institute farm; C = Cotton-tract

**Present address:* S.R.S., N.D.R.I., Bangalore.

TABLE 2. METHYLENE BLUE DYE REDUCTION BEHAVIOUR OF ADULTERATED GHEE SAMPLES

Type of ghee	Adulterants		Optical density at 650 nm			
	Source of body fats	%	0 min.	10 min.	20 min.	30 min.
Buffalo (C)	—	—	0.06	0.06	0.06	0.07
Cow (I)	—	—	0.44	0.44	0.41	0.38
	Pig	5	0.44	0.44	0.39	0.36
		10	0.42	0.42	0.37	0.34
		20	0.40	0.40	0.34	0.30
		50	0.31	0.31	0.26	0.24
	Sheep	5	0.42	0.42	0.38	0.35
		10	0.40	0.40	0.37	0.34
		20	0.34	0.34	0.30	0.28
		50	0.30	0.30	0.27	0.25
	Goat	5	0.42	0.42	0.38	0.35
		10	0.40	0.40	0.36	0.34
		20	0.35	0.35	0.31	0.28
		50	0.30	0.30	0.26	0.25
	Buffalo	5	0.42	0.42	0.38	0.34
		10	0.39	0.39	0.36	0.33
		20	0.32	0.32	0.32	0.30
		50	0.28	0.28	0.26	0.24
Buffalo (I)	—	—	0.42	0.42	0.40	0.38
	Pig	5	0.40	0.40	0.38	0.36
		10	0.36	0.36	0.35	0.33
		20	0.34	0.34	0.34	0.31
		50	0.32	0.32	0.30	0.28
	Sheep	5	0.38	0.38	0.38	0.34
		10	0.36	0.36	0.34	0.32
		20	0.34	0.34	0.32	0.30
		50	0.30	0.30	0.28	0.26
	Goat	5	0.38	0.38	0.36	0.36
		10	0.36	0.36	0.34	0.32
		20	0.34	0.34	0.32	0.30
		50	0.30	0.30	0.28	0.27
	Buffalo	5	0.40	0.40	0.38	0.37
		10	0.38	0.37	0.36	0.34
		20	0.36	0.36	0.34	0.32
		50	0.30	0.30	0.27	0.25

C = Cotton tract; I = Institute farm



Absorption spectrum of colour Produced with Halphens, reagent

(1) Cotton-tract ghee ×—×—×
(2) Cotton seed oil Δ—Δ—Δ
(3) Sterculia foetida oil O—O—O

sample (5 g) was taken in a test tube, 0.1 ml of the dye solution was added and the two mixed by gentle shaking avoiding air bubbles. Immediately after shaking, the optical density was recorded in a colorimeter using a red filter (650 nm). The samples were then incubated at 60°C and the intensity of the blue colour was measured at regular intervals to follow the discharge of colour. The blank used was the same fat sample without the dye.

(b) Assay system with methylene blue added to the fat solution in iso-amyl alcohol: Since it was not possible to dissolve the dye solution in a small volume of cottonseed oil or sterculia oil, the dye was added to the fats taken in iso-amyl alcohol. 5 g of the liquid CTG, 1 g cottonseed oil and 0.5 g sterculia oil samples were dissolved in 5 ml of iso-amyl alcohol in separate test tubes. 0.1 ml of the dye solution was then added, the contents of the tubes were mixed and the tubes were securely corked. The tubes and their contents were then placed in a bath of boiling saturated sodium chloride solution and the disappearance of the methylene blue colour was noted.

Halphen test for cottonseed oil: The Halphen test was originally developed as an empirical method to detect the adulteration of various vegetable oils with cottonseed oil[6]. Though the tissues or eggs of hens fed on cottonseed oil meal show a positive Halphen test (Lorenz and Almquist[7], Shenstone and Vickery[8], it is not known with certainty whether ghee from the milk of animals fed on cottonseeds or cottonseed oil will exhibit a similar positive Halphen test. Accordingly, samples of CTG were tested for the Halphen colour reaction. The method originally developed by Halphen (1897) and described by the A.O.A.C.[9] was used. Carbon disulphide containing one per cent sulphur in solution was mixed with an equal volume of isoamyl alcohol. To 5 ml of the mixture in a test tube, an equal volume of the liquid fat sample under examination was added and the tube was securely corked. The tube with its contents was then placed in a bath of boiling saturated sodium chloride solution for one hr. A charcteristic crimson colour was produced in the presence of CTG.

The Halphen colour reaction given by CTG was compared with those of both refined cottonseed oil and standard sterculia oil. The latter contains, a high proportion of fatty acids with cyclopropene ring structures (Nunn[10]) which are responsible for the Halphen colour reaction (Nordby *et al*[11]).

Results and Discussion

Discharge of methylene blue colour when added directly to the fat samples: The capacity for reduction of methylene blue by different fat samples and adulterated ghee samples is depicted in Tables 1 and 2. The colour of methylene blue was dramatically reduced in the presence of CTG in comparsion with NG and body fats. The difference in the colour reduction was so marked that one could easily detect the change even visually. Similarly (5, 10, 20 and 50 per cent) with buffalo, goat, pig and sheep body fats could be distinguished from CTG as these fats did not reduce methylene blue (Table 2) as quickly as did CTG.

Component responsible for Halphen reaction in CTG: All the CTG samples from the Saurashtra-Gujarat area, ghee samples from animals fed different quantities of cottonseeds under controlled conditions, and a few samples of buffalo body fats showed a positive Halphen test. That depot fat and milk fat from animals fed cottonseeds often show a positive Halphen test, has been reported by Woodman[12], and Cocks and Rede[13]. These observations suggested that the component responsible for the Halphen colour reaction, namely cyclopropene fatty acids[11] present both in cottonseed oil (Craven and Jeffrey[14], MacFarlane *et al*[15], and Shenstone and Vickery[16]) and in sterculia oil (Nunn[10]) may be present in CTG samples as well. The absorption maxima (Fig. 1) of the colour produced by CTG, cottonseed oil and sterculia oil with Halphen reagent was the same (550 nm) for all the three fat samples. This observation taken with those of Lorenz and Almquist[7], and Shenstone and Vickery[8] confirm that cyclopropene fatty acids when ingested in animal diets are incorporated into the body and milk fats.

Component present in CTG responsible for the discharge of methylene blue colour: It was observed that methylene blue was reduced only in CTG, cottonseed

oil and sterculia oil, all of which contain cyclopropene fatty acids as a common component. Cyclopropene fatty acids carry a strained ring, and it is possible that they may reduce methylene blue.

Heating of CTG or cottonseed oil with methylene blue or with the Halphen reagent for 30 min in a boiling bath of saturated sodium chloride was found to destroy their ability to develop the Halphen colour or reduce methylene blue. On the other hand, similar heating of these fats without dye or the Halphen reagent did not markedly affect these abilities. It is tentatively concluded that the reduction of methylene blue by CTG may be due to the cyclopropene fatty acids present.

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Synergistic Pesticidal Action of Juvenile Hormone Analogue and β-Ecdysone on Diapausing Larvae of Khapra Beetle *Trogoderma granarium* Everts

PREM LATA BHATNAGAR-THOMAS
Biology and Agriculture Division,* Bhabha Atomic Research Centre, Trombay, Bombay-400085, India

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Diapause in 90 per cent of khapra beetle larvae is terminated in about 11 days with high concentrations of β-ecdysone (MH). Within the range of lower dosages also, diapause is terminated but after about 20 to 50 days. The duration of diapause is significantly reduced when various concentrations of MH are mixed in the larval diet. Diapause in larvae exposed to juvenile hormone analogue (JHA) Altozar(R) is terminated only in the presence of food in about 31 to 63 days. However, simultaneous treatment of the larvae with JHA and MH, synergistically reduces the duration of diapause in comparison to that obtained with either MH or JHA alone. Upon termination of diapause the subsequent metamorphosis of the larvae is arrested at high concentration of MH, while similar results are obtained even at low doses of MH when JHA is included. It is discussed that due to their synergistic pesticidal action, JHA and MH together, may be more efficient pesticides than either of them individually for khapra beetle.

Earlier studies from this laboratory[1-3] and elsewhere[4,5] have shown that larvae of the khapra beetle, *Trogoderma granarium* Everts undergo diapause, or prolong it if already in that state, when exposed to high levels of juvenile hormone analogues (JHA). These larvae are able to moult and feed intermittently and can survive for several months on JHA treated diet: and once JHA is removed, the larvae pupate and develop into normal fertile adults[1]. Such adaptability of khapra beetle larvae towards exogenous JHA, makes them tolerant to the pesticidal action of the hormonal analogue[2]. It was, therefore, of interest to examine if diapause of *T. granarium* larvae could be precociously terminated by application of exogenous β-ecdysone (MH) so that the larvae become more responsive to applied JHA. Results of these studies are presented in this paper.

Materials and Methods

Larvae for the experiments were obtained from the standard culture of *T. granarium* maintained at 34±1°C and 50-60 per cent R.H., using semolina as the diet. Diapause was induced by the over crowding of the last instar larvae: at the time of experiments the larvae were in diapause for about 6 months. β-ecdysone was purchased from Schwartz/Mann, USA and JHA Altozar(R) was a generous gift from Zoecon Corporation, U.S.A.

Effectiveness of these hormones in terminating larval diapause and arrest of metamorphosis was ascertained both in the presence of food as well as its absence. Procedure for mixing the hormones with the food was more or less similar to the one described earlier[6]. One gram samples of semolina were treated with 0.3 ml of solvent mixture (acetone and ethanol, 10:1) alone or with various concentrations of MH and/or JHA dissolved in it, in 2 inches diameter petridishes. The solvent was allowed to evaporate at room temperature, before the test larvae were released on it. In another series, diapause larvae were subjected to a similar hormone exposure but without food. For this, the inner surface of a petridish of the same size was treated either with the solvent alone or with various concentrations of the hormone(s).

Dosages employed varied from 100 to 700 μg for MH and 1 to 5 μg for JHA. Combined effect of these hormones was examined using mixtures of 1 or 2 μg of JHA with varying quantities of MH ranging from 100 to 700 μg. Treatment at each dose level was replicated four times with ten larvae in each replicate. Observations on the terminations of diapause were generally made every 24 hr for the first 15 days and thereafter on alternate days upto 70th day of the experiment.

Results

Diapause was considered terminated when the larvae pupated and an arbitrary limit of 90 per cent pupation served as the basis for the comparison of various treatment effects.

Effects of JHA: When the larvae were exposed to different concentrations of JHA, without any food,

*Bio-Organic Division

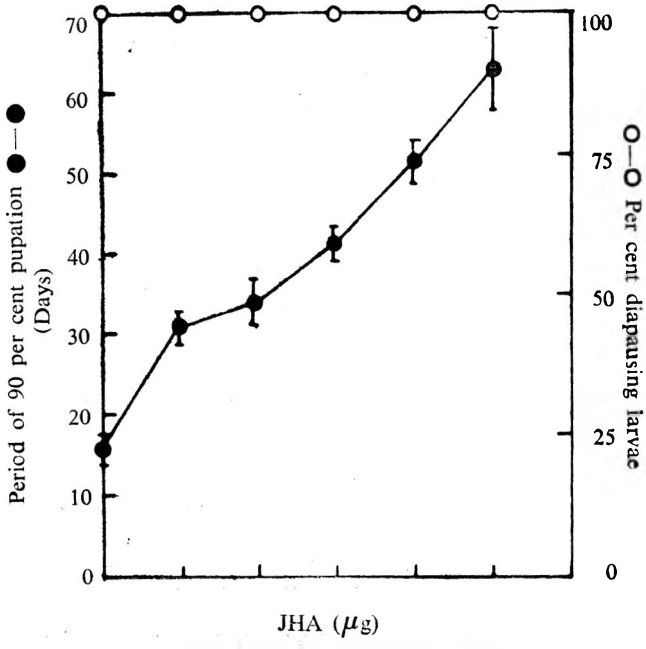

Fig. 1. Termination of diapause in *T. granarium* larvae by juvenile hormone analogue altozar(R)

●—● with food; O—O without food

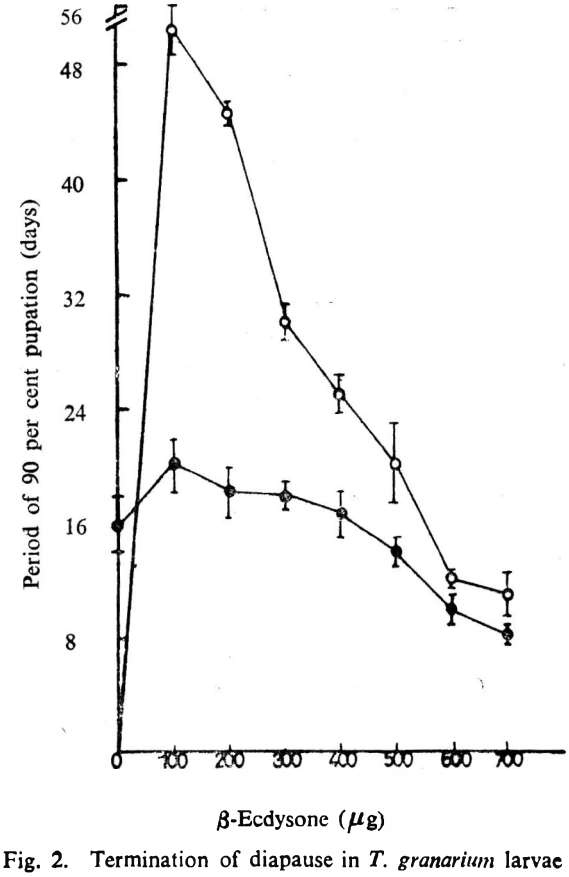

Fig. 2. Termination of diapause in *T. granarium* larvae by ecdysone

●—● with food; O—O without food

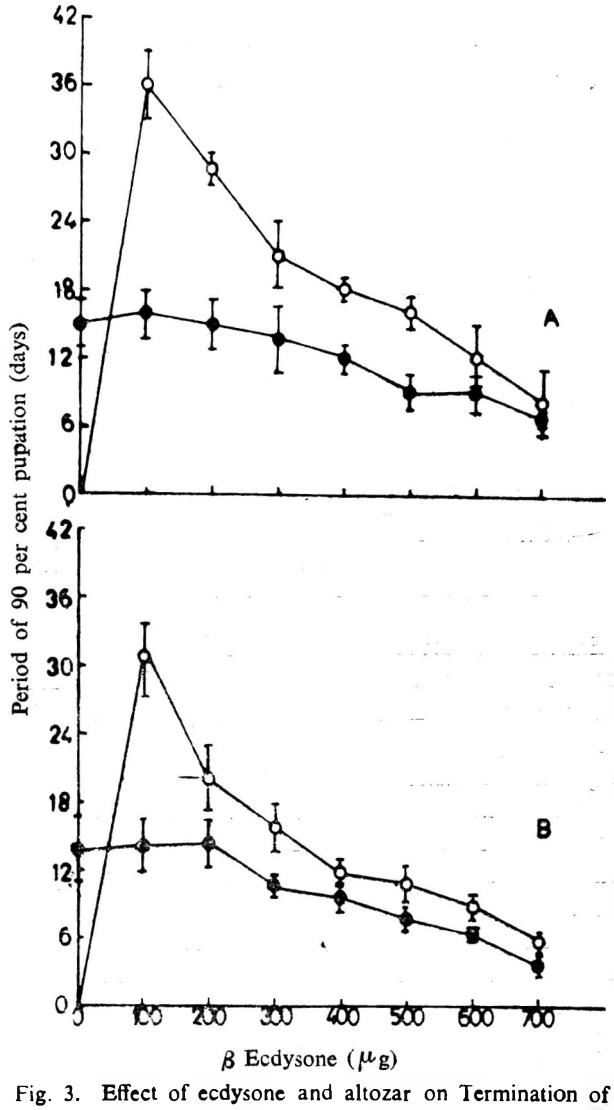

Fig. 3. Effect of ecdysone and altozar on Termination of diapause in *T. granarium* larvae

●—● with food; O—O without food;
A—1μg altozar; B—2μg altozar

they continued to remain in diapause state as in the control (Fig. I). However, when JHA was mixed in the diet the larvae pupated, per cent pupation being inversely proportional to the dose at any given time interval. Fig. 1 shows the time taken for 90 per cent pupation at different hormonal dose levels. It was observed that with increasing dose, there was a progressive delay in pupation. Compared to the control, 1 μg JHA induced about 15 days delay in pupation, while with 5μg the delay was 65 days.

Apart from its effect on delaying pupation, JHA totally inhibited metamorphosis at all the concentrations tested[3].

Effects of β-ecdysone: Unlike as in JHA treatments in the absence of food, in experiments with ecdysone, the termination of diapause was dependent upon

hormonal dose and nearly 100 per cent larvae pupated within two-weeks of exposure to the highest dose, whereas in the control none pupated till 70 days experiment (Fig. 2). At the dose of 100μg, 90 per cent larvae pupated in 50 days, while with 700 μg the same effect could be obtained even within 11 days.

Apart from its effect on termination of diapause, MH also adversely affected subsequent metamorphosis. With doses of 300 μg and above imaginal ecdysis was severely inhibited. In most of the cases, although a definite puparium was formed, further developmental steps towards the imaginal stadium appeared to be hampered. With the lower doses, however, about 15 per cent of the pupae developed into normal fertile adults.

In the presence of food, even the lowest dose induced about 90 per cent pupation within 20 days, which was more or less similar to what was observed in the control (Fig. 2). The same trend was discernible with almost all the doses tested excepting at 70 μg MH, where 90 per cent pupation was obtained within 8 days compared to about 16 days in the control. It is thus apparent, that feeding itself stimulates pupation and, therefore, the effect of MH is not evident excepting at very high concentrations. Nontheleess, the influence of MH was clearly evident on the post-pupal development as in the case of hormone treatment without food, and there were extreme developmental aberrations with doses above 300 μg.

Combined effect of MH and JHA: Observations on the combined effect of both the hormones on larval diapause are presented in Fig. 3. Surprisingly, in the presence of MH, the influence of JHA was not evident, especially in the light of the results presented in Fig. 1. At all the concentrations, the effect of MH was more pronounced: in fact, one could even suspect synergistic effect of JHA and MH activity. In the presence of JHA, termination of diapause was accelerated from 50 days in treatments with 100 μg MH alone (Fig. 2) to 35 days with 1 μg JHA added to it (Fig. 3A). Also when the JH component was increased to 2 μg (Fig. 3B), there was a further decrease in the time taken for 90 per cent pupation. Further, the combination treatment resulted in the total absence of any viable progeny even at the lowest dose.

In the presence of food, the synergistic effect of 1 and 2μg of JHA respectively on MH activity was more pronounced; with increase in MH concentration from 100—700 μg, the period for 90 per cent pupation was reduced from about 14 - 6 days and the entire resulting progeny was non-viable. In order to understand the interaction between food, JHA and MH, the basic underlying physiological mechanisms[12] however, needs to be ascertained further.

Discussion

Present observations on the extension of larval diapause as a result of JHA treatment support the earlier reports on the induction and maintenance of diapause in khapra beetle larvae by high JH/JHA titres[1,2,5]. Dose dependent acceleration of termination of diapause with MH in *T. granarium* larvae, together with the exhibition of stationary moulting cycles[1,2,7] further suggest that as in the case of *Blattella germanica* the immediate cause for larval diapause in khapra beetle may also be due to the inadequate synthesis and/or release of moulting hormone rather than its total absence. Inhibition of adult development with MH may be attributed to the inability of the larvae to gain physiological competence to react favourably to MH. The delayed effect of low doses of MH might have resulted in the development of some of the larvae into normal adults, since they have had time to acquire the needed physiological competence. The earlier termination of diapause by MH in the presence of JHA suggests that JHA, in some way, promotes the action of MH. Such synergism has been previously reported in *Tenebrio*[10] and *Sarcophaga crassipalpis*[11].

Thus it appears that combination of these two hormones or their analogues may prove to be excellent pesticides especially for the control of insects infesting stored grain and grain products in general and *T. granarium* in particular. The prospects of hormone based pesticides seems encouraging, particularly with the availability of highly effective JHA (s) and the amazing amounts of ecdysone-like materials from certain weeds, ferns and evergreen plants[12].

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Changes in Keto Acid Concentration in Bananas During Storage and Ripening

S. B. RAMA AND M. V. PATWARDHAN
Central Food Technological Research Institute, Mysore-13

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Musa paradisiaca ('Robusta' variety) bananas were harvested at commercial maturity and stored at 12.8 and 8.3°C respectively. It was noticed that during storage oxaloacetic acid concentration increased under controlled and storage conditions. The changes in ∝-ketoglutaric acid and pyruvic acid concentrations were different at 12.8°C as compared to storage at 8.3°C. When the stored fruits were transferred to 23°C for normal ripening the changes during ripening in fruits which were stored at 12.8°C, were different than those in fruits which were stored at 8.3°C. It is already known that fruits stored at 12.8°C ripen normally when transferred to room temperature, whereas those stored at 8.3°C are affected by chilling and do not ripen normally.

Green bananas when stored below their optimum storage temperature of 12.8±0.5°C[1] develop in the course of storage, severe browning of the cortical tissue due to chilling injury. The fruits affected by chilling injury become desiccated and fail to ripen normally. The skin becomes brittle, dull brown to black during ripening and highly susceptible to infection. The physiological and bio-chemical aspects of chilling injury in bananas have been studied by many workers[2-4]. Relationship between the accumulation of oxaloacetic acid in the tissue and subsequent development of low temperature breakdown in cold stored apples has been studied by Hulme and workers[5,6]. Accumulation of ketoacids such as ∝-keto glutaric acid and pyruvic acid in the peel of pepper fruits during their storage has been studied by Ogata and workers[7]. Murata[8] has studied the accumulation of keto acids such as pyruvic, ∝-keto-glutaric and oxaloacetic in the peel of chilled tissues in bananas.

The present report indicates a comparative study of keto acids in peel and pulp during storage of banana at 12.8±0.5°C (RH 85 per cent) and 8.3±0.5°C (RH 85 per cent) as well as during ripening of the normal and stored fruits at 23°C.

Materials and Methods

Green unripe banana bunches of "Robusta" variety without blemishes and bruises were harvested at commercial maturity and cut into hands. To the cut surfaces antifungal paste[1] was applied. The hands were transferred to cold chambers maintained at 12.8±0.5°C RH 85 per cent and 8.3±0.5°C RH 85 per cent for storage and to 23°C, 85-90 per cent RH for ripening respectively.

TABLE 1. CONCENTRATION OF KETO ACIDS IN THE PEEL OF BANANA FRUITS DURING STORAGE AND SUBSEQUENT RIPENING (μg/100 g. FRESH WT.

Keto acid	Control fruits		Storage period[a]		Subsequent* ripening	Storage period[b]	Subsequent* ripening
	Raw (initial)	Ripe*	1 wk.	3 wk.		1 wk.	
Pyruvic acid	177**	66	116	422	365	127	56
	(170–181)	(60–78)	(110–126)	(415–428)	(350–370)	(110–135)	(55–58)
∝-Ketoglutaric acid	152	302	1287	3148	1676	894	991
	(143–165)	(300–304)	(1267–1312)	(3066–3230)	(1600–1720)	(821–966)	(981–1002)
Oxaloacetic acid	120	140	220	240	720	220	330
	(118–122)	(135–148)	(210–232)	(220–260)	(700–740)	(160–270)	(224–334)

*Ripened at 23±0.5°C.

**Each value represents mean of 4 determinations The figures in the parenthesis indicate the range.

[a]Stored at 12.80.±5°C at 85 % R.H.

[b]Stored at 8.3±0.5°C at 85 % R.H.

TABLE 2. CONCENTRATION OF KETO ACIDS IN THE PULP OF BANANA FRUITS DURING STORAGE AND RIPENING (μg/100g FRESH WEIGHT)**

Keto acid	Control fruits						
	Raw (initial)	Ripe*	Storage period[a] 1 wk.	Storage period[a] 3 wk.	Subsequent ripening	Storage period[b] 1 wk.	Subsequent* ripening
Pyruvic acid	103 (100–107)	77 (58–98)	68 (66–70)	63 (58–69)	177 (175–180)	863 (840–886)	230 (211–249)
α-ketoglutaric acid	160 (120–188)	282 (245–328)	113 (100–127)	201 (167–235)	206 (200–210)	834 (818–883)	247 (237–257)
Oxaloacetic acid	150 (140–170)	150 (145–155)	160 (150–170)	140 (130–150)	290 (285–296)	340 (330–345)	80 (78–84)

*Ripened at 23 ± 0.5°C.

**Each value is the mean of 4 determinations. The figures in the parenthesis indicate the range.

[a]Stored at 12.8 ± 0.5°C and 85% R.H.

[b]Stored at 8.3 ± 0.5°C and 85% R.H.

Estimation of keto acids: Peel and edible portion were cut into small pieces separately. Extraction and estimation of keto acids were done according to the method of Hamdy and Gould[9].

Results and Discussion

Data on concentration of keto acids in tissue (fresh weight) during storage and ripening before and after exposure to low temperature are given in Tables 1 and 2.

During storage at 12.8°C pyruvic acid concentration in the peel increases whereas at 8.3°C it decreases. Similarly in the pulp at 12.8°C pyruvic acid decreases whereas at 8.3°C it shows an increase. There is an increase of α-keto-glutaric acid during storage both at 12.8 and 8.3°C in peel as well as in pulp. In the case of oxaloacetic acid there is considerable increase both in the peel and pulp at 8.3°C and in the peel at 12.8°C.

During ripening pyruvic and α-keto glutaric acid follow the same trend in the peel of stored and unstored fruits.

There is considerable build up of oxaloacetic acid in the peel of stored fruits compared to control. In the pulp oxaloacetic acid increases in fruits kept at 12°C whereas in the fruits kept at 8.3°C it shows a drop. The α-keto-glutaric acid show decrease during ripening at 8.3°C whereas there is an increase during ripening of controlled fruits. The decrease in pyruvic acid concentration as noticed in controlled ripened fruits is also maintained in the case of fruits ripened after storage at 8.3°C.

Thus it is observed that during low temperature storage the oxaloacetic acid concentration builds up under all conditions except in pulp at 12.8°C. Similar trend of accumulation is noticed in the case of α-keto-glutaric and pyruvic acid concentrations. An increase in the concentration of oxaloacetic acid during storage has already been demonstrated for apples by Hulme[5]. It is not clear however whether this increase in concentration is the result or cause of chilling injury in low temperature stored fruits. One interesting observation can however be made regarding the behaviour of fruits at 12.8 and 8.3°C. The trend of changes at 8.3°C is in the reverse direction compared to trend in changes at 12.8°C. Thus it appears likely that the metabolic changes in low temperatures stored fruits may in some cases be different when the storage temperature gets lowered enough to cause chilling injury to the fruit.

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Enterotoxigenic Staphylococci in Goat Meat[1]

C. C. PANDURANGA RAO[1]
Indian Vetrinary Research Institute, Izatnagar, U.P.

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One hundred and twenty seven goat meat samples were examined for coagulase postive *Staphylococci* using 10 percent sodium chloride broth for enrichment followed by surface streaking on ETGPA medium of Baird-Parker. Twelve (9.45 per cent) of the meat samples revealed coagulase positive isolates, which were then examined for enterotoxigenicity employing cellophane—over-agar method and immuno-diffusion on microslides. Seven of the isolates were found to be enterotoxigenic out of which six isolates produced enterotoxin 'C' and one isolate enterotoxin 'D'. Enterotoxigenic staphylococci from goat meat are reported for the first time in this country.

Staphylococcal food poisoning has been one of the most common food-borne illnesses. In India, there have been a few scattered reports of staphylococcal food poisoning, all attributed to milk products.[1–4] The only method of ascertaining the food-poisoning potentialities of staphylococcal isolates has been the test for enterotoxigenicity since no other characteristic serves as a reliable indicator of enterotoxigenicity[5].

No study has so far been carried out to ascertain the prevalance of enterotoxigenic staphylococci in meats in this country. The present study was undertaken to obtain this information in respect of goat meat so as to assess the potential health hazard to consumers from staphylococcal intoxication through goat meat.

Materials and Methods

One hundred and twenty seven goat meat samples collected from Bareilly (U.P.) market were employed in this study.

Samples were initially enriched in 10 per cent sodium chloride broth for about 18 to 24 hr at 37°C after which surface streaking on ETGPA medium of Baird-Parker[6] was carried out. Following incubation of the plates at 37°C for 24 to 48 hr, colonies resembling *Staphyloicoccus aureus* were picked on to Nutrient agar slants for further study.

Morphology and anaerobic breakdown of glucose employing the medium of Hugh and Leifson[7], in respect of the isolates were studied. Cultures, identified as staphylococci, were tested for coagulase production by the tube test using rabbit plasma. Coagulase positive isolates were then examined for enterotoxigenicity.

Enterotoxigenicity of the coagulase positive isolates was tested by a slightly modified method of Hallander.[8] Brain heart infusion agar (Difco) plates were prepared and the medium was covered with autoclaved cellophane before inoculation of plates. Two milli-litres of the saline suspension of the culture, matching Burroughs Wellcome (B.W.) opacity number 9 was poured over the cellophane and was spread uniformly with a sterile cotton swab. After incubation for 48 hr at 37°C, growth on the cellophane was harvested with 5 ml of sterile normal saline. The suspension was centrifuged at 5000 rpm for about 45 min and the supernatant was tested for enterotoxin.

Crude enterotoxins A,B,C,D and E and corresponding antisera, procured from Prof. M.S. Bergdoll, Food Research Institute, Wiscons in (U.S.A.) were employed in the present study. Culture supernatants were tested by immuno-diffusion on microslides as per the technique of Casman *et al*[9]., with slight modification. Wells punched out in agar gel with a cork borer were used in place of the plastic template for depositing the reagents. Sodium barbital agar was replaced with one per cent agar in phosphate buffered saline. The slides were subjected to enhancement technique before final examination and for this purpose, Amido black was substituted for Thiazine Red R.

Results and Discussion

Out of 127 goat meat samples examined, 12 (9.5 per cent) revealed coagulase positive staphylococci. When compared with the observations in respect of buffalo meat, pork and bovine milk[10], it appears that coagulase positive staphylococci occur more often in meats than in milk and among meats of goat, buffalo

1. *Present address:* Senior Bacteriologist, Central Institute of Fisheries Technology, KAKINADA-2, (A.P.)

and pork, these organisms are encountered less often in goat meat than in buffalo meat and pork. Jay[11] noted that 32 per cent of 209 unfrozen market meat samples contained coagulase positive staphylococci. Nair and Sengupta[12] observed that 11 per cent of goat meat samples collected from Calcutta market revealed coagulase positive staphylococci, while Gupta and Choudhuary[13] also reported a lower frequency of coagulase positive staphylococci in goat meat as compared to cattle and pig.

Out of 12 coagulase positive isolates, 7 (58 per cent) were found to be enterotoxigenic. In terms of meat samples, 5.51 per cent of 127 samples revealed enterotoxigenic staphylococci. Six out of 7 enterotoxigenic staphylococcal isolates produced enterotoxin 'C' while only one produced 'D'. None elaborated A, B or E. In the present study, enterotoxin 'C' was found to be the toxin elaborated by almost all the enterotoxigenic strains from goat meat. Similarly, preponderance of enterotoxin 'C' producing isolates was observed in bovine milk, buffalo and pig meat (Panduranga Rao[10]). In U.S.A., Casman *et al*[14]., reported predominance of staphyloccocal isolates elaborating enterotoxin 'D' in frozen foods, while in U.K., Simkovicova and Gilbert and Payne and Wood[16] reported predominance of entertoxin 'A' producing strains in meat products. Studies on isolates from nasal carriers among human population in this country also revealed a preponderance of enterotoxin 'C' producing *Staphylococci*[10] Enterotoxin 'C' is unique among staphylococcal enterotoxins in that while it is constantly emetic for the monkey, it is variably so or relatively innocuous for the cat. If kitten test alone is employed for detection of enterotoxin 'C' producing isolates, they are likely to be missed as it happened with strain No. 1 of Casman *et al*[14]., who were able to detect it by serological tests, but not by the kitten test.

Acknowledgement

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Survival of Some Freeze-dried Lactic Cultures Stored in Gelatin Capsules

MADAN LAL, M. P. TIWARI, R. N. SINHA AND B. RANGANATHAN
Division of Dairy Bacteriology, National Dairy Research Institute, Karnal, Haryana, India.

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The viability and activity of freeze-dried culture powders of *Streptococcus diacetilactis* DRC_1, *S. lactis* C_{10}, *S. thermophilus* (H), *Lactobacillus bulgaricus* (W) and *L. acidophilus* (R) after individually filling in gelatin capsules, have been examined immediately after freeze-drying and after subsequent storage upto 3 months at 5° and 37°C. The percentage cell survival of these five cultures immediately after freeze-drying was 21.4, 19.5, 14.5, 10.3 and 3.0, respectively. Out of these cultures, *S. diacetilactis* DRC_1 showed maximum viability and activity, the rate of cell survival being higher at 5° than at 37°C, while survival was minimum with *L. acidophilus* (R) at both storage temperatures. The role of cell morphology and moisture content in relation to viability and activity of such cultures has been discussed.

Lactic starter cultures are generally freeze-dried under vacuum and stored in glass ampoules. The viability of such cultures extends for several years[1]. But during routine handling, glass ampoules present some problems since pieces of glass fall into milk during breaking of glass ampoules while inoculating the cultures in milk. In view of the above difficulties, the possibility of preserving some selected starter cultures as freeze-dried powder in gelatin capsules have been investigated.

Materials and Methods

Streptococcus diacetilactis DRC_1, *S. lactis* C_{10}, *S. thermophilus* (Hansen), *Lactobacillus bulgaricus* (Wisconsin) and *L. acidophilus* (Russian) were grown in appropriate growth media (Table 1). In each case, cells were harvested after incubation (at 30°/37°C) at the maximum logarithmic growth phase (Table 1). After three washings with normal saline, cells were centrifuged and resuspended in sterile reconstituted non-fat milk (pH 6.8) and transferred in the form of thin films to anodized aluminium trays. The trays were then loaded into the shelves of a shelf freeze-dryer (Model 30 P 2T of M/s Edwards High Vacuum Co., England). The method of freeze-drying involved initial freezing at -30°C for 2 hr followed by desiccation under vacuum at 70 microns of Hg for 8 hr and finally warming at 30°C for 2 hr. Two hundred mg sample of the freeze-dried culture powder was filled in each gelatin capsule. The capsules were stored at 5° and 37°C upto 3 months. The cell survival during freeze-drying was determined by estimating number of viable cells before and immediately after freeze-drying. The viability (cell survival) and activity, were studied by taking two gelatin capsules containing freeze-dried culture powder at each stage. The contents of one gelatin capsule were used for determining the viability [by standard plate count[2] in agar media (Table 1)] while the contents of the other were used for determining

TABLE 1. GROWTH MEDIA* USED FOR SELECTED STARTER CULTURES

Organisms	Medium	Incubation temp. °C	Max. log. growth phase (hr)
S. diacetilactis DRC_1	Yeast Dextrose Broth	30	7
S. lactis C_{10}	,,	30	8
S. thermophilus (Hansen)	Lactose Yeast Extract Phosphate Broth	37	9
L. bulgaricus (Wisconsin)	Tomato Juice Broth	37	10
L. acidophilus (Russian)	,,	37	10

*The above media with addition of 2% agar were used for determining cell viability by standard plate count.

TABLE 2. EFFECT OF FREEZE-DRYING ON THE SURVIVAL OF SELECTED LACTIC CULTURES

Organism	% survival
S. diacetilactis DRC_1	21.4
S. lactis C_{10}	19.5
S. thermophilus (Hansen)	14.5
L. bulgaricus (Wisconsin)	10.3
L. acidophilus (Russian)	3.0

TABLE 3. EFFECT OF PERIOD OF STORAGE ON VIABILITY OF SELECTED FREEZE-DRIED LACTIC CULTURES

Culture	Period of storage (months)					
	1		2		3	
	5°C	37°C	5°C	37°C	5°C	37°C
S. diacetilactis DRC_1	11.5	9.0	2.5	1.40	0.42	0.05
S. lactis C_{10}	9.2	8.4	2.1	1.30	0.35	0.03
S. thermophilus (Hansen)	8.5	6.4	1.9	1.10	0.20	0.01
L. bulgaricus (Wisconsin)	3.6	1.5	0.9	0.08	0.15	Nil
L. acidophilus (Russian)	2.5	0.8	0.4	0.02	Nil	Nil

TABLE 4. ACTIVITY OF FREEZE DRIED LACTIC CULTURES AFTER 3 MONTHS OF STORAGE

Organism	Titratable acidity (% lactic acid)	
	5°C	37°C
S. diacetilactis DRC_1	1.04	0.83
S. lactis C_{10}	1.01	0.82
S. thermophilus (Hansen)	1.02	0.80
L. bulgaricus (Wiscons in)	1.00	—
L. acidophilus (Russian)	—	—

the activity (per cent titratable acidity) after inoculation into milk. Viability was determined immediately after freeze-drying and after subsequent storage at 5° and 37°C at the end of 1, 2 and 3 months. Titratable acidity was determined only at the end of 3 months of storage at 5° and 37°C. The experiments were carried out in triplicate and the results represent average of three trials.

Results and Discussion

The percentage cell survival of freeze-dried *Streptococci* immediately after freeze-drying varies between 21.4 and 14.5. Almost similar[3] values have been reported earlier from this laboratory in case of freeze-dried *S. lactis* C_2 stored in glass ampoules. Survival was maximum (21.4 per cent) in *S. diacetilactis* DRC_1 and minimum (3.0 per cent) in *L. acidophilus* Russian (Table 2).

These results are comparable to those of Gavin[4] who reported higher survival of freeze-dried cells of *S. thermophilus* as compared to those of *L. bulgaricus*. The *lactobacilli* exhibited lower viability as compared to cocci, thereby indicating higher resistance of cocci than long rod forms during freeze drying.

It may be seen from Table 3 that viability of freeze-dried cells in gelatin capsules decreased during storage in all the five cultures, irrespective of the temperature of storage (5° and 37°C). The extent of loss of cell survival at the end of 3 months of storage was about 8 to 10 folds at 37°C, as compared to storage at 5°C. There was a proportionate decrease in activity (Table 4) of freeze-dried culture powder stored in gelatin capsule with a corresponding decrease in cell survival (Table 3) during storage.

Diffusion of moisture and/or air during storage is known to be detrimental to viability and activity of freeze-dried bacterial cultures. It is likely that the above factors may be responsible for the low viability noted in the freeze-dried culture powder filled in gelatin capsules[5-7]. In earlier trials[3] with glass ampoules, cell survival of freeze-dried *S. lactis* C_2 was significantly reduced when stored in presence of air. Hence, strip-packing of gelatin capsules containing freeze-dried culture powder may, perhaps, improve the viability of the lactic cultures by preventing the diffusion of moisture and/or air.

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CONTROL OF MADERIZATION IN INDIAN WHITE WINES

Maderization in white wines can be minimized by pretreatment of juice with phenol complexants such as polyvinyl pyrrolidone (PVP), formaldehyde (HCHO) and casein either alone or in combination. A combination of 50 ppm each of PVP and HCHO appears suitable. Wines prepared by such treatment of juice have been found to be less brown and acceptable.

Maderization (nonenzymatic browning) of white wines is the most undesirable change with regard to quality of white wines. The mechanism and factors involved in maderization and its prevention have been extensively examined[1-3]. It is generally agreed that maderization is mainly due to the nonenzymatic oxidation of catechins and leucoanthocyanins and this can be prevented by treating wines prior to bottling with phenol complexing agents such as gelatin, casein, polyvinyl pyrrolidone (PVP), etc. which have the ability to precipitate selectively catechins and leucoanthocyanins[4-6]. In warmer wine producing countries particularly in India, maderization begins immediately after the juice is extracted because of prevailing high temperature. We have, therefore, examined whether maderization can be prevented or minimized by treating the juice with phenol complexing agents prior to fermentation instead of prior to bottling.

Fresh grapes of 'Perlette' variety (50 kg) from the Haryana Agricultural University farm were destemmed, crushed and pressed through cheese cloth to extract the juice. The juice was treated with 100 ppm of SO_2 (as potassium metabisulphite) and ameliorated to 24° Brix with cane sugar. The juice (1.5 l) was transferred to 2.5 l capacity Winchester bottles and treated with phenol complexing chemicals as follows:

Chemical	Quantity used
HCHO	50 and 100 ppm
PVP	50 and 100 ppm
HCHO+PVP	50 ppm each
HCHO+PVP	100 ppm each
Casein	250 ppm

The juice and chemicals were mixed and stored at 8-10°C for 12 hr, racked and inoculated with actively growing wine yeast (*Saccharomyces cerevisiae* var. *ellipsoideus*) at 2 per cent level and allowed to ferment at 23±2°C. The course of fermentation was followed by measuring the Brix at regular intervals. After fermentation, the wine was treated with 100 ppm of SO_2 and clarified. The chemical composition of wines was determined according to Amerine[7], total phenolic content with Folin-Ciocalteu reagent[8] and total aldehydes by the method described by AOAC[9]. The extent of maderization was determined by measuring the optical density at 420 nm[10] using a Bausch and Lomb spectronic-20 colorimeter, and accelerated maderization test by the method of Cantarelli *et al*[6].

The different treatments did not significantly affect the time course of fermentation except at higher concentration (100 ppm) of HCHO. The extent of maderization of wines treated with different chemicals was determined by the difference in the colour intensities of fresh and four-month old wines. The wines prepared by 50 ppm PVP had undergone less maderization than others (Fig. 1). Quantitative measurement of residual phenol suggests that the wines prepared from juice pretreated with phenol complexing chemicals had lower amounts of total phenols than control (Table 1).

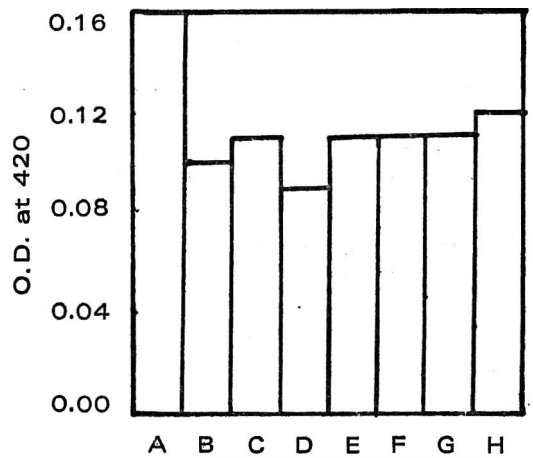

Fig. 1. Extent of maderization of white wines after four months.

A, Control; B, HCHO 50 ppm; C, HCHO 100 ppm; D, PVP 50 ppm; E, PVP 100 ppm; F, HCHO+PVP 50 ppm each; G, HCHO+PVP 100 ppm each; H, Casein 250 ppm.

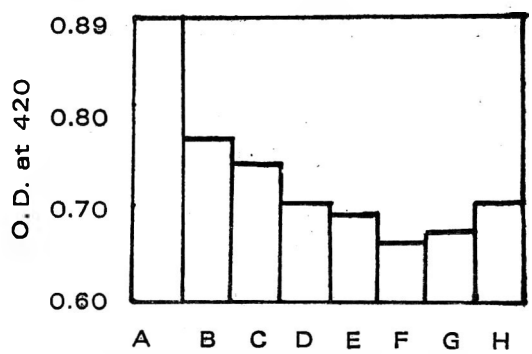

Fig. 2. Accelerated maderization test

Legend as in fig. 1.

TABLE 1. CHEMICAL COMPOSITION OF WINE

Treatment	pH	Total acidity (g.HTa/100ml)	Volatile acidity (g.HAc/100ml)	% alcohol (v/v)	% sugar	Total aldehydes (mg/l)	Total phenols (ppm)
Control	3.15	0.68	0.036	12.8	0.13	57.2	200
HCHO 50 ppm	3.20	0.64	0.056	13.1	0.18	50.6	160
HCHO 100 ppm	3.20	0.62	0.053	13.1	0.20	57.2	170
PVP 50 ppm	3.20	0.62	0.053	13.1	0.13	55.0	170
PVP 100 ppm	3.15	0.64	0.050	12.6	0.15	55.0	180
HCHO+PVP 50 ppm each	3.20	0.60	0.053	12.6	0.18	52.0	170
HCHO+PVP 100 ppm each	3.20	0.60	0.048	13.1	0.18	55.0	170
Casein 250 ppm	3.20	0.64	0.059	12.8	0.18	59.4	170

The total potential of white wines to undergo browning during aging was determined by subjecting the 4-month old wines to accelerated maderization test which showed that pretreatment of juice with formaldehyde and PVP each at 50 ppm is sufficient to minimize browning of 'Perlette' white table wine (Fig. 2).

The chemical composition of fresh wines prepared by different treatments did not differ significantly (Table 1). No residual odour of these chemicals was noticeable. These studies show the possibility of minimizing maderization by pretreating the juice with phenol complexing agents and this technique could be applied for prevention of maderization in warmer wine producing regions over the conventional procedure.

Department of Microbiology,
Haryana Agricultural University,
Hissar (Haryana) 125004.

M. C. BARDIYA
B. S. KUNDU
P. TAURO

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HEAT INDUCED CHANGES IN PROTEINS OF RENNET WHEY FROM BUFFALO MILK

Gel filtration patterns on Sephadex G-100 revealed that heat treatment at 95 and 100°C for 10 min caused the proteins of rennet whey from buffalo milk to undergo considerable increase in the molecular size.

Polyacrylamide gel electrophoresis of heat treated buffalo rennet whey before and after dialysis exhibited extensive denaturation of the individual serum proteins. Heat treatments at 100°C induced maximum change in the serum proteins. These results indicate that buffalo milk rennet whey proteins are more vulnerable to heat treatment than the proteins of cow rennet whey.

Various investigators[1-4] have studied in detail the changes in serum proteins of bovine milk on heat treatment. On the other hand, very limited information is available in similar lines on buffalo milk[5]. Stephen and Ganguli[6] recently reported about heat induced changes in serum proteins obtained after the removal of casein from buffalo milk by acid precipitation. In the present study, rennet-whey from buffalo milk was heat treated and the changes were monitored by both gel filtration and polyacrylamide gel electrophoresis.

Buffalo milk was collected from the Murrah breed and cow milk from Tharparkar, Red Sindhi and Sahiwal breeds. Prior to the preparation of rennet whey, fat was removed by a cream separator. The temperature of the skim milk was adjusted to 37°C, and treated with a solution of Hansen's rennet (50 mg/ml) at the rate of 0.1 ml rennet per 10 ml of skim milk.

After the coagulam had set, whey was drained and centrifuged for 30 min at 1000×g. The clear supernatant was passed through a pack of cotton wool. Samples of whey were heated from 65 to 100°C for 10 min and cooled immediately in ice cold water to room

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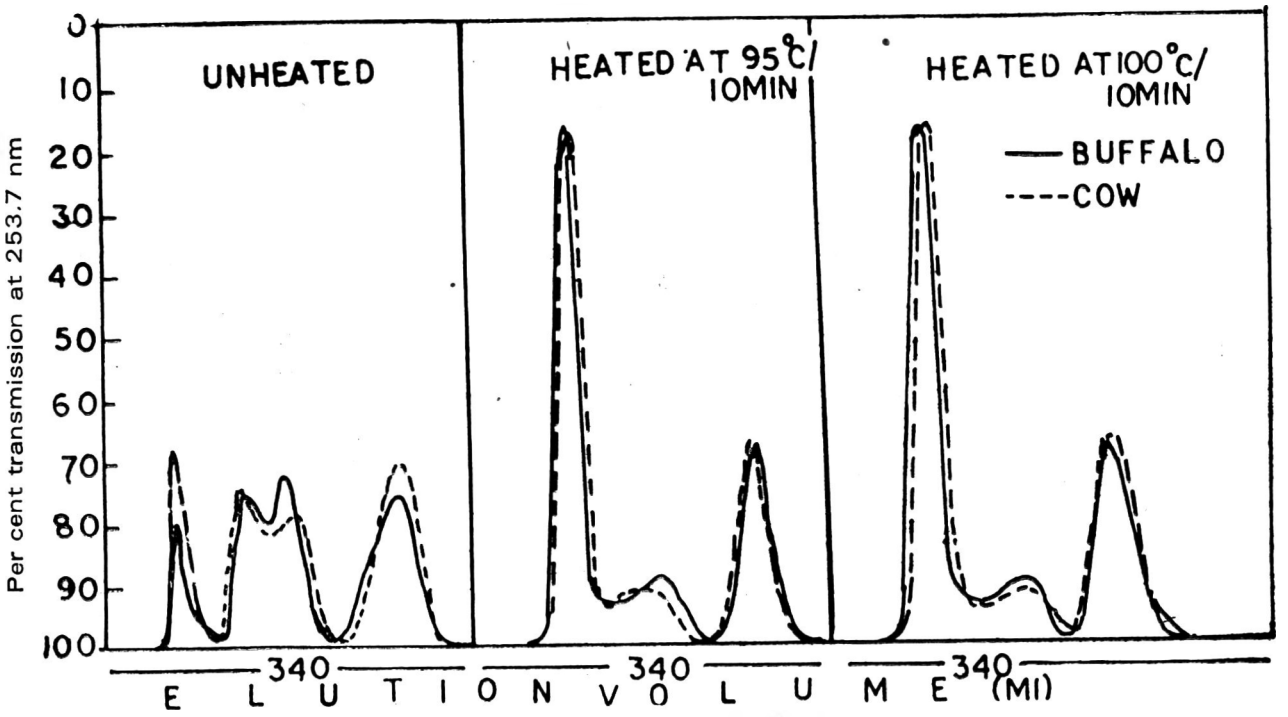

Fig. 1. Gel filtration patterns of unheated and heated rennet whey on Sephadex G-100 (3×50 cm column). Elution with phosphate buffer pH 6.9 containing NaCl.

temperature. The destabilized proteins were removed in accordance with the procedure of Kenkare *et al.*[3] Gel filtration of the unheated and heated rennet whey was carried out on Sephadex G-100 and elution of the protein fractions was done using phosphate buffer[6], at pH 6.96 Polyacrylamide gel electrophoresis was performed on a 8 per cent gel in a cell assembled in this laboratory[7]. Protein zones were stained with Amido Black.

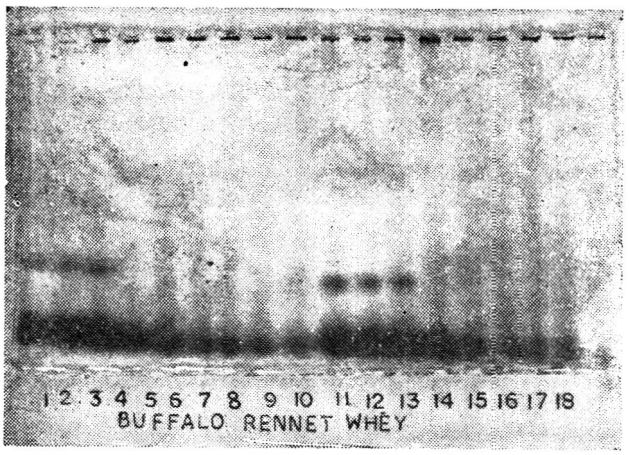

Fig. 2. Zonal electrophoresis patterns of buffalo unheated and heat treated rennet whey. 1-9, unheated and heated at 65, 70, 75, 80, 85, 90, 95 and 100°C for 10 min respectively. 10-18, unheated and heat treated as above, after the removal of the destabilized whey proteins by sedimentation.

Gel filtration patterns of unheated and heat-treated rennet wheys (from buffalo and cow milk) are shown in Fig. 1. Unheated rennet whey resolved into four fractions, three protein fractions and a non-protein fraction having very strong UV absorption at 253.7 nm. Heat treatments at 95 and 100°C caused drastic reduction in the second and third protein fractions. At the same time, the non-protein fraction remained the same. Between the heat treatments, the maximum reduction of the 2nd and 3rd fractions took place at 100°C. Instead of two separate fractions they emerged as a

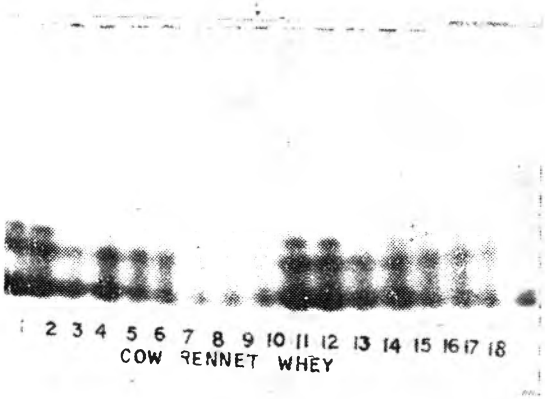

Fig. 3. Zonal electrophoresis patterns of cow unheated rennet whey. 1-9, unheated and heated at 65, 70, 75, 80, 85, 90, 95 and 100°C for 10 min respectively. 10-18, unheated and heat treated as above, after the removal of the destabilized whey proteins by sedimentation.

single protein fraction. The reduction in these fractions were compensated by the increase in the first protein peak. It was observed from the area of the protein peaks, that buffalo milk rennet whey underwent greater changes than cow rennet whey proteins.

The course of heat-induced changes in rennet whey proteins was followed using polyacrylamide gel electrophoresis of the whey heated from 65 to 100°C for 10 min. In another set of experiments, the electrophoresis of the heat-treated whey was carried out after removing the destabilised whey protein by centrifugation. Such electrophoretic patterns are shown in Figs. 2 and 3.

Dialysis of the rennet whey was carried out prior to heat treatment to find out whether the dialysable constituents exert any influence on the heat induced changes of the rennet whey proteins of buffalo milk. Polyacrylamide gel electrophoresis of such systems revealed considerable reduction in the individual serum proteins.

These results show that heat treatment of buffalo rennet whey caused considerable change by way of denaturation as well as change in the molecular size distribution of the individual serum proteins. Between buffalo milk and cow milk rennet wheys, serum proteins from buffalo milk underwent greater change than from cow rennet whey. Our previous studies[6] with acid whey from the milk of these two species indicated that serum proteins of buffalo milk was more vulnerable to heat treatment than cow milk.

The authors are grateful to Dr. D. Sundaresan, Director, for his kind interest in this study.

National Dairy Research Institute
Karnal 132001
JOSE STEPHEN
N. C. GANGULI

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BOOK REVIEWS

Commercial Processing of Vegetables: by L. P. Hansen, Noyes Data Corporation, Park Ridge, New Jersey, 1975, pp 448.

The United States patent literature in food technology is scattered over in food technology journals. Furthermore, the details of patent literature covered in these journals are often incomplete and do not disclose sufficient details for the research workers in the field. The only way to get sufficient information of a U.S. patent is from the U.S. Patent Office. Noyes Data Corporation has been doing yeoman service by publishing U.S. patent literature in food technology, which is the largest in the world, in the form of books. These books supply detailed technical information and can be used as a guide to U.S. patent literature. By giving sufficient information and eliminating legal jargon and juristic phraseology, these books present advanced, commercially oriented review of modern methods of processing as given in U.S. patents.

The present book covers U.S. patent literature on commercial processing of vegetables.

Altogether 226 processes are covered by 237 patents issued mostly from 1965 to 1975. These pertain mainly to the processing of potatoes, tomatoes and legumes.

The table of contents is organised in such a way so to serve as a subject index. In addition to the subject classification by chapter, additional references are indicated in chapter or section introductions to patents in other chapters which are applicable to the vegetable being reviewed. Other indices by company, inventor and patent number help in providing easy access to information contained in this book.

In total there are twelve chapters, each containing a number of sections. The first chapter "on general dehydration processes for vegetables" is covered by 21 patents on varied aspects such as freeze drying and other methods of dehydration, dehydration by reverse osmosis, fluidized bed drying, etc. A few patents cover drying agents and additives, compression of dried vegetable products and irradiation of dehydrated vegetables. The second chapter on "other general processes for vegetables" includes various canning systems, freezing processes, preservatives, coatings and films for vegetables, sauces for frozen vegetables and miscellaneous processes. These are covered by 31 patents.

The third, fourth and fifth chapters are on varied processes for instant mashed potatoes, processing of fried potato products and other potato processes. A total of 74 patents, the largest in the book, are covered by the above processes.

The sixth chapter on "processing of other root vegetables" includes sweet potato, carrot and radish and is covered by 11 patents.

Chapter seven on "bulbs" pertains to lye peeling of onions, dehydrated bulb products, allium condiments and french fried onion rings. A total of 13 patents cover the above processes and products.

"Stems, leaves and flower heads" form the subject matter for the chapter eight. These include lettuce, spinach, cabbage type vegetables viz. Kim chee (pickled Nappa) and asparagus. The processes covered for the above are packaging, freezing drying and dehydration in 10 patents.

"Processing of vegetable seeds" covered in chapter nine comprise improving flavour and texture of legumes, production of quick cooking legumes, freezing, freeze drying and other dehydration methods, stabilization of colour in legumes and other miscellaneous processes. The above are covered by 36 patents. Besides, three patents cover processing methods for corn.

"Tomato processing methods" are amply covered in chapter ten. These include vacuum peeling process for tomato, packaging and coating methods, production of high consistency tomato juice by acid or alkali treatment, methods for the manufacture of tomato ketchup by minimizing heat damage and inactivating pectin splitting enzymes, etc. Manufacturing tomato juice concentrate by separation of insoluble and serum fractions and evaporation of serum, dehydration process viz. foam mat drying and spray drying for tomato juice powder, quick freezing of tomato slices, tomato enhancers and products such as tomato salad, tomato sauces are covered in 23 patents.

Processing of pumpkins in the form of flakes, pie mix and pickle and cucumbers for pickles and their various ramifications are covered in chapter eleven in 9 patents.

The last chapter on "Processing of mushrooms" comprises handling methods of fresh mushrooms, freezing and canning methods and preparation of puffed mushroom snacks which are covered in 7 patents.

The get up of the book is good. However, the letters used are small and is rather strenuous for the reader to persue. This serves as a very useful book of reference for research workers and industrialists engaged in vegetable processing.

L. V. L. SASTRY

Evaluation of Certain Food Additives—Some Food Colours, Thickening Agents, Smoke Condensates and Certain Other Substances: Technical Report Series, 576, World Health Organization, Geneva, 1975, pp 23.

Food additives are required for the purposes of preserving, texturizing, flavouring and colouring of foods. This report gives the proceedings of the 19th meeting of the joint FAO/WHO Expert Committee on food additives convened (*i*) to prepare specifications and carryout the toxicological evaluation of certain food additives; (*ii*) to review and adopt general principles and methods for specifications; and (*iii*) to revise the specifications for certain food additives.

The report includes general considerations for establishing and revising the specifications; information on impurities or transformation products of intentional and unintentional food additives; smoke, smoke condensates and liquid smoke and revision of specifications for 31 substances including acids, bases and salts, as well as azodicarbonamide, benzoyl peroxide, calcium oxide, oxystearin and sodium carboxymethyl cellulose. Comments are made on particular substances like amaranth, ferrous gluconate, quinoline yellow, guar, locust bean and tara gums, microcrystalline cellulose, amydated pectin, aspartame, dichlorodifluoromethane, sucrose acetate isobutyrate, tertiary butylhydroquinone, tin compounds and triacetin.

The FAO/WHO documents on food additives, acceptable daily intake for many chemicals, information on specifications and toxicological information required on some of the additives are also given.

S. RANGANNA

The Market Potential for Papain: by G. Flynn, Tropical Products Institute, Publication No. G-99, 56/62, Gray's Inn Road, London WC 1×8 LU, 1975, pp. 58. Price: £ 1.20, (free to official bodies in developing countries.)

The enzyme papain finds extensive use in the chill-proofing of beer, tenderisation of meat, preparation of protein from fish waste to replace milk in the diet of calves and to a lesser extent in other foods. In addition, it is also used in phrmaceutical preparations, leather bating, etc. About 95% of the papain is utilised in the food and beverage industry.

The principle sources of crude papain are Zaire, Tanzania, Uganda and Sri Lanka. Small quantities are produced in other countries. Following the decline of trade with Uganda and of production in Sri Lanka resulting from the outbreak of mosaic disease, Tanzania and Zaire are the major exporters of papain. The U.S.A., Japan, U.K., Belgium and France are the major importers. Most of the material finds its way eventually to U.S.A. where it is refined, formulated into preparations and re-exported.

The present report contains four chapters. The first chapter outlines the characteristics, functions and utilization of papain; the second examines in detail the technological options available to the principal industrial users and makes an assessment of potential use of papain; the third describes the existing and changing pattern of world trade which is well supported by tabulated data and the fourth outlines the procedures for the measurement of enzyme activity.

A brief description of the commercial methods of manufacture, refining, quality grading and packaging are also included in the report. The report is well documented with 86 references, addresses of importers, processors, users, etc., and tables giving papain uses, world beer production and consumption, meat consumption papain exports and imports, etc.

The report would be of topical interest to all those engaged in production, consumption, trade and research in the field of papain.

S. RANGANNA

Food Composition Tables—Updated Annotated Bibliography, by the Nutrition Policy and Programme Service, Food Policy and Nutrition Division, FAO, Rome, Italy, 1975, pp. 181+xxii.

An Annotated Bibligraphy on Food Composition Tables was issued by FAO in 1969 on Nutrition Informations Documentation Service No. 1. It has now been updated, covers 72 countries and contains 169 titles.

The bibligraphical articles include: (*a*) title of the book; (*b*) its background; *c*) the portion of food analysis; (*d*) the nutrients covered; (*e*) presentation and grouping of foods; and (*f*) additional information that may be found in the book, such as dietary requirements of the country, list of foods with their scientific names, methodology of analysis, altitude, rainfall and other physical features of country, etc.

The aim of the bibliography is to provide brief information of the contents of the current food composition tables of different countries. It is a useful list of sources for nutritionists and food planners and policy makers for their research work, dietary and food consumption survey, and regional planning.

SAROJINI K. DASTUR

Commercial Processing of Fruits: by L. P. Hanson, Noyes Data Corporation, Park Ridge, New Jersey, USA, England, 1976. pp 301+x.

This book, which forms number 30 in the series of Food Technology Reviews, the earlier one being No. 27 on Commercial Processing of Vegetables, 1975, is a welcome addition to the reference library of the food scientist and technologist. With detailed technical information from US patent literature in the field of fruit handling and processing, the book presents an advanced review of modern methods with a commercial orientation. The US patent literature is perhaps the largest and most comprehensive collection of technical information of inestimable value to the advanced research scientist and technologist. The table of contents itself serves as a subject index. Company, Inventor and Patent Number indices are of great value in providing easy access to detailed information in the book. The book highlights the importance of patent literature in relation to new information, data and ideas, providing an excellent starting point to the next investigator so that he is kept aware of the latest technologies in the specialised field. Such information is not readily available from periodicals or books.

The book reviews nearly 174 processes issued during the last ten years, and the term Processing includes the handling of fresh fruit for storage and transportation and their selected pre-harvest treatment to improve their quality after harvesting and during subsequent processing. The major items dealt with refer top rocesses such as handling of fresh fruit, general dehydration and other general processes common to several of the fruits. Taking typical fruits as a class, citrus fruit, pomes, drupes, berries, grapes, bananas and miscellaneous fruits such as avocado, date, melon, pineapple, coconut, etc., have been dealt with in considerable detail by referring to various processes of great economic importance and commercial significance, developed during recent years. Some of the processes and technologies are quite novel and capable of wider extension in related fields other than processing of fruits only.

In the case of handling of fresh fruit, conditions for storage and transport occupy an important place. Transport of fruit under controlled atmosphere, use of reduced pressure to remove volatile respiration products, humidity control with special emphasis on the degreening of citrus fruits, ethylene ripening of fruit, uniform wax coating of fruits, other hydrophilic polymer coatings, etc. are described in full detail with regard to equipment as well as technique.

Film packaging for control of O_2 and CO_2 has been dealt with special reference to strawberries and melons, 2(4 thiazolyl) benzimidazole (TBZ) and ortho-polyphenol tetrahydrate (AOPP), an alkali metal salt, have been found to be highly useful in preventing decay of fruits. Both of these inhibit molds and fungi without adverse side effects. They are highly effective in the case of citrus fruits and also in the case of apples, peaches, etc. Their useh as been approved by the F & DA of USA.

Under General Dehydration Processes, the freeze drying process, vacuum dehydro-freezing, combined freeze-drying-air drying process, use of molecular sieves in freeze drying, etc., have been dealt with in great detail. Drum drying, using thickening agents in the case of purees, and construction of modified drum dryer have been described. Drying of fruit cereal purees and of banana puree, employing wheat flour, cane sugar, skim milk powder and lecithin, is of particular interest in connection with mango cereal flakes and baby foods, Mangotine from mango and Banatine from bananas, developed at the Central Food Technological Research Institute, Mysore.

Dehydration by reverse osmosis is a recent addition to the several dehydration methods in vogue. Other drying methods referred to are spray drying of aqueous dispersions, microwave radiation drying, use of nitrous oxide as drying agent, drying using vegetable oils as in the case of citrus peels, etc. Amylose ester protein coatings, protective coatings of solid shortenings, pregelatinised starch coatings, and addition of sweeteners and flavour enzymes are described in some detail to bring out their importance.

Vacuum peeling of fruits, use of ammonia salts in combination with surface active substances, calcium carrageenan as gelling agent, colour and flavour stabilization by using a synergistic mixture of phosphoric acid and sodium sulphite or bisulphite, vacuum treatment to remove gases from the flesh of fruits, prevention of browning using nitrous oxide, use of enzymes in candied fruits to quicken the process, artificial fruits, using calcium lactate with the alginate sol, after first adding suitable additives to the alginate, etc., are some of the important processes highlighted under "Other General Processes".

Reduction of sour taste in citrus fruits with foliage application of at least one of protease, lipase, naringinase and hesperidinase to citrus fruit trees at the period of ovary enlargemnt, is an interesting phenomena. Cooling of containers after canning of orange segments and prevention of clouding in the canned product by an enzymatic process form the subject of some of the patents. Citrus and grape fruit puree, isolation of citrus chromoplasts for use in enhancing natural colour, upgrading of citrus peel to a tasty nutritious prodcut

for human consumption by dehydrating the peel with hot oil, are some important processes developed in recent years.

Pomes occupy an important place among fruits. Preservation of fresh apple slices using chemicals such as ascorbic acid and calcium chloride, lye peeling of apples using a wax solvent such as ethyl alcohol, drying of fruit after impregnating it with sugar syrup, reducing the hygroscopicity of dehydrated fruits by replacing part of the hygroscopic sugars with less hygroscopic sugars such as sucrose prior to dehydration, dried apple sauce resistant to cracking by partial removal of the juice from the fruit and supplementing with the addition of malic acid, sucrose and apple flavour to the dehydrated sauce powder at the time of packaging, etc, are some of the important commercialised processes.

Among drupes, olives form the subject of a number of patents covering holding solutions, pure culture fermentation, chlorination of freezing brine for ripe olives, etc., pitting cherries with prior freezing of the outer layer only to minimise loss of juice, use of sodium metaphosphate along with papain, in preparing maraschino cherries, removal of sulphur dioxide by aerating the brine, while maintaining its pH below the maximum bisulphite pH of 4.5 are some of the latest advances in the field. Cherry pits can be processed into an edible flour.

Cranberry sauce in different forms, immersion freezing of strawberries, freeze-dried strawberries as component in breakfast cereal, and quick freezing of raspberries, are some important patented processes. Raisin paste from grapes, uniform colouring of raisins, cleaning of raisins in acqueous baths containing silicone polymers such as Dow Corning Antifoam AF Emulsion, etc., are of topical interest.

Processing of bananas is of special interest to this country. Controlled ripening of bananas using ethylene from molecular sieves such as Zeolitic molecular sieves based on aluminium silicates, packaging in box or bag maintaining a stable controlled atmosphere, manufacture of homogenised banana puree and the dehydrated product, drying of limed bananas, deep-fried unpeeled banana chips, banana paste and frozen banana pulp are subjects of considerable interest and importance to us at the moment, when great emphasis is placed on the commercial exploitation of some of the important varieities of bananas for export as fresh fruit as well as in the form of processed products, which are finding increasing demand and acceptability in several countries abroad. The use of a highly hygroscopic biscuit just below the cover of the frozen pulp container, directly before it is sealed, to absorb the exuding milky fluid, is an interesting technique similar to the use of inpackage desiccants in the case of hygroscopic fruit juice powders, of low moisture content.

Other fruits: Avocados can be successfully frozen using liquid nitrogen. An interesting observation has been made that full strength, or somewhat diluted lemon juice, is a particularly effective antioxidant and can maintain especially cut or sectioned fruit against oxidation degradation.

Melons or cantaloupes can be preserved by a simple injection of a charge of inert gas into the seed cavity. A mixture of nitrogen and carbon dioxide is particularly effective and is commercially feasible. Patent literature covering delaying of senescence of the harvested pineapple fruit and use of ethylene on plants for differentation, employing an aqueous suspension of activated charcoal in water, which is presaturated with ethylene, have been discussed. Surface active agents have to be added to water to reduce its surface tension so that a smaller volume of the suspension could be used per hectare. There is greater differentition and also the fruit is more uniform and advanced in maturity. Processing aspects of this important commercial fruit are covered earlier under general methods.

The book is on the whole a highly important addition to the armoury of the food scientist and technologist, and it is to be welcomed as a creative source of ideas for those with imagination.

G. S. SIDDAPPA

Food Colorimetry: Theory and Applications: by F.J. Francis and F.M. Caldesdazle. The AVI Publishing Company, Inc., Westport, Connecticut, 1975, pp. 477.

Research publications in the field of colour measurement both in theory and applications to food products have been extensive. However, books published in this field are very few. The last book published by Mackinney and Little on "Colour of Foods" was in 1962 and by the same company. Thus, the present book is a welcome addition in the field. The authors have published extensively in the field of colour measurement applied to food products and are eminently suited to write the present book. A particularly noteworthy feature of the book is a balanced coverage of the theoretical aspects of colour and its measurement and applications to food products.

The first 18 chapters have been devoted to theoretical aspects of colour including instrumentation. Measurement of colour of various food products have been covered in the next 21 chapters. Continuous colour

measurement and computer programming are given in the last two chapters.

In the first chapter, the nature of light has been briefly discussed with particular reference to corpuscular, wave and dualistic theories. Chapters 2 to 5 comprise discussion on the nature of colour, colour solids, notably Munsell, and colour charts by Ridgway, and Maerz and Paul. In a logical sequence, the eye, anatomy of vision and the colour perception by the eye are discussed. Chapter 6 deals with the measurement of colour by additive mixing of coloured lights making references to works of Newton, Grassman and Maxwell in the field. Chapter 7 introduces the concept of tristimulus values and the primary lights and the mathematical specification of colour in three-dimensional space by vectorial diagrams. Development of the CIE system (Commission Internationale de l'Eclairage, i.e. the International Commission on Illumination), based on the use of standard illuminants, viz. A,B,C (also called standard sources), conditions for the measurement of sample colour, units used for measuring colour and the tristimulus values for the equal energy spectrum representing the standard observer, are briefly covered in chapter 8. This chapter also gives the relation between X,Y,Z tristimulus values and the x,y,z chromaticity coordinates and how all real colours may be described by using positive amounts of CIE primaries and the concept of chromaticity diagram.

Chapter 9 describes unambiguously, how by using transmission or reflectance spectrophotometry the colour of an object may be specified in the CIE system. In particular, the schematic representation of the Weighted Ordinate Method for computing tristimulus values is praiseworthy, as it enables easy understanding of a difficult subject. This is followed by mathematical methods for calculating the same. Then the method using Selected Ordinates is discussed with a sample calculation. The chapter ends with a note on tristimulus colorimetry which is simpler to follow and easy to comprehend and is largely adopted by the food industry in measuring the colour of foods.

Chapters 10 to 13 deal with instruments for colour measurement, such as tristimulus colorimeters, viz. Gardner and Photovolt Colorimeters, the Colour-Eye, the Colormaster and the Hunter Color Difference meter, etc. Reference has also been made to various manually operated spectrophotometers and the General Electric Recording Spectrophotometer (now called as Diano-Hardy Spectrophotometer), which has been unofficially accepted as a reference instrument for colorimetry. Goniophotometry for measuring gloss has been briefly described and instruments for measuring the same is indicated. Visual colorimeters such as Macbeth-Munsell Disc Colorimeter based on additive colorimetry and Jones colorimeter, as well as Lovibond Tintometer which are based on subtractive colorimetry and a note on colour television are included in chapter 12. Wide range spectrophotometry described in chapter 13 does not specifically involve colour measurement. It does, however, involve the use of light transmittance to predict the colour and quality of agricultural commodities, as in detection of internal browning in apples and automatic detection of blood in eggs.

Chapters 14 and 15 give equations for conversion of colour readings from one system to another, development of colour scales and colour diffrences. Chapter 16 deals with colour tolerance, with regard to food and other articles. Chapter 17 introduces the Kubelka-Munk colourant layer concepts which have been widely applied in the paint industry for colour matching problems. The senior author and his students have done extensive work in the application of Kubelka-Munk theory, which is based on light scattering and absorption, in the colour measurement of some food products. Facets such as sample presentation, viz. horizontal, vertical or direct viewing and physical attributes, transparency, turbidity or opacity which influence colour measurement are covered in chapter 18.

Chapters 19 through 39 discuss colour measurement related to a variety of food products, standards for the same and problems inherent and instruments for colour. Food products discussed in these chapters are tomatoes and their products, orange coloured vegetables, green vegetables, cranberry products, citrus products, potato products, cereal products, meat products, tuna, salmon, sugar, beer, wine, tea and coffee, caramel, egg yolks, fats and oils, dairy products, cocoa, chocolate and peanut butter, apples, peaches, cherries and watermelon, honey, maple syrup, sugar syrups, molasses, etc.

Chapter 40 gives a brief account of instruments used for continuous measurement of colour of foods as applied in industry for colour grading of various items, viz. apples, peaches, oranges, shelled peanuts, coffee beans, grains, dehydrated potato slices, potato chips, Instruments mentioned are FMC Spectrosort, Sortex Colour Sorters (various models for diffrent products), etc.

The last chapter consists of computer programmes which may be used to interconvert both colour data and colour scales, as well as colour diffrences. All the programmes have been written in Fortran IV computer language and have been adopted for use in a CDC 3600 computer, except one which uses IBM 360/65 instrument.

In chapter 9, tristimulus values of only the source A spectrum (Table 9.1) are given. It would have been more useful if tables for sources B and C were also included. In the same chapter, selected ordinates for only illuminant C are given (Table 9.2). However, the same for illuminants B and C do not find a place. Similarly, it would have been more useful if tables for CIE equivalents of Munsell value scales were also given. Although such data may be found in otherbooks, inclusion of these would certainly enhance the value of the book. These data are necessary for those not having sophisticated instruments to measure colour or colour difference.

The get up of the book is good. References are included practically under each chapter for further study. Subject index is given at the end of the book. The book, being latest in the field is highly useful for students, research workers, and industrialists concerned with colour problems in food products manufacture, grading of raw materials for colour, etc.

LAKSHMI NARAYANA SETTY

Acidulants for Foods and Beverages: by M.H.M. Arnold, Food Trade Press Ltd. 7 Garrick Street London WC2, 1975, pp. 137.

Information on acidulants is scattered in the literature as they are always treated under food additives. No author has so far attempted to treat the subject independently as has been done in this concise book.

The book consists of 21 chapters which deal with all aspects of acidulants. As rightly pointed out by the author in the Ist Chapter there are just three important sources of edible acidulants, the first is fruit, the second lactic fermentation and vinegar, the third butyric fermentation in some cheeses. Information on production figures, the problem of molecular structure, use of acid salts is also included.

The next chapter deals with the acids of fruit viz. citric acid, malic acid, tartaric acid, ascorbic acid, isocitric acid, succinic acid, quinic acid, shikimic acid and their significance.

Chapter III deals with the major acidulants important of which are vineagr and acetic acid, citric acid and phosphoric acid. Methods of production of these acids are briefly described and markets for ceratin acidulants indicated. Similarly Chapter IV is devoted to secondary acidulants like acetic acid, tartaric acid, malic acid, fumaric acid and Chapter V for minor and marginal acidulants like adipic acid, glutamic acid, succinic acid, ascorbic acid, isocitric acid, shikimic acid, quinic acid, oxalic acid, hydrochloric acid, sulphuric acid, etc. The use of lactones and various others are examined in Chapter VI.

Very useful information on the metabolism and toxicity of various acids has been brought out and their importance in the basic verebs cycle discussed in Chapter VII. Indeed the food technologists and food manufacturers are interested to know about the qualitative and quantitative aspects of sourness and thus Chapter VIII is devoted to the nature of sourness and how it can be measured. Information on the amount of sourness in unit weight of some food acids has also been given.

The secondary effects of acidulating the foods are discussed in Chapter IX. Preservation, sequestration and plasticisation of protein are some such effects which are valuable properties of the acidulants.

The chemical and biological properties, the flavour and the solubility problems and the problems involved in handling and storage of different acidulants are examined respectively in the next two chapters (X and XI).

Chapter XII is devoted for discussion on food regulatory measures on acidulants and other legal aspects.

The uses of various acidulants in various products like sugar confectionery, preserves, canned, dried and frozen fruit and vegetables, desserts and powder mixes, soft drinks, fermented beverages, pickles and sauces, animal protein products and cereal products are discussed in detail in the next nine chapters and the useful information on the particular requirement of the products and the characteristics of the acidulants that can be used are indicated.

Although the author has put forward his reasons in the preface, the literature references if included would have been useful. The book with its excellent printing and presentation will prove to be a valuable source of information not only to food scientists and technologists, students of food technology but also to the food manufacturers. The book will be a welcome addition to the food science and technology libraries.

L. V. L. SASTRY

Commercial Vegetabale Processing: by B. S. Luh and J. G. Woodroof, The AVI Publishing Co., Inc., 1975, pp. 755.

This is a treatise surveying "the entire vegetable processing industry and the vegetable processing operations" prepared by the editors in collaboration with 17 specialists from Universities, research institutions

and industry, intended to be "a valuable reference for plant managers, superintendents, quality assurance managers, food chemists, researchers, and those interested in technical knowledge of the food processing industry". The book aptly answers this description and is indeed, a valuable addition to the AVI list on Food Science and Technology.

The book comprises of 18 chapters, five of which of general nature that can fit into any book of this type concerning any food commodity. These include chapters on general principles, microorganisms, containers, quality control, plant sanitation and waste disposal and nutrition labelling. The rest of the eleven chapters cover the entire sequence of operations from harvesting, pretreatment, to processing by canning, freezing, dehydration, freeze-drying and production of juices, pickles and soups. Chapters on grades and standards, nutritive value and quality criteria fill in the relevant details. Each of the chapters ends with an exhaustive bibliography and important data are tabulated or diagramatically represented for conveying the concepts effectively as well as to serve as reference.

The introductory chapter summarises the recent trends in processing methodology. In Chapter II the microbiological problems associated with food in general and vegetables in particular are surveyed. The possible hazards of the use of effluent water in vegetable cultivation are, however, not touched. The chapter on containers discusses the salient features of tin, glass and plastics as packaging materials. Details about fabrication of the containers given appear to be beyond the scope of the subject matter. Toxicity aspects of plastic materials are not discussed. Chapter IV deals more with the benefit of mechanical harvesting and gives special procedures for different crops. However, storage and sorting aspects are dealt with rather cursorily Chapter V similarly gives a sketchy account of blanching and peeling and only a few vegetables are discussed in detail. In fact, these details are repeated unnecessarily in chapters on canning, dehydration and freezing.

In the otherwise exhaustive chapter on canning the problems of discolouration, corrosion and chlorophyll degradation do not appear to have received due attention. In the discussion on freezing aspects of microbiology of frozen vegetables, pigment stability, oxidative changes, freezing injury, etc. have not received consierdation. Similarly, in the chapter on dehydration, the points conspicuously omitted or just touched upon include packaging of dehydrated vegetables, spray- and drum-dried products, sulphuring devices, vegetable cuts for dehydration, etc. These three main chapters on processing give adequate details about processing of important vegetable varieities. Freeze-drying receives special attention in a chapter which is well-documented giving adequate details on processing and packaging. The chapter on pickles and saurkraut gives a fair account of the processes and problems except that it deals mainly with the Western types of fermented preparations only.

Information useful for international trade has been presented in the chapter on Grades and Standards giving details about important processed vegetables. The chapter on quality control by Luh and O'Neal though of a more general nature rather than pertaining to vegetables, offers an exhaustive account of the subject in all its aspects. The same is true about the discussion on plant sanitation and waste disposal. The chapter on composition and nutritive value covers the aspects on flavour, colour, pesticide residues as well. A table of composition is a good addition. The discussion on storage life and objective criteria for quality is rather scanty. The chapter on soups gives some useful recipes.

In such an exhaustive treatise a few pertinent aspects of vegetables, however, are conspicuous by their omission. These include pre- and post-harvest chemical treatments, details on chemical composition in relation to texture, enzymic browning systems and recovery of by-products. However, inspite of these omissions, this offers an excellent reference book on the subject.

D. V. REGE

1974 *Evaluations of Some Pesticide Residue in Foods:* WHO Pesticide Residue Services No. 4, Series No. 574. World Health Organisation of U.N.

The evaluations contained in these monographs were prepared by the joint meeting of the FAO working committee of experts on pesticide residues and the WHO expert committee on pesticide residues that met in Rome during 1974. For about 34 pesticides, acceptable daily intakes have been established. These information, along with the other agricultural data, presented will help in fixing tolerance limits in foods. Under each monograph, the important parameters, such as, the identity of the compound, acceptable daily intakes, toxicological studies, fate of residues in plant, soil, water, food and analysis comprising of extraction, clean-up, isolation, detection and purification are discussed. Evaluation for acceptable daily intake consists of biochemical aspects like biotransformation, effects on enzymes and studies on man and rats.

Under toxicological studies, data on carcinogenicity in mouse, rats, rabits, studies on mutagenicity, tetragen. city and reproduction in mouse and rats are presented-

Short term studies on rats and dogs and long term studies like dermal, inhalation, are covered under acute toxicity.

The pesticide residues, resulting from pre-harvest and post-harvest treatments, in raw agricultural commodities, horticultural products are covered.

The monographs are available with WHO Pesticide Residues Services.

The present volume provides excellent compilation of data on the toxicity, acceptable daily intake and fate of pesticides residues on different materials and conditions. It will serve as a useful guide to pesticide residue laboratories, toxocologists and institutions administering pesticide residue regulation act.

K. VISVESHWARAIAH

Research on Cassava and Legumes

The International Development Research Centre (IDRC), Ottawa, Canada, will provide grants totaling £ 759 000 over three years to support the establishment of regional networks in Latin America and Asia to improve the production and utilization of the root crop cassava.

The IDRC has been supporting cassava research in Africa, Asia and Latin America for the past four years, and already some remarkable results have been achieved. Although many of these advances are still at the laboratory stage, their promise is especially significant for the millions of people of those developing countries where cassava is an important part of the staple diet. Research is also progressing on the potential of cassava as a source of cattle and livestock feed.

The two regional projects will be centered on the International Centre of Tropical Agriculture (CIAT) in Cali Colombia, where much of the research in recent years has been carried out. Each will aim at further development of the recently discovered techniques to adapt them to the different conditions found in the two regions.

In addition to encouraging cooperative research, the projects will provide regional training facilities, technical guidance and support for trainees returning to their home countries, and will attempt to speed-up the rate of adoption of improved production and utilization techniques among small-scale producers and processors of cassava.

Crop scientists and soil scientists at the University of Nairobi, are launching two research programmes designed to improve legumes and other crops that may be grown in sequence with maize and so help balance the diet of rural families in Kenya.

The first is a breeding and agronomic programme concentrating on improvement of pigeon peas that are grown as a source of protein in dry, marginal areas of the country.

The second is a study of crop rotations in densely populated highland areas. The object is to analyze the interactions when maize is paired with any of seven other crops and to learn what effects these cropping patterns have upon soil conditions, nutrients and pests.

A New International Agricultural Research Centre (ICARDA)

A New International Agricultural Research Centre, to serve the special needs of countries in North Africa and West Asia, was formally launched. Canada played a large part in setting up the Centre, which it is hoped will help to solve the food problems for countries in the area.

Active planning for the International Centre for Agricultural Research in the Dry Areas (ICARDA) has been under way for more than two years. But on 29 July ICARDA came formally into being when the 16 donor governments and institutions who are members of the Consultative Group on International Agricultural Research (CGIAR) sub committee for the Establishment of ICARDA handed over responsibility for the development of the centre to its own Board of Trustees. The CGIAR sub committee took this step at a meeting in Washington.

Chairman-elect of the nine members on the Board at present is Dr. Taher Obaid of Saudi Arabia, a former deputy minister of agriculture. Four other Board members come from countries in the region—Algeria, Iraq the Sudan and Syria—while the other four come from Australia, Canada, the Netherlands and the United States. The Canadian member is Dr. Omond Solandt, former chairman of the Science Council of Canada.

The Acting Director General of ICARDA is Dr. William Pelton, who is assistant director of Lethbridge research station in Alberta. His main role will be to plan development of the sites near Aleppo, in Syria, and Tabriz, in Iran.

ICARDA will concentrate on the improvement of durum wheat and barley and of grain legumes such as chickpea, broadbean and lentil and of farming systems. Research will also be done on sheep husbandry. Scientists at the Tabriz station will specialize in cereals and legumes that perform well in high altitude, winter rainfall conditions.

Prof. V. Subrahmanyan Industrial Achievement Award

Prof. V. Subrahmanyan Award was instituted by the Association of Food Scientists & Technologists (India), to be given to any Indian who has made notable contributions for the development of agro-based food and allied industries or for contributions to food science and technology with immediate prospects and/or future potential for industrial application. This Award for 1975 goes to Dr. H.S.R.Desikachar.

Dr. Headathale Srinivasa Ramanuja Desikachar was born in 1924 at Hedathale, Mysore District. He had his early education at the Central College, Bangalore and after taking his B.Sc (Hons) in Chemistry, he joined the Dept of Biochemistry, Indian Institute of Science, Bangalore, as a research student. Subsequently he obtained Associateship of the Indian Institute of Science for his researches, and Doctorate degree from the University of Bombay, mainly in the area of processing soybean milk and on factors affecting the nutritional value of soybean. For a brief period he worked in the National Chemical Laboratory, Poona and in October 1950, joined the Central Food Technological Research Institute, Mysore. Presently, he is the Project Coordinator of the Rice and Pulse Technology Discipline in CFTRI.

For over 20 years, Dr. Desikachar has been actively pursuing research and development activities in the area of cereal technology. His special contributions have been towards the development of improved methods of parboiling of paddy, a method of commercial curing of new paddy and a process for the stabilization of rice bran. In addition, several processes for the production of convenience foods have been developed under his direction. All these processes have found wide application in food industries all over the country. Dr. Desikachar has over 75 research publications to his credit.

Dr H. S. R. Desikachar, receipient of Prof. V. Subrahmanyan, Industrial Achievement Award

Dr. Desikachar is widely travelled and has participated in many international conferences. He spent an year at the University of Toronto, Canada, during 1951-52 on a post-doctoral fellowship. He attended two international conferences on rice in Japan (1966 and 1975), a symposium on rice by-products in Spain (1975) and a conference on sorghum and millets in Mexico (1976). In the year 1974, he visited several West African countries, Canada, the Philippines and Japan on deputation to study the problems of grain processing. He is a member of the Scientific Advisory Committee of the International Crops Research Institute, for the Semi Arid Tropics (ICRISAT), Hyderabad and also several technical committees of the Government of India.

Southern Regional Branch, Madras

Dr. K. Krishnamurthy, Deputy Commissioner (S&R) Minister of Agriculture and Irrigation, Department of Food, Krishi Bhavan, New Delhi, spoke on Scientific Grain Storage in the Country on 11th September 1975. Following is the summary of the talk.

The storage of food grains at the level of farmers is very important in our country as more than 70 million tonnes are stored in the villages at present. On examination of post harvest handling and storage in the country it is revealed that considerable quantities of food grains are lost during these operations. It is estimated that losses are to the extent of atleast 10% which means more than 10 million tonnes of food grains worth more than thousand crores of rupees are lost every year. This is because of non-scientific and old methods followed at all points between production and consumption. Proper storage of foodgrains till consumption is as important as taking steps to increase production. The storage techniques adopted by the Government and their agencies are modern and therefore, the losses are negligible. However, the storage at the level of traders and farmers in rural areas is far from satisfactory. Qualitative losses occur as a result of contamination with excreta of insects, rodents, birds and also contamination with mycotoxin produced by moulds. The losses in quality also occur as a result of changes in constituents in the grain like gluten in wheat. There-

fore it needs no emphasis that scientific storage methods have to be applied for preserving the grain from quantitative and qualitative losses.

The Indian Grain Storage Institute at Hapur and other ICAR and CSIR Institutes are engaged in developing scientific storage pratices like use of improved storage structures, use of insect control and rodent control methods, prevention of damage due to moisture and proper storage management. The Save Grain Campaign in the Department of Food is engaged in popularising the recommended scientific storage practices in the country. At present it is working with six field offices located at Madras, Hyderabad, Bombay, Bhopal, Ghaziabad and Patna. Shortly five more offices will be opened at Udaipur, Chandigarh, Lucknow, Calcutta and Bhubaneshwar. These work in close collaboration with the State Governments in popularising scientific storage practices. About Rs. 120 lakhs have been given to different state governments for fabrication and sale of small capacity metal bins for use in rural areas. Anti-cogulants, ALP tablets and zinc phosphide are being popularised for use in rodent control in and around houses. EDB samples are recommended for insect control in stored foodgrains. Intensive demonstration and publicity programmes are being carried out for bringing the technical know-how to the doors of farmers and traders. Apex level training for the trainers, stipendary and non-stipendary training programmes to the educated youth drawn from farmers and traders, review and orientation training programmes to the extension staff of the Centre and State Governments are carried out regularly in modern methods of post harvest handling, storage and preservation of food grains. The application of science and technology to traditional agriculture in recent years is resulting in a steady increase in the production of foodgrains in the country. The benefits of increase in production of foodgrains will not be enjoyed if the losses are allowed to continue. A coordinated approach is needed to implement the programme. Research institutes extension departments, industries and voluntary organisations have to play an important role in modernisation of storage in the country.

Technical Session on "A New Development in Paddy Parboiling and Drying" by M. Kuppuswamy, Chief Engineer (Rice Mills), Food and Nutrition Board, Ministry of Agriculture and Irrigation, Department of Food, Krishi Bhavan, New Delhi.

Several methods of parboiling have been developed and used in recent years. The improved parboiling methods developed at Jadavpore and the CFTRI, Mysore have no doubt made an impact in the country. Although they are useful they have some inherent defects. The hot soaking method gives a highly coloured rice with a long cooking time.

A new scheme of soaking in which the kernel is moistened with humid hot air has been devised to overcome these defects. The high temperature combined with high relative humidity tend to produce accelerated soaking effect on the grain. As it is being done under controlled temperature conditions, there is no discolouration of the grain. In this method the leaching losses are avoided. The humid hot air that passes through the grain can be recirculated which results in considerable saving in energy. The process could lend itself to a continuous operation by setting up suitable lay-out in which hot air passage is arranged in a closed circuit while paddy moves down the wetting unit. Theoretical calculations to determine the time of soaking in the light of equilibrium moisture values show the practicability of attaining desired moisture levels within about 3 hours.

In an actual set-up it is possible to develop hot air directly from husk fired furnace. Into this hot air, duct nozzles can be fitted for spraying warm water. Since the humid hot air is again reused, the husk furnace as well as the spray nozzles would need to be only of very modest proportions. Only heat and water have to be added into this ducting system as and when required. Thus, there is no wastage of heat in this process, nor is there any leaching from paddy; fungal damage is also obviously eliminated. As the temperature will be around 60°-70°C there will not be any discolouration of grain.

After soaking the grain as described above, the grain can be gelatinised in about 2-3 min. This can best be accomplished in cylindrical perforated tube inside of which is fitted with another cylindrical perforated tube of smaller size. The wet grain passes down through annular space while steam is passed through the inner tube and escapes through the perforations and comes in contact with the flowing mass of soaked paddy. In this way, there is uniform parboiling, as the penetration of steam is uniform throughout. The steaming unit is very simple in design and small in size. Steam requirements are minimal as gelatinisation is accomplished as a flash process. This will be followed by mechanical drying in the usual way.

Laboratory studies have confirmed the usefulness of the above process. It now remains to be seen whether and in what manner, we can instal a pilot parboiling plant based on the above principles. The leaching losses taking place in all the existing praboiling systems (traditional or modern) would by itself justify the need

for such an approach since we can hope to realise about 2 per cent rice through avoidance of wash water losses. Considering that more than 20 million tonnes of paddy are being parboiled each year, this amount of saving is indeed quite substantial.

The Annual General Body Meeting of the Southern Region was Held at Madras on 25th August 1976. Technical Sessions on "Extrusion Cooked Foods" were held. Three papers entitled (*i*) Project profile on cooker extruder based on snack foods, by Shri K. Raghunandan, (*ii*) project aspects of cooker extrusion processing by Shri Varada Seshamani, and (*iii*) extrusion cooked foods in social welfare feeding programmes by Shri M. R. Chandrasekhara were presented.

Shri K. Raghunandan, Total Engineering Consultants, 33 Queens Road, Bangalore-51, presented complete project profile for a snack foods' plant using a cooking extruder. All aspects concerning plant capacity, raw-materials, machinery personnel, packing, pricing and marketing and financial inputs were covered.

Shri Varada Seshumani, Executive Director, Bangalore Tool Works Pvt. Ltd., 33, Queens Road, Bangalore-51 analysed general food processing systems and the unit operations involved. He elaborated on the S100 cooker extruder developed at BTW with financial assistance from NRDC.

CEP is now a general purpose Food Processing method. Its raw materials are basically cereals and oilseeds, but even in this, its full capabilities as a process have yet to be explored. It can produce expanded, semi-expanded, cooked and semi-cooked products of various shapes and sizes. It is not a specific processing method for a specific product, like 'bread' or 'biscuits'. It is a compact system with easily adjustable variables and a number of control options. And what is unreplaceable, it can if I may use the expression 'pasteurise' the food into long shelf life and palatability.

According to present technology, the process of cooker extrusion, is based mainly on the plasticity of the processed product; 'machine design' considerations were thus based on comparisons to plastics processing technology. With the development of extensive experimentation on various materials this relationship has become very tenuous. We now have, for example, products like:

Expanded snacks, which are produced by CEP directly for packing, with a moisture content of 4 to 6 per cent, without the use of a drier.

Weaning foods with easy dispersability and therefore higher digestibility, due to efficient and irreversible processing of the starch and protein.

Oil-cake-based high protein products easily and economically processed due to the almost complete elimination of shelf life inhibitors.

Textured vegetable protein (TVP) products.

This range of capabilities of CEP combined with its other advantages like process integration, production economy, general purpose capability and versatility etc., make it an extremely simple break-through of the 1970s useful for every food processer. He pointed out that Shri N. H. Vyas, in his paper 'The Scope for Extruded Foods in India ' (PFNDAI), has estimated the potential beneficiaries under nutrition programmes in India, to be about 47.6 million. Deficiencies such as the protein-calorie gap, can only be overcome by high-volume low-cost processing of self-contained formulations, and increasing the availability of such foods. This should be done by better processing as well as the processing of non-traditional materials.

Shri M. R. Chandrasekhara, President of AFST, gave a talk on Extrusion Cooked Foods in Social Welfare Feeding Programmes, and also recounted his recent experience as an FAO Advisor in Sierra Leone, Africa, wherein trials for production and popularisation of weaning food mixtures based on locally available ingredients are under way.

The feeding programmes of the country are aimed at improving the nutrition of the vulnerable groups. It is now realised by many countries that no state can afford to neglect malnutrition in growing children and in pregnant and lactating mothers.

Only three types of foods can be included in such programmes: (*i*) When the ingredients are purchased locally, cooked centrally and distributed (*ii*) powdered and dry blended foods like Bal Ahar or Indian Multipurpose Food, and (*iii*) foods where processing is minimal like energy food. CSM is a food wherein in its preparation the ingredients soyabean and corn are separately processed. In our experiment we mixed the ingredients and passed the mixture through the Wenger. The two blends which we used then were (*a*) Indian Multipurpose Food blend, and (*b*) Bal Ahar type blend. Since then other organisations have taken up using the Wenger extruder for preparation of foods for the social feeding programmes. The advantage in extrusion cooking are: (*i*) When optimum conditions are achieved, the process is simple - excluding of course such contigencies like power shut-down. (*ii*) A production of 1200 to 2400 tonnes per annum could be easily achieved. There are very few other processes where this volume of food could be processed with such little effort. (*iii*) The product is ready-to-eat. The extruder is ideally suited for the production of snack foods. The materials are passed through high temperature - short time

cooking. Hence the raw flavour of the ingredients is over-come and pleasant flavours and tastes develop. Because of the volume expansion the texture is also highly acceptable. (*iv*) Antinutritional factors like anti-trypsin factor in soy is inactivated. (*v*) The extrusion process is versatile it can produce different shapes and sizes catering to the needs of different age groups, i.e., a varied product profile.

Along with these there are a few disadvantages. The major criticism is that the equipment is very costly. The cost per kg of food at the consumer level is estimated to be Rs. 6/- or for 75 g/child/day it is Re. 0.45, which is too high for the feeding programmes.

The next problem is the cost of packaging. Only HD polythene bags or multiservice containers are suitable. Multilayer pouches could be used for bringing in consumer appeal.

We have tested the effect of extrusion cooking on the nutritive value of the product - IMPF (Indian Multi Purpose Food). In our animal experiments we found that extrusion cooking did not affect the protein efficiency ratio adversely though extruded Bal Ahar was slightly affected.

Extruded Bal Ahar and IMPF for making *Kesari Bath* (a sweet preparation of India) and *Uppamav* (breakfast food) are used. Extrusion cooking appears to be a very favourable processing technique for social feeding programmes.

Shri M.R. Chandrasekhara referred to the World-Bank project in Chitradurga District of Karnataka wherein energy food of CFTRI is used. The concept behind this is that when the nutrition of the child is protected, the motive for having more children is overcome. Energy food is a blend of toasted wheat, puffed gram, roasted groundnut flour with some jaggery and vitamins. Processing costs are minimum and the food is very highly acceptable.

Development and Marketing of Weaning Foods in Sierra Leone

Sierra Leone is a small country with a total area of about 28,000 sq. miles and is about half that of Karnataka. Agriculture is poorly developed, the main crops being rice, tapioca, sweet potato, groundnut and some pulses. The diet of the population consists mainly of tapioca, rice and cooked and pounded leaves of tapioca and sweet potato with plenty of palm oil, fish and some meat. There is plenty of malnutrition and undernutrition amongst children, as a result of food taboos and misconceptions. Eggs, fish and milk are not given to children, milk of course is produced in very little quantity in the country.

The idea of production of weaning food for small children was evolved as early as 1961, when a U.N. home science adviser formulated a mix based on rice, groundnut and sesame, all available locally. The mixture was tested and recipes were evolved for its production in the homes. Later from 1973, a project for centrally preparing the food in a factory was started with the aid of FAO/UNDP. The blend was slightly altered, the present formula being parboiled rice 49.5 per cent dehulled-expeller pressed sesame flour 27 per cent cowpea dehulled and powdered 13.5 per cent, sugar 9 per cent, vitamin premix 1 per cent with vitamin and l-lysine added. Bennimix is the name of the weaning food—as sesame is called benniseed. It was only in January that production started. The cost of production is about Rs. 8.80 per lb. Now it is being sold at Rs. 4 per lb.

The constraints for the project are the small capacity of the plant (about 1 tonne per week) inefficiency of some of the equipment and lack of knowledge among the public throughout the country. Special weaning food for children, as in most developing countries, is a new concept. However, with the over-riding enthusiasm of the Social Welfare Department the project would be sure to become a success.

List of New Members

Mrs. R. Lakshamma, 207 IIIrd Block, Sita Krupa, Rajajinagar, Bangalore-560 010.

Mr. D. Gurumurthy Reddy, Technical Assistant, T. N. Nutrition Project, 7A Gopalapuram Ist Street, Madras-600 086.

Mr. P. Prakasha Lal, C/o B. Y. Rao, Professor, Department of Food Technology, Laxminarayana Institute of Technology, Nagpur-440 010.

Mr. A. N. Srirangarajan, D 14 Ajantha. Anushakthi Nagar, Bombay-400 094.

Dr. Narendra Narain, 13-C Nandadevi, Anushakthi Nagar, Bombay-400 094.

Dr. D. S. Pradhan, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Dr. Kulkarni Suhas Bhalchandra, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. C. K. Krishnan Nair, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. Konkimalla Gurumurthy Chetty, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085

Mr. P. S. Chauhan, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. Khambata Gev Kersasp, School Building No. 5, Cusrow Baug, Colaba, Bombay-400 039.

Mr. P. P. Khardekar, C/o P. G. Khardekar, Campaign Officer (Agri), Collectorate Compound Z.P., Thana.

Dr. Modi Jitendra Dhirajlal, 1/32 Kalyan Building, 156 Khadilkar Road, Bombay-4.

Miss. Sabira Haiderali Dharsi, IF5, Jail Road East, 3rd Floor, Bombay-400 009.

Mr. Oza Dinesh Shankerlal, 12 University Hostel, R. A. Kidwai Road, Matunga Bombay-400 019.

Dr. Ashok Champakalal, Plot No. 235, Block-6, 'Purushottam Nivas', Jawaharnagar, Goregaon (West), Bombay-400 062.

Mr. Sitangsu Chakraborty, 4P Nandaram Sen Street, Calcutta-700 005.

Mr. K. Gangadhara Rao, C/o Directorate of Sugar & Vanaspati, Jamnagar House, Block No. 11, Gallery No. 16, Room No. 19, Shajahan Road, New Delhi-110 011.

Miss Nilu Chakraborty, Office of the Food & Nutrition Board (Eastern Region) Ministry of Agriculture & Irrigation, Department of Food, 8 Esplanade East Calcutta-700 069.

Miss Rashmi Chandra, 'Gokul', (Near State home for Women), Hyderabad-500 873.

Miss D. Gayathri, C/o D. K. Dorairaj, 2-2-647/274 Srinivas Nagar, Bagh Amberpet, Hyderabad-500 013.

Miss Zakia Fatima, 5-8-38 Fatch Sultan Lane, Station Road, Hyderabad-500 001.

Miss Manorama Rayaprol, 6-4-481/2 Krishnanagar, Bholakpur, Secunderabad-500 003.

Miss Anurag Chaturvedi, 4-1-1232 Bogulkunta, Hyderabad-500 001.

Miss. Renuka Rama Rao, 75 Sarojini Devi Road, Secunderabad.

Miss. V. Manorama, College of Home Science, Hyderabad.

Miss. L. Anuradha Reddy, C/o Dr. Y. V. Reddy, IAS, 12 Banjara Hills, Hyderabad.

Dr. B. Y. Rao, Professor of Chemical Technology, Laxminarayana Institute of Technology, Nagpur-400 010

Mr. P. A. S. Subramanian, Assistant Director (Chemical) S.I.S.I., Rajajinagar, Bangalore-560 044.

Mr. K. Rajagopala Kurup, M/s. Food Concentrates (India), B-49 Industrial Estate, Rajajinagar, Bangalore-560 044.

Mr. T. V. Subramanyan, Deputy Technical Advisor, Department of Food, Sastri Bhavan, Madras-600 006.

Mr. K. Narasimha Murthy, U 14 Ganesh Block, Sheshadripuram, Bangalore-560 020.

Mr. G. S. Mohan, 23/1 Richmond Road, Bangalore-560 025.

Mr. M. S. Parthasarathy, Karnataka Agro Corn Products Ltd., Hebbal, Bangalore-24.

Mr. R. L. Reddy, C/o Indian Standards Institution, Southern Regional Office, CIT Campus, Adyar, Madras-600 020.

Mr. V. Vaidyanathan, 2/40 Konda Reddiar Street, Reddiarpalyam, Pondicherry-10.

Dr. M. Prabhakara Rao, Assistant Professor, College of Home Science, Hyderabad-4.

Mrs. K. M. Jainamma, CFTRI Experiment Station, CSIR Complex, Industrial Estate P.O. Trivandrum-695- 019.

Miss. M. A. Sumathy Kutty, CFTRI Experiment Station, CSIR Complex, Industrial Estate P. O., Trivandrum-695 019.

Mr. M. N. Jayadeva, 869/A Karnataka State Agro Corn Products Ltd., Vani Vilasa Road Siddappa Square, Mysore-570 004.

Mr. Subhas Dattatreya Arve, At & Post: Borgaon, Tal-Tasgaon, Sangli District, Maharashtra.

Mr. E. P. Appavoo, No. 50, 3rd Main Road, R. M. Puram, Madras-600 028.

Mr. B. K. Rout, Assistant Director (St. & Res.), 43-Model School Road, Madras-600 006.

Mr. A. K. Anandan, Aerated Water Factory, Spencer & Co. Ltd., Madras-600 002.

Mrs. V. A. Gangolli, Principal, Food Crafts Institute Opp. S.S.C. Board Office Shivaji Nagar, Poona-411 005.

Dr. A. K. De, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. Shreekrishna Dattaram Pandit, C/o Larsen & Toubro Limited, Food Processing Division. L & T House, Bombay-400 038.

Dr. Koppikar Satish Venkatrao, Biochemistry & Food Techonology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. A. G. Deshpande, Room No. 134, New Hostel, University Department of Chemical Technology, Matunga, Bombay-400 019.

Dr. M. N. S. Rao, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. Sadananda Vishnu Ghadi, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. Mohan Waman Nulkar, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. Alur Madhavesh Dadacharya, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. Arun Kumar Sharma, FIPLY, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. R. P. Pande, Assistant Production Manager, MAFCO Limited, P.O. Box. No. 55, Nanded-431 601.

Mr. Desai Kiran Mangubhai, 31-Lajpatrai Road, Prabhunivas, Ville Parle (West), Bombay-400 056.

Mr. Parekh Rajnikant Manilal, 31-Lajpatrai Road, Prabhunivas, Ville Parle (West), Bombay-400 056.

Miss. Rannade Shah, E-12 Government Girls Hostel, Netaji Subhas Road, Bombay-4.

Mr. D. R. Ranganath, C/o Foods Scction, Department of Chemical Technology, Matunga, Bombay-400 019.

Dr. Dilip Bhattacharjee, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. K. K. Surendranathan, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. M. Meyyappan, Amrut Distilleries (P.) Ltd., 36 Sampangi Tank Road, Bangalore-27.

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Editor
V. B. OBEROI

ANNOUNCEMENT

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Nominations for the above award for the year 1976 are invited. The guidelines for the award are as follows:

1. To begin with only Indian Nationals (one or group of persons) engaged in the field of Food Science and Technology will be considered for the award.

2. The Awardee / Awardees should have contributed to the field of Food Science and Technology, for the development of Agro-based food and allied industries or to basic food science and technology with immediate prospects and / or future potential for industrial application.

3. The Awardee / Awardees should be proposed and seconded only by the members of the Association; the bio-data of the candidate should be given in detail including the work done by him and for which he is to be considered for the award.

4. The Awardee will be selected (from the names sponsored) by an Expert Panel constituted by the Executive Committee for the above purpose.

Nominations with particulars of the awardee along with his bio-data and contributions, should be sent by **Registered Post,** *so as to reach Shri M. M. Krishnaiah, Honorary Executive Secretary, Association of Food Scientists and Technologists (India), Central Food Technological Research Institute, Mysore-570013 latest by 31st of January 1977.*

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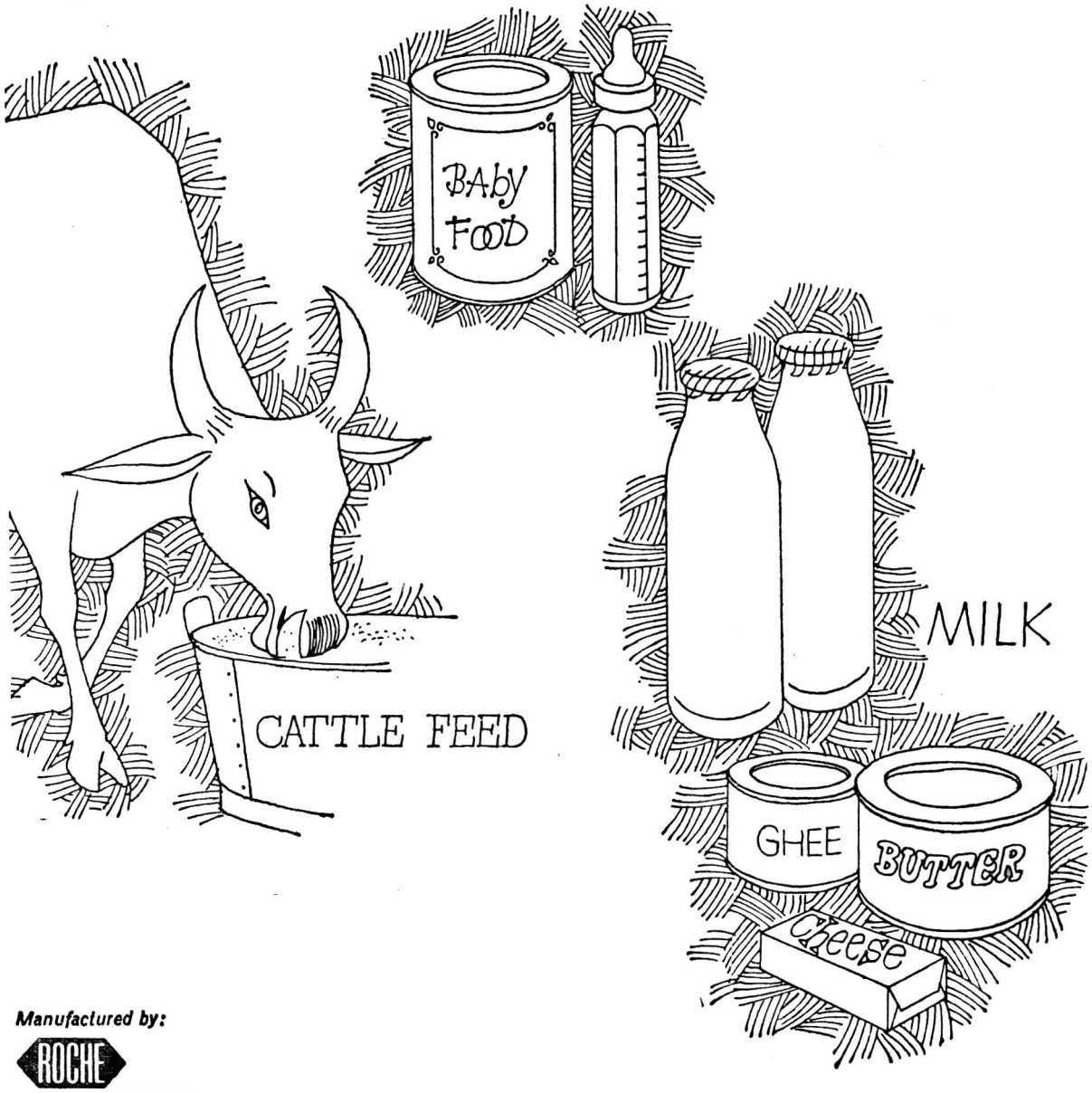

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   (*b*) *Book:* Venkataraman, K., The Chemistry of Synthetic Dyes, Academic Press, Inc., New York, 1952, Vol. II, 966.

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

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

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

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

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