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Research Notes

The Effect of Ante-mortem Epinephrine Injection on Plasma Glucose, Post-mortem Glycolysis and Meat Quality Factors of West African Dwarf Ewes

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Eight West African dwarf ewes were allotted into two equal groups in an attempt to study the effect of epinephrine on plasma glucose, post mortem glycolysis and some aspects of meat quality factors. One group was injected subcutaneously a one-shot dosage of 0.1% adrenaline solution at a level of 1.8 mg/10 kg live body weight 6 hr prior to slaughtering. Plasma glucose levels were estimated during the first hour post injection. The other group of ewes which was injected with an equal volume of water served as control. Following death, the pH of the longissimus dorsi was followed as an index of glycolysis. Subsequently, brine diffusion distance, salt uptake, drip loss, cooking loss, total moisture and the organoleptic attributes of tenderness, juiciness, flavour and overall acceptability were estimated in selected muscles.

An overall elevation of plasma glucose by epinephrine was observed during the one hour period of blood collection a lower than normal initial pH was observed in both groups since both struggled prior to death. The ultimate 24 hr pH was higher in the epinephrine treated mutton (6.43) than in the control group (5.63). Mean drip loss and cooking loss were 1.86 and 30.88% in the epinephrine treated samples as against 1.63 and 33.38% respectively in the control samples.

Higher estimates of diffusion lengths were obtained in the treated samples (P <0.05) although between muscle variations were observed. Slight differences were observed as a result of treatment effect on tenderness, juiciness, flavour and overall acceptability. Between muscle variation in these parameters were highly significant.

Prevalent methods for slaughtering animals in Nigeria necessarily involve a lot of energy utilization during the death struggle. Previous studies by Aberle and Merkel[1] suggest that the stress of slaughtering produce enough endogenous epinephrine to cause a maximum rate of glycolysis and that the rate of post mortem pH fall and ultimate pH values were not altered by injection of exogenous epinephrine prior to slaughter. Other results obtained by Pearson *et al.*[2] have also shown that injection of epinephrine only 5 min prior to slaughter did not significantly alter glycolysis or other muscle properties although such muscles were slightly toughened.

In this paper, the effects of injecting epinephrine subcutaneously 6 hr pre-mortem into West African dwarf ewes have been investigated. These effects have been quantitated through the estimation of blood plasma glucose levels within one hour post injection, and post mortem, through the measurement of changes in muscle pH as an indication of muscle glycolysis. Other quality indicating characteristics of the raw and cooked meat were also estimated.

Materials and Methods

Eight West African dwarf ewes of unknown ancestry, previous feeding regime or management were obtained from the regular commercial outlet at Sango, Ibadan in Western Nigeria. It is believed that these animals were raised somewhere in Northern Nigeria and have just been delivered for sale at the commercial outlet. The liveweight of ewes ranged from 18.15 to 28.48 kg with an average of 23.2 kg. The average age was estimated to be roughly 36 months based on the scheme of Starke and Pretorious[3].

The ewes were allotted to two groups each containing four animals. Prior to slaughter, feed was witheld overnight from each group of ewes although they had access to water. Exactly six hours before they were slaughtered, one group of ewes was injected subcutaneously in the right flank with enough 0.1 per cent adrenaline hydrochloride solution to give final dosage of 1.8 mg/10 kg liveweight. Equal relative volume of water were injected into the other group. Blood samples (10 ml) were withdrawn immediately from the jugular vein of each ewe and again at 30 min and 1 hr after the initial sampling.

Blood samples were transferred into centrifuge tubes containing some grains of ethylenediaminetetraacetic acid (EDTA) and immediately mixed to prevent coagulation. The plasma was separated by centrifugation at 3,000 rpm for 30 min, removed by suction into capped vials and frozen until analysed for glucose.

The glucose content of the thawed plasma was estimated by the glucose oxidase method using o–dianisidine[4].

The ewes were exsanguinated without being previously rendered insensible and drained as much as possible. Dressing of the carcasses were completed within 20 min of death. The initial pH of the longissimus dorsi (LD) muscle was taken by inserting the electrode of a pH meter into two slits made in the fiber direction on the muscle at the location between the 12th and 13th ribs and opposite the last lumbar vertebra. The observation was again repeated after 30 min, 3, 6, 12, 18 and 24 hr.

Immediately after taking the initial pH, the carcasses were weighed and transferred into a cold room at 0°C. After 24 hr the two longissimus dorsi, biceps femoris, semimembranosus and semitendinosus muscles were carefully dissected from all carcasses, individually packed in polythene bags and frozen at -29°C. One of each pair of muscle from each carcass was subsequently used for brine diffusion measurements while the other was used for estimation of palatability traits.

In estimating brine diffusion characteristics, a 7.5 cm length of muscle was taken from the thickest portion of each frozen muscle such that the fibres run approximately from one end to the other. A 2 cm cylindrical meat core was taken in the fiber direction using a well bevelled stainless steel meat core borer. Each meat core was carefully inserted into a clean 2-cm diameter cylindrical glass tube with a clearance of approximately 1 cm from one end of the glass tube. This space was filled with a spongy foam material which also extend slightly beyond the glass rim. Each glass tube was then clamped upright in a 250 ml beaker containing 50 ml of a brine solution prepared by dissolving 175.0 g of NaCl, 89.6 g of commercial sucrose, 2.4 g of KNO_3 and 0.56 g of L-ascorbic acid in 1,100 ml of distilled water. Clearance from the base of the beaker was provided by the spongy foam while the other open end of the glass tube was plugged with cotton wool.

The curing solution was allowed to diffuse into the meat for 24 hr at room temperature. All the glass tubes containing the meat cores were then packed into a home kitchen pressure cooker and steamed for 5 min to fix the cured colour. Subsequently, the meat cores were removed, cooled in air, cut lengthwise symmetrically into two and the length of fixed visual colour estimated as an index of brine diffusion distance.

Two sections were removed starting from the cured end of each core. The lower section contained all the cure diffusate while the next section appeared devoid of cured meat colour. Each section was manually mined. The total amount of salt in aliquots from each section was determined by the acid digestion method based on A.O.A.C. procedure.[5]

The remaining member of each pair of muscles was rapidly sliced into chops of 2.5 cm thick while still frozen. The two largest chops from each muscle were rapidly weighed, then thawed completely at room temperature, mopped dry with paper and again weighed. The difference in weight was taken as the drip loss. Subsequently, the two chops were broiled in a kitchen gas oven previously pre-heated. Each chop was broiled on each side for 12 min at the end of which time it was judged to be of medium "doneness". The chops were cooled to room temperature, dried as before, weighed and the difference was taken as the cooking loss.

The cooked chops were sliced into cubes roughly 2.5 cm thick. Cubes from each muscle were subjectively evaluated for tenderness, juiciness, flavour and overall acceptability by a six member taste panel using a nine-point hedonic scale rating.

Results

The changes in the plasma glucose levels in the experimental ewes are shown in Fig. 1. The initial plasma glucose levels varied from 19 to 33 mg/100 ml of blood in the eight ewes studied. Thirty minutes post injection, there was a significant increase (P<0.01) in the plasma glucose level from an average of 27.25 mg per cent to an average of 64 mg per cent in the epinephrine injected ewes. The comparative values for the control ewes were 22.75 and 29.75 mg per cent respectively. At 60 min post injection when blood collection was terminated, the glucose level rose further to a mean of 81 mg per cent in the epinephrine injected ewes while those for the control ewes fell to 24.50 mg per cent.
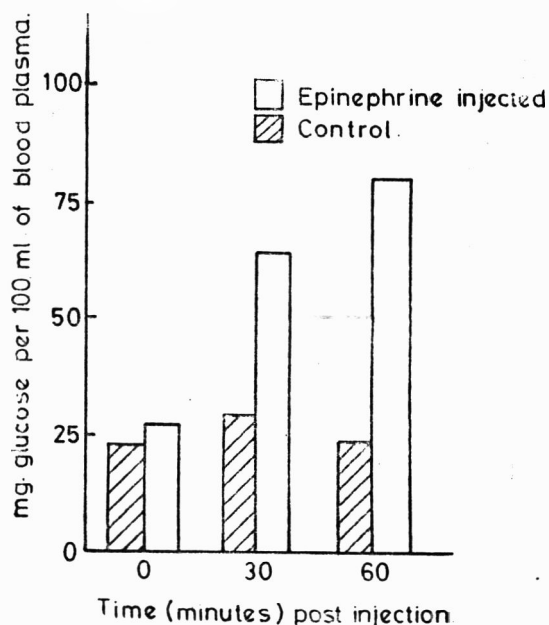

Fig 1.   The effect of subcutaneous epinephrine injection on blood plasma glucose levels.

TABLE 1. THE EFFECT OF PRE-MORTEM EPINEPHRINE INJECTION ON SALT CONTENT AND CURE DIFFUSION DISTANCE IN SELECTED EWE MUSCLES

Muscle type	Epinephrine injected				Control			
	L.D.	B.F.	S.M.	S.T.	L.D.	B.F.	S.M.	S.T.
NaCl content (mg %) (cured meat)	35.80	36.23	35.75	36.27	35.61	35.84	35.72	36.19
NaCl content (mg %) (uncured meat)	27.56	28.55	28.16	29.64	24.04	26.13	26.13	26.43
Cure diffusion distance (cm)	1.55	1.68	2.18	1.60	1.23	1.35	1.81	1.43

L.D.—Longissimus dorsi;   B.F.—Biceps femoris: S.M.—Semimembranosus;   S.T.—Semitendinosus.

The mean pH values taken on the longissimus dorsi at the location between the 12th and 13th ribs and opposite the last lumbar vertebra in both groups of ewes at various time intervals post mortem are shown in Fig. 2. The initial pH of 6.66 obtained in the control group is considerably lower than in the treated group which had an initial pH of 6.87. The pH values did not change appreciably ($\triangle$pH of 0.44) during the 24 hr post mortem period in the epinephrine treated carcass while in the control group, the corresponding change ($\triangle$pH of 1.02) was very significant ($P<0.01$). The major part of the pH drop appeared to have taken place within the first 12 hr post mortem.

Fig 2.   The effect of pre-mortem administration of epinephrine on post-mortem muscle pH.

Mean values presented in Table 1 indicate that pre-slaughter injection of epinephrine significantly ($P<0.05$) enhanced the extent of cure diffusion as measured by the cure diffusion distance. Further, significant variations ($P<0.01$) were noted among the different values for the four muscles. In both experimental groups the semimembranosus exhibited the highest diffusion distance followed in decreasing order of magnitude by the biceps femoris, semitendinosus and longissimus dorsi.

No significant treatment effect or between muscle differences were observed in the per cent salt content of the lower cured meat section. However, in the upper uncured meat section, the per cent salt content was significantly higher ($P<0.05$) in all the muscles from the epinephrine injected ewes. Between muscle differences were also significant ($P<0.01$).

Values for drip loss, cooking loss and moisture content are shown in Table 2. There was a significant ($P<0.01$) treatment effect with respect to drip loss. Variation in the muscle characteristics was also evident especially in the semimembranosus which lost more fluid as drip in both the treated and control samples.

The average cooking loss of 30.88 per cent obtained in the treated muscles was significantly ($P<0.05$) less than the corresponding values of 33.38 per cent obtained in the control samples. This difference was reflected in the values for all the muscles except the biceps femoris in which 37.09 per cent cooking loss was obtained as against 35.90 per cent in the control. No statistically discernible difference in the moisture content of the raw

TABLE 2. EFFECT OF PRE-MORTEM EPINEPHRINE INJECTION ON DRIP LOSS, COOKING LOSS AND MOISTURE CONTENT OF SELECTED EWE MUSCLES

Muscle type	Epinephrine injected				Control			
	L.D.	B.F.	S.M.	S.T.	L.D.	B.F.	S.M.	S.T.
Drip loss %	1.74	1.68	2.09	1.91	1.60	1.59	1.82	1.51
Cooking loss %	26.41	37.09	30.33	29.72	30.57	35.90	34.32	32.78
Moisture content % (raw muscle)	75.83	78.97	77.93	76.67	74.43	76.80	75.91	74.79

L.D.—Longissimus dorsi;   B.F.—Biceps femoris;   S.M.—Semimembranosus: S.T.—Semitendinosus.

TABLE 3. EFFECT OF PRE-MORTEM EPINEPHRINE INJECTION ON TASTE PANEL SCORES FOR VARIOUS ORGANOLEPTIC ATTRIBUTES OF SELECTED EWE MUSCLES

Muscle type	Epinephrine injected				Control			
	L.D.	B.F.	S.M.	S.T.	L.D.	B.F.	S.M.	S.T.
Tenderness	7.15	4.85	6.25	6.55	5.15	3.70	4.90	6.05
Juiciness	6.90	5.55	6.15	6.15	6.50	5.75	5.90	6.25
Flavour	5.85	5.05	5.95	6.02	6.60	5.25	5.80	6.30
Overall acceptability	5.80	5.05	5.60	6.25	6.80	5.25	5.80	6.60

L.D.—Longissimus dorsi;   B.F.—Biceps femoris;   S.M.—Semimembranosus;   S.T.—Semitendinosus.

muscle was noted although the slight increase in the treated muscles approached significance.

The treatment effect was significantly $(P < 0.05)$ reflected in the taste panel tenderness scores (Table 3) with the muscles from the epinephrine injected ewes having an overall mean rating of 6.20 against 4.95 in the control. All muscles were rated higher in the former treatment group. When considered between muscles, the tenderness differences were significant $(P<0.05)$ with the longissimus dorsi receiving the highest scores in both treatment and control groups. Significant differences in flavour and juiciness were not detected as a result of the treatment effect or between muscles. On the overall acceptability, the epinephrine injected ewes were less preferred than the respective controls although this lacked significance.

Discussion

The initial glucose values observed in the present study for the eight ewes appear to be in good agreement with previous values of 18-49 mg per cent determined in sheep of mixed British and Australian breeds on low plane of nutrition[6] and of 26.6 mg per cent determined in the blood plasma of starved western or dorset ewes[7]. The initial low level of plasma sugar observed in this investigation may be expected considering the fact that the ewes were obtained during the middle of the dry season when natural feed was scarce and this may have been compounded by a previous exposure to the drought condition.

The drastic rise in plasma glucose following epinephrine injection may be attributed to the glycogenolytic effect of the hormone in the liver. This action is known to be mediated through the stimulation of adenylcyclase and subsequent activation of phosphorylase necessary for the conversion of liver and muscle glycogen to glucose. In the control group the observed increase in plasma glucose to 29.75 mg per cent at 30 min post injection would be expected considering the stress imposed on the ewes during water administration and

blood sample collection. As suggested by Aberle and Merkel[1] such a stress may cause some production of endogenous epinephrine. The observed levels of plasma glucose at all sampling periods however remain within previous range of values reported for starved ewes[6-7].

That blood glucose remained high in the treatment group after one hour post injection of epinephrine would seem to indicate that epinephrine has a transient inhibitory effect on glucose induced release of insulin or on the effectiveness of insulin in regulating blood glucose levels. This may be a peculiar trait in ruminants in which blood glucose levels fall more slowly even in response to exogenous insulin[8]. Glucose arising from epinephrine induced glycogenolysis in the skeletal muscles is in the short run necessarily metabolised. A significant decrease in the activity of glycogen synthetase in skeletal muscle is enhanced by epinephrine[9] and might have inhibited the reconversion of such glucose back to glycogen while the action of the epinephrine remained effective. This then may manifest itself in a depletion of the muscle glycogen reserve.

Preslaughter glycogen depletion with the present dosage of epinephrine was probably effective in preventing appreciable pH drop in the treated group as compared with control. Previous work by Bendall and Lawrie[10] has shown that epinephrine accelerates post mortem glycolysis although when injected shortly before slaughter, neither the rate of pH drop nor the ultimate pH are affected[2]. Repeated injection of epinephrine spaced over a prolonged period of 12–24 hr have been found to raise the ultimate pH of meat and thereby produce dark cutting beef. The present finding shows that repeated injection of epinephrine over a long period is not necessary to exhaust glycogen reserve but rather, this could be achieved in the case of sheep by a one-short dosage of 1.8 mg epinephrine per 10 kg body weight injected six hours prior to death.

The struggling necessarily involved in the slaughtering procedure used in this experiment and which is the normal method generally used in Nigeria in the absence

of any humane slaughtering facilities may account for the initial pH being well below 7.0 which is normally associated with muscle soon after death[11]. Such struggling is known to elicit the production of endogenous epinephrine which subsequently accelerates glycolysis and in the short run enchances the accumulation of lactic acid.

Greater uptake of curing brine as reflected by the higher cure diffusion distance in the muscles from the epinephrine injectcd ewes was enhanced by the higher ultimate pH in keeping with previous results by Bouton et al.[12] with minced mutton in which it was shown that the water holding capacity of meat was superior at high ultimate pH than at low ultimate pH. In a previous work on porcine muscles by Arganosa and Hendrickson[13] the cure diffusion distance in these muscles were in the same order as observed in this investigation using ovine muscles. The differences between muscles may be due partly to differences in the degree of closeness of the microstructure of each muscle which affects the capillary movement of the brine in the spaces between its fibres and partly to the inherent myoglobin and total pigment in the fresh tissue as suggested by Hornsey[14].

The lack of difference in the salt content of the lower cured meat section of the meat cores would be expected if all the muscle were completely saturated with the curing brine. The upper section in all the epinephrine injected muscles contained mcre salt than the control due to the ionic disturbance in the former.

The higher ultimate pH value resulting from epinephrine injection enhanced water retention during cooking. Hamm and Deatherage[15] showed an increase in water holding capacity with increase in pH of beef homogenates cooked at 60° C. Since there is an inverse relationship between cooking loss and water holding capacity, an increase in water holding capacity would result in a decrease in cooking loss such as has been obtained with the epinephrine injected muscles. The present result is further supported by the finding of Khan and Nakamura[16] who observed a pronounced decrease in cooking loss with increase in pH of meat epinephrine treated chickens.

The improvement in tenderness rating was expected from previous work of Khan and Nakamura[17]. Consumer studies have indicated that tenderness is perhaps the most determining attribute of meat. It is therefore not clear whether the lack of acceptability by the panel for the more tender meat from epinephrine injected ewes was due to the apparent preference of the average Nigerians for fairly tough chewable meat. More research in this direction is needed.

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Studies on Biochemistry of Higher Fungi. I. Submerged Growth of *Volvariella volvacea* in Synthetic Medium

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Paddy straw mushroom, *V. volvacea* grows well in synthetic medium in submerged culture. Starch and KNO_3 are well utilized as carbon and nitrogen sources. It required boric acid, Fe^{++}, Mn^{++}, Ca^{++}, Mo^{++}, Zn^{++} as micronutrients for growth. The growth period continues upto 15 or 16 days. Biochemical analyses show fall in protein and fat but gain in fibre, carbohydrate and ash content of cultured mycelia in synthetic medium compared to whole fruiting body.

The success of growing mycelia of higher fungi in shake culture has largely facilitated the studies of their biochemistry of growth. The technique is largely adopted for the steady production of edible mushrooms for human consumption. During the last twenty years, there had been various studies on their submerged growth. In addition to their growth, investigations have also indicated the possession of different industrially important enzymes like proteolytic or specific amino peptidases[1-5], different carbohydrases[6-9] or different pharmacologically potential compounds like anticholesteroloemic factor[10-12], or carcinostatic polysaccharides[13-15] in the mushroom.

In this context, study was undertaken to grow under submerged culture some of the edible mushrooms and to study their biochemistry of growth. *Volvariella volvacea* or paddy-straw mushroom, one of the common edible mushrooms, is largely consumed by the people of China. Japan, Philippines and India, is taken for study. The mushrooms are now produced on composed bed only.

This paper describes the attempts at the submerged growth of paddy-starw mushroom in shake culture and determination of their growth requirements.

Materials and Methods

Mushroom: The fresh fruiting body of *Volvariella volvacea* was collected from Rice Research Station, Chinsura, Hooghly (West Bengal).

Chemicals: All the chemicals used are of AR or GR quality with the exception of malt extract (OXOID) and potato extract, the latter being prepared in the laboratory from fresh potato. Special precautions were taken in the study of micronutrient requirements to avoid all chances of metal contamination. For this purpose triple glass distilled water and highly cleaned corning glass wares were used throughout this experiment.

Growth on solid agar: For initial mycelial growth of the mushroom, fresh fruiting body was first lightly washed with absolute alcohol, and small pieces from the inner body were cut from the different portion of the mushroom (gill, cap, hymenium) and inoculated aseptically on solid agar medium in 250 ml conical flask. The composition of the medium is based on the different nutrients suggested by Humfeld and Sugihara[16] and Jennison *et al.*[17] for mushroom growth. The medium composition in per cent is: glucose, 5; malt extract, 1; potato extract, 10; $CaCl_2$, $2H_2O$, 0.037; KH_2PO_4, 0.087; $MgSO_4$. $7H_2O$, 0.05; boric acid, 0.057; $CuSO_4$. $5H_2O$, 0.0039; $FeSO_4$. $7H_2O$, 0.025; $MnCl_2.4H_2O$, 0.0036; $NaMo O_4$ $4H_2O$, 0.0032; $ZnSO_4$. $7H_2O$, 0.03; pH, 5.0, and agar, 2.5 per cent. Mycelial growth commencing from the inoculated piece was observed after 40 to 48 hr of inoculation. The filament elongation continues for 7–8 days. The growth was purified from bacterial or fungal contaminants, if there be any, by re-inoculating on the same medium containing streptomycin (30 γ/ml) and mycobacillin (20 γ/ml), the two broad spectrum antibacterial and antifungal antibiotics.

Submerged growth: For the studies of different nutrient requirements of the mushroom, mycelial growth from the flask was transferred into the same liquid broth (100 ml/500 ml) flask and shaken on a rotary shaker (350 rpm with throw of 3.5 cm) for 15 days. Mycelial balls appear within 2-3 days after inoculation and characteristic fruity flavour appears after 6 to 7 days of growth.

Studies on nutrients: The mycelial pellets from each flask, after 15 days of growth, was freed from the broth by repeated washings, suspended in 100 ml sterile water in a Waring blender and cut into small pieces. All the

operations were done aseptically. Such suspension (2 ml) was used as inoculum per 100 ml of broth.

Carbon nutrition: The composition of the medium as described earlier, was slightly altered by replacing glucose by 5 per cent each of dextrin, soluble starch, amylum. straw lignin and carboxymethyl cellulose (soluble form). Growth was studied in shake flask containing 100 ml broth per 500 ml flask for 15 days. The mycelial balls were then filtered, washed and dry weight was taken after dehydration at 60-70°C for 48 hr. Each set of experiment was always done in triplicate.

Nitrogen nutrition: After proper selection of carbon source, potato and malt extract from the same medium was replaced by different inorganic nitrogen sources including urea. The concentration of different salts used was on the basis of their nitrogen content. Growth after 15 days of inoculation was measured in the same way as described earlier.

Determination of C:N ratio: After proper selection of carbon and nitrogen sources in the medium, attempts were made to determine proper concentration of carbon and nitrogen sources of the medium for best growth. The concentration of carbon source was varied keeping that of nitrogen constant and *vice versa.* Growth in each experiment was determined in the same way as described earlier.

Micro-nutrients: In growing mycelia of *V. volvacea,* initially a complete medium was suggested containing different minerals commonly required for mushroom growth. After selection of proper carbon and nitrogen sources, and their proportion, it was assessed whether the different micro nutrients are essential or not for the proper growth. Hence the composition of the medium was adjusted by keeping carbon source, nitrogen source, KH_2PO_4 and $MgSO_4$ constant and omitting serially different micronutrients *viz.* Ca^{++}, Cu^{++}, Fe^{++}, Mo^{++} Zn^{++}, Mn^{++} and boric acid from the mixture.

Yields of the mushroom mycelia in the different media so constituted, were determined in the same way as described earlier.

Selection of optimum pH: It is well known that basidiomycetes grow in liquid culture in the acid pH. Aafter formulating proper synthetic medium, based on the selection of carbon, nitrogen and minerals, initial pH of the medium was altered from 1 to 7 and the growth of mushroom as well as change in pH were determined.

Growth curve of the mushroom: With proper determination of medium composition and pH, a growth curve for *V. volvacea* was prepared by determining mycelial dry weight in regular day interval. Simultaneously change in pH of the medium was also noted during the course of fermentation.

Biochemical analysis of mushroom fruit body and mycelial growth: Analysis was made for protein, carbohydrate, fibre, fat and ash content of both fresh fruiting body (whole) and mycelia from growth maximum.

Protein was estimated by micro-Kjeldahl method. Fat, fibre and ash were estimated according to the standard methods[18]. Carbohydrate was estimated in terms of glucose unit by Anthrone method[19].

Results and Discussion

Carbon and nitrogen sources: It is obvious from the Table 1, that *V. volvacea,* utilizes soluble starch more preferentially than amylum, dextrin or its monomer glucose whereas lignin or cellulose is very poorly utilised. Monosaccharides other than glucose were not tested because of their higher cost they were not suitable for mushroom fermentation. In replacing potato and malt extract by other simple nitrogen sources like amino acids and other costly nitrogen sources were omitted. It is observed that among the different nitrogen sources tested (having same nitrogen content), KNO_3 serves excellently as a source of nitrogen for mushroom mycelial growth retaining its full flavour (Table 2).

C:N ratio for growth: It is well known that C:N ratio has profound effect on the mushroom growth as well as on the amount of protein, fat or carbohydrate content of mycelia[22]. In our preliminary experiment, we have considered the first factor and hence determined the appropriate C:N ratio for best growth. In one set of experiment nitrogen percentage was kept constant (0.73 per cent KNO_3) and carbon source, namely starch concentration was varied. After proper selection of carbon concentration again KNO_3 concentration was varied to determine proper starch/KNO_3 ratio for maximum growth. The maximum growth attained is about 1.6 g dry wt/100 ml of medium having C:N ratio of approximately 13:1 (Fig 1).

TABLE 1. EFFECT OF DIFFERENT CARBON SOURCES ON THE GROWTH OF *V. VOLVACEA* IN SUBMERGED CULTURE

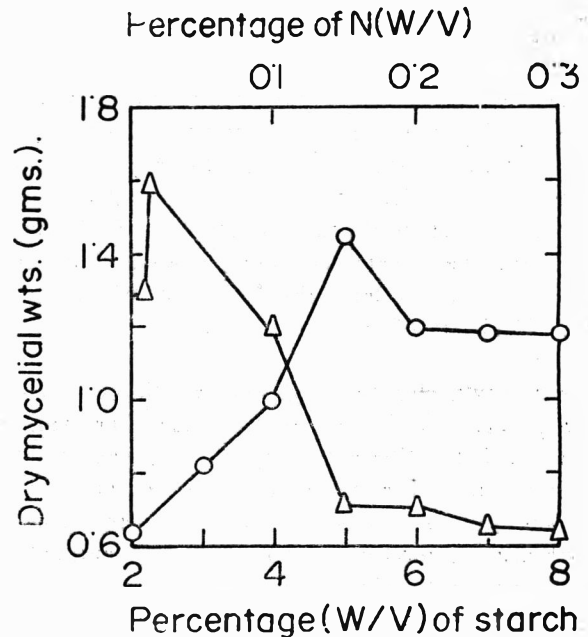| Carbon source (5% w/v)	Av. growth (dry wt.) (g/100 ml)
Glucose	1.061
Dextrin	0.950
Soluble starch	1.601
Carboxymethyl cellulose	0.013
Straw lignin	0.341
Amylum	0.881

Composition of the medium is the same as described in the text with the omission of glucose only. Growth, always in triplicate was measured 15 days after incculation.

TABLE 2. EFFECT OF VARIOUS NITROGEN SOURCES ON THE GROWTH OF *V. VOLVACEA* IN SUBMERGED CULTURE

Nitrogen source (w/v)	Carbon source (w/v)	Av. growth (dry wt.) (g/100 ml)
NH_4NO_3 (0.3%)	Starch (5%)	1.143
$(NH_4)_2SO_4$ (0.5%)	,,	0.938
KNO_3 (0.73%)	,,	1.603
$NH_4H_2PO_4$ (0.9%)	,,	1.213
NH_4Cl (0.4%)	,,	0.902
Urea (0.22%)	,,	1.513

Composition of the medium is the same as described in the text with the omission of glucose, potato extract and malt extract. Growth, always in triplicate, was determined 15 days after inoculation.

Micronutrients requirement for growth: In assessing the requirements of different micronutrients for growth, instead of making lots of combinations of those seven ingredients, experiments were only done to study the effect of omission of any metal ion from the mixture on the growth of the mushroom (Table 3). It is obvious from the table that omission of any metal ion except Cu^{++}, showed some inhibitory effect on the mycelial growth of the mushroom. They can be arranged according to their degree of essentiality on the mushroom growth in decreasing order as Ca^{++}, Fe^{++}, boric acid, Mn^{++}, Mo^{++}, Zn^{++}, and practically Cu^{++} has no effect on the growth.   It is also interesting to note that omission of Ca^{++} from the medium causes synthesis of pink pigment in the broth but the mushroom flavour is completely lost. No such drastic effect on the flavour or commencement of pigment production was observed in the case of omission of any other metal ion. The observation that the mushroom produces pink pigment but looses its flavour in absence of Ca^{++} in the medium was found to be very interesting.   The experiment was repeated with graded doses of Ca^{++} in the medium and it has been observed that flavour production initiated by the addition of Ca^{++} ion in the medium and the pigment production still continues in presence of Ca^{++} upto the concentration of 0.037 per cent of $CaCl_2$, $2H_2O$. The mushroom flavour was just identified physically and it appeared that maximum flavour production has taken place at a concentration of 0.1 per cent of $CaCl_2$, $2H_2O$ where there is also no pigment formation. In absence of any standardised method for the quantification of flavour and pigment at the present, it has not been possible to correlate them with Ca^{++} concentration.

Fig 1. Effect of C/N ratio on the growth of *V. volvacea*

TABLE 3. EFFECT OF OMISSION OF MICRO-NUTRIENTS ON THE GROWTH OF *V. VOLVACEA* IN SUBMERGED CULTURE

Nutrients omitted	Av growth (dry wt.) (g/100 ml)	Flavour	Pigmentation of broth
Boric acid	0.815	+	Reddish tint
$CuSO_4,5H_2O$	1.655	+	Straw yellow
$FeSO_4,7H_2O$	0.785	+	,,
$MnCl_2,4H_2O$	0.860	+	,,
$NaMoO_4,4H_2O$	0.996	+	,,
$ZnSO_4,7H_2O$	1.310	+	,,
$CaSO_4,2H_2O$	0.764	—	Pink
None	1.660	+	Straw yellow

Composition of the medium (% w/v): Starch-5, KNO_3-0.108, KH_2PO_4,-0.087, $MgSO_4.7H_2O$-0.05, boric acid-0.057, $CaCl_2$, $2H_2O$-0.037, $CuSO_4$, $5H_2O$-0.0039, $FeSO_4$, $7H_2O$-0.025, $MnCl_2$, $4H_2O$-0.0036, $NaMoO_4$. $4H_2O$-0.0032, $ZnSO_4$. $7H_2O$-0.03 Growth, always in triplicate, was measured 15 days after inoculation.

TABLE 4. DETERMINATION OF PH OPTIMUM FOR GROWTH OF *V. VOLVACEA* IN SUBMERGED CULTURE

Initial pH	Final pH	Av growth (dry wt.) (g/100 ml)
1.0	1.0	0.015
2.0	2.5	1.034
3.0	4.0	1.459
4.0	4.0	1.869
5.0	4.0	1.820
6.0	4.0	1.700
7.0	4.0	1.705

Composition of the medium (% w/v): Starch-5, KNO_3-0.108, KH_2PO_4-0.087, $MgSO_47H_2O$,-0.05, boric acid-0.057, $CaCl_2$. $2H_2O$-0.037. $FeSO_4.7H_2O$-0.025. $MnCl_2, 4H_2O$-0.0036, $NaMoO_4$. $4H_2O$-0.0032, $ZnSO_4$, $7H_2O$-0.03, Growth, always in triplicate, was measured 15 days after inoculation.

Fig 2. Growth curve of *V. volvacea*

TABLE 5. BIOCHEMICAL COMPOSITION OF FRUITING BODY AND SUBMERGED GROWTH OF *V. VOLVACEA* (DRY WEIGHT BASIS)

Components	Fruiting body ($\%$ w/w)	Submerged mycelia ($\%$ w/w)
Fat	3.27	2.83
Protein (N$\times$6.25)	26.25	9.01
Ash	0.64	7.18
Fibre	14.37	25.05
Carbohydrate (glucose)	48.3	54.3

Composition of the medium for submerged growth is the same as described under Table 4. Data represent the average of analyses made in triplicate.
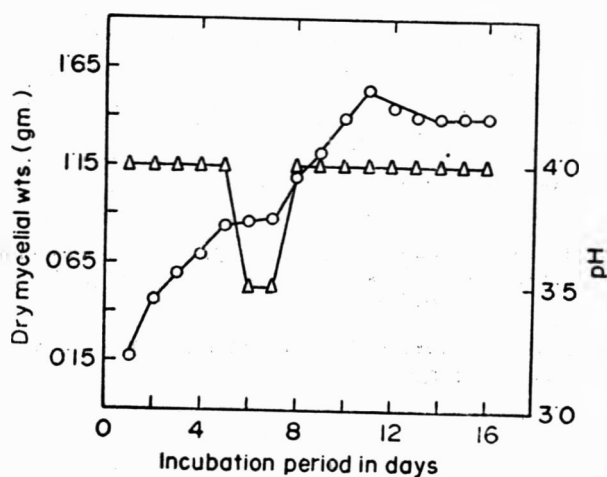
Growth pattern of mushroom: It is obvious from Table 4 that optimum pH for mushroom growth is 4 and it also appears that where the initial pH varies from 3 to 7, the final pH comes to 4 although their growth maximum is differently altered. Growth curve of *V. volvacea* is shown in Fig 2. It is obvious from the curve that in this mushroom initial lag phase continues upto 2 days after inoculation and then it enters in logarithmic phase which continues for 11 days when it reaches maxima, after which there is a slight loss of mycelial weight and then growth reaches a steady state upto 16 days. The pH course studied during the growth period is relatively steady, only there is a slight increase in acidity of the medium in mid-logarithmic phase, accompanied by retardation of broth which may be due to high acid production causing growth retardation. Later probably the organism controls the acid production and growth continues upto 16 days.

In determining growth maximum of mushroom, the effect of temperature on the growth could not be studied accurately due to the lack of proper temperature controlling system, but it was found that best growth takes place in the temperature range of 28-30°C.

Biochemical composition: Repeated analyses of fat, protein, ash, carbohydrate and fibre content of both of a fruiting body and mycelial growth of *V. volvacea* are shown in Table 5. It is obvious from the data that on average there is fall in fat and protein contents and rise in ash, fibre and carbohydrate contents of fruiting body in submerged culture. The fibre, ash and carbohydrate contents of mycelia are about 1.8, 11.0 and 1.13 times higher than those of the fruiting body. But on the other hand, protein and fat contents of the fruiting body are lowered to about 0.3 and 0.87 times respectively in submerged culture with respect to what we have considered only growth and it has been found that protein content of the cultured mycelia is highly influenced by the alteration of its C:N ratio in the medium[20].

Conclusion: It is found that *V. volvacea* can be cultured in a simple synthetic medium containing (per cent) starch, 5; KNO_3, 0.108; KH_2PO_4, 0.087; $MgSO_4$. $7H_2O$, 0.05; $CaCl_2$. $2H_2O$, 0.037; Boric acid, 0.057; $FeSO_4$. $7H_2O$, 0.025; $MnCl_2$. $4H_2O$, 0.0036; $NaMoO_4$. $4H_2O$, 0.0032; $ZnSO_4$. $7H_2O$, 0.03 and pH, 4.0; and temperature 28-30°C. Their biochemistry now can be easily studied in laboratory. Further attempts to increase nutritive value of the cultured mycelia similar to fruiting body can also be undertaken either by using different cheap organic nitrogen source (i.e., vegetable waste, fruit waste, etc) or by altering C:N ratio and mineral concentration of the synthetic medium.

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Residues of Some Contact Soil Insecticides in Potatoes

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Insecticidal schedules involving application of aldrin dust, heptachlor dust, carbaryl (Sevin) bait and chlorfenvinphos (Birlane) granules in the soil @ 2.25, 1.5, 1.25 and 4.0 kg active ingredient/ha, respectively, at planting time and separately at first earthing time for the control of cutworms, *Agrotis* spp. were evaluated, in field experiments. It was observed from the data that excessive residues of aldrin, dieldrin, heptachlor and heptachlor epoxide persisted upto 151 days following the application of aldrin and heptachlor on/in raw whole, unboiled and unpeeled potatoes. Processing of potatoes could bring down the toxic residues of aldrin and dieldrin below the tolerance level in all the cases and from all the stages but not of heptachlor and heptachlor epoxide. Carbaryl and chlorfenvinphos seem to be safe insecticides as no detectable residues of these two insecticides could be found at any stage in any case on/in processed as well as unprocessed potatoes.

In India, the losses in potato yield due to cutworms, *Agrotis* spp. are estimated to be 35 to 40 per cent.[1,2] A number of new organic insecticides have been tried against different species of cutworms with varying consequences. Existing literature on the control of cutworms with the help of contact soil insecticides in potato fields reveal aldrin (dust), heptachlor (dust), carbaryl (Sevin bait), chlorfenvinphos (Birlane granules), toxaphene (dust) and DDT + pyrethrum (dusts) to be effective[1,3]. As these insecticides are applied and mixed with soil, there may be the chances of their toxic residues on/in potatoes grown in such soils. It is, therefore, essential that recommended and commonly used contact soil insecticides on/in potatoes be evaluated from toxic residues angle to safeguard consumers. In the present investigation, aldrin, dieldrin, heptachlor, heptachlor epoxide, carbaryl and chlorfenvinphos residues on/in potato tubers following insecticidal treatments have been studied.

Materials and Methods

Potato variety "Kufri Jyoti" was planted in April 1975 at the farm of Central Potato Research Institute, Simla in 3.0 × 2.5 m plots. Each plot contained 6 rows of 12 plants/row. There were 9 treatments including control which were replicated 3 times in randomised block design.

Four contact soil insecticides viz. aldrin dust, heptachlor dust, carbaryl (Sevin) bait and chlorfenvinphos (Birlane) granules were applied and mixed with the soils of experimental plots @ 2.25, 1.5, 1.25 and 4 kg active ingredients/ha, respectively, in two different sets. In the first set these insecticides in the above dosages were applied in furrows at planting time before seed tuber placement while in second set, insecticides in said full dosages were applied near the base of plants at first earthing time with the view to see the fate of dissipation of these soil insecticides on/in potatoes, when applied in single full dosages separately at two different timings. Control plots were left untreated.

Potato tuber samples corresponding to each treatment and replicate were collected on 136 (15 days before harvest) and 151 (at harvest) days after planting time and on 97 (15 days before harvest) and 112 (at harvest) days following the earthing time insecticidal applications for residue studies.

During the experimental period, the average maximum temperature was 22.3°C, average minimum temperature 15.3°C and average relative humidity 66.8 per cent. There was 108.3 cm total rain fall.

Extraction of potato tuber samples, collected at different intervals following the insecticidal applications was done after dividing them in two sets (i) unwashed, unboiled and unpeeled (unprocessed) and (ii) washed, boiled and peeled (processed) potatoes. Extraction procedures used by Dewan *et al.*[4] and Awasthi *et al.*[5] were followed. After weighing 100 g of representative samples from each set of each treatment and replicate the tubers were cut into small pieces and were stripped with 200 ml of solvents for one hour. Acetone solvent was used for carbaryl and chlorfenvinphos while n-hexane was used in case of aldrin and heptachlor residues study.

Acetone extract was filtered and a known volume evaporated in Kuderna Danish evaporator to remove acetone. The aqueous concentrate containing a little

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acetone was then diluted with water and extracted 3 times successively with 50 ml lots of chloroform. The combined chloroform extract in each case was dried over anhydrous sodium sulphate, made to a known volume and a suitable aliquot cleaned up by passing through a 2 cm diameter column containing 1.25 cm layer of anhydrous sodium sulphate overlaid with 10 g of adsorbant mixture (2:2:1, activated charcoal : magnesium oxide: celite 545) and elution with chloroform. The eluate was then concentrated to 1 ml in Kuderna Danish evaporator and chlorfenvinphos residues were determined following the colorimetric procedure of Getz and Watt.[6]

For carbaryl residue determination, the extracts were processed for clean up by the procedure detailed in AOAC[7] and the residues were determined by using diazonium salt colorimetric method reported by Miscus and Law[8] as modified by Benson and Finnocchiaro[9].

As regards the analysis for the residues of aldrin, dieldrin, heptachlor and heptachlor epoxide, a known volume of n-hexane extract was cleaned up as was done in case of chlorfenvinphos sample but the elution was done by extra pure n-hexane (washed with 5 per cent sulphuric acid and distilled over silver nitrate.) The eluate was then made to a known volume and the insecticidal residues were estimated through GLC. Using column OV—1 at inlet temperature of 225°C and detector

temperature of 250°C in a electron capture detector for aldrin, dieldrin (Dieldrin residues are from the conversion of aldrin to dieldrin in the soil and plant system through oxidation and enzymic reaction processes), heptachlor and heptachlor epoxide (similarly heptachlor epoxide residues are due to the conversion of heptachlor to heptachlor epoxide in soil and plant through oxidation, enzymic reactions and photodegradation processess) residues. Residues of chlorinated insecticides and their respective metabolites were also confirmed by TLC techniques.

Results and Discussion

Table 1 presents average residue (ppm) data of aldrin, dieldrin, heptachlor, heptachlor epoxide, carbaryl and chlorfenvinphos on/in potatoes (both unprocessed and processed) grown in treated plots during main crop season (April-September) of 1975 at Simla.

Aldrin and dieldrin residues on/in potaoes: It is evident from the data (Table 1) that average residues (ppm) of aldrin and dieldrin on/in unwashed, unboiled and unpeeled (unprocessed) potatoes, collected from aldrin treated plots on 136 and 151 days following the planting time and on 97 and 112 days after earthing time insecticidal applications persisted above the tolerance limit of 0.1 ppm[10] in all cases though a decreasing trend of these toxicants was noticed with the increase of time

TABLE 1.   RESIDUES OF SOME CONTACT SOIL INSECTICIDES ON/IN POTATOES (MEAN OF 3 REPLICATIONS)

| Insecticides | No. of days after planting time application | | | | No of days after earthing time application | | | |
| | 136 | | 151 | | 97 | | 112 | |
	Raw	Processed	Raw	Processed	Raw	Processed	Raw	Processed
Aldrin	0.19	0.03 (84.21)	0.15	0.03 (80.00)	0.27	0.06 (77.78)	0.21	0.04 (80.95)
Dieldrin*	0.25	0.05 (80.00)	0.21	0.04 (80.95)	0.41	0.08 (80.49)	0.32	0.07 (78.13)
Heptachlor	0.18	0.06 (66.67)	0.15	0.06 (60.00)	0.23	0.07 (69.57)	0.21	0.07 (66.67)
Heptachlor epoxide**	0.31	0.08 (74.19)	0.26	0.08 (69.23)	0.37	0.11 (70.27)	0.27	0.08 (70.37)
Carbaryl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chlorfenvinphos	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = Residues not detected.

*Dieldrin residues are from the conversion of aldrin to dieldrin in soil and plant system through oxidation and enzymic reaction processes.

**Heptachlor epoxide residues are due to the conversion of heptachlor into heptachlor epoxide in soil and plant through oxidation, enzymic reactions and photodegradation processes.

Figures in parentheses denote the percentage of reduction of chlorinated insecticides after washing, boiling and peeling of potatoes.

Raw refers to unwashed, unboiled and unpeeled. Processed refers to washed, boiled and peeled.

gap. However, the residues of both these toxicants (aldrin and dieldrin) were found below the tolerance level (0.1 ppm) during both the treatment timings on/in processed (washed, boiled and peeled) potatoes. Processing of potatoes eliminated aldrin residues by 77.78 to 84.21 per cent and that of dieldrin by 78.13 to 80.95 per cent. Further it may be seen from Table 1 that average residues in the form of dieldrin were more than that of aldrin in all the cases both on/in unprocessed and processed potatoes, collected from aldrin treated plots at different intervals following the insecticidal applications. Singh and Kalra[11] have also reported aldrin and dieldrin residues above the tolerance limit in potatoes harvested 3 months later from aldrin treated (2 kg active ingredient/ha) plots.

Heptachlor and heptachlor epoxide residues on/in potatoes: Average residues of heptachlor and heptachlor epoxide on/in raw (unwashed, unboiled and unpeeled) and processed (washed, boiled and peeled) potatoes, collected on 136 and 151 days after planting time and on 97 and 112 days following the earthing time insecticidal applications from heptachlor treated plots were determined above the tolerance level of 0.05 ppm[10]. Though, the processing (washing, boiling and peeling) of potatoes eliminated the residues of heptachlor by 60.00 to 69.57 per cent and that of heptachlor epoxide by 69.23 to 74.19 per cent, even then the residues of both these toxicants did not come down below the tolerance limit at anytime on/in processed potatoes (Table 1). As compared to heptachlor, heptachlor epoxide residues (average) were found in higher quantities every time both in unprocessed and processed potatoes, collected from heptachlor treated plots at different intervals following the insecticidal applications. Further it may be seen from data (Table 1) that excessive residues of heptachlor and heptachlor epoxide persisted upto 151 days on/in unprocessed as well as on processed potatoes grown in heptachlor treated soils. Lichtenstein and Schulz[12] have also reported that in general more residues were found in crops grown on heptachlor treated soils than in those from aldrin contaminated soils.

Carbaryl residues on/in potatoes: Considering the tolerance limit of carbaryl as 5.0 ppm[13], it may be concluded from the data in Table 1 that this insecticide is quite safe to use for the control of soil insect-pests of potato crop from toxic residues point of view as no detectable residues of carbaryl could be found on/in unprocessed as well as on processed potatoes grown from carbaryl bait treated (1.25 kg active ingredient/ha) soil at any sampling from any treatment timing (i.e. neither from planting time application of carbaryl nor from first earthing time application).

Chlorfenvinphos residues on/in potatoes: As was found to be the fate of carbaryl residues, no detectable residues of chlorfenvinphos (Birlane granules) could be found in raw (unwashed, unboiled, and unpeeled) and in processed (washed, boiled and peeled) potatoes collected on 136 and 151 days after planting time and 97 and 112 days following the first earthing time insecticidal applications from chlorfenvinphos treated (4 kg active ingredient/ha) soils (Table 1). Like carbaryl bait, this insecticide also appears to be safe from toxic residue as it did not leave any detectable amount of residues even in raw potatoes at any sampling date. Tolerance limit for this insecticide has been reported as 0.05 ppm[13].

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The Application of Essential Oils and their Isolates for Blue Mould Decay Control in *Citrus reticulata* Blanco

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Application of essential oils like lemongrass, sweet basil, eucalyptol, peppermint and mentha (*Mentha arvensis*), and isolates like citronellol, geraniol, citral and camphor was tried as preservatives for oranges. Both *in vitro* and *in vivo* tests were carried out. Geraniol and mentha oil have been found to be fully effective to check the growth of *Penicillium italicum*. The other essential oils in descending order of effectiveness are sweet basil oil and lemongrass oil.

In India, the production of fresh fruits and vegetables is estimated to be around 20 million tonnes. However, the spoilage has been reported to be of the order of 25-30 per cent in India with world average of about 9 per cent on account of various factors, including improper storage facilities. Low temperature storage, which is widely used by industry, has two limitations: firstly some fruits like, lemon and banana, are injured by storage at a temperature sufficiently low to inhibit growth of pathogenic fungi; secondly, fruits like, apples and grapes which hold well at 0-2°C are often stored for several months in order to maintain a favourable marketing condition. The growth of some of the important micro-organisms such as, *Botrytis cinerea, Penicillium expansum,* etc., which are considered responsible for post-harvest diseases, is suppressed but not halted at the proper storage temperature. Generally in the case of chemical treatments, micro-organisms responsible for spoilage are totally killed which eliminates the difficulty in retail merchandising.

The antimicrobial activity of essential oils toward animal and human pathogenic micro-organisms have been investigated by several workers[1-9]. Dabbah *et al*[3] studied the antimicrobial action of some citrus oils on selected food borne bacteria. Maruzzella and Balter[4] made an *in vitro* study of the antifungal activity of 119 essential oils against 12 phytopathogenic fungi. The present investigation deals with *in vitro* and *in vivo* study of the effect of some essential oils, like, peppermint mentha, sweet basil, lemongrass, etc. and isolates, viz., geraniol, citronellol₂ etc., on *Penicillium italicum* which is considered as one of the micro-organisms responsible for spoilage of citrus fruits in India.

Materials and Methods

Sound and healthy oranges (*Citrus reticulata* Blanco) of uniform size were brought to the laboratory within two days after harvesting. *Penicillium italicum* (blue mould) was isolated from the orange and maintained in potato dextrose medium. Its pathogenicity was also tested. All the essential oils and isolates used were commercially available pure forms.

In vivo *test:* The fruits were artificially inoculated with *Penicillium italicum* by scratching the peel with a sterile pin at the stem end and swabbing the picked portion with the inoculum. A spore suspension of the mould from a 7-day old culture on potato dextrose medium was used as inoculum. Spore suspension was prepared in sterile distilled water. Spore suspension (1 ml) was added to scratched portion of each fruit. After 24 hr of inoculation, fruits were dipped for 2 min in the emulsion containing the essential oil. The fruits were then dried and stored in a sterile glass chamber at 30°C and 70-80 per cent relative humidity. Emulsion was prepared by taking 10 ml oil, 2 ml Tween 20 as emulsifying agent and 88 ml water. Ten milli litre of the emulsion, thus prepared, was further diluted in one litre of water. Thus, solution used for treatment contained 0.1 per cent oil. Inoculated but untreated fruits served as control sample. Three replications were maintained and each batch consisted of twenty fruits. Samples were inspected daily for decay, physiological rind break down and chemical injury. Results have been expressed as percentage of fruits affected.

In vitro *test:* Oil emulsion was prepared by dissolving 2 ml liquid emulsifier (Tween 20) in 88 ml water and 10 ml oil. Geraniol, camphor, lemongrass oil, eucalyptol oil, mentha oil, sweet basil oil, peppermint oil, citral and citronellol were used in this investigation. The Czepex's Dox medium was prepared and 25 ml of culture medium was apportioned in 150 ml culture flasks constituting the two sets of treatments. Fractional sterilization was accomplished in Arnold's steamer. In one set of experiment 0.25 ml of oil emulsion was added and

in another set 0.5 ml was used. Seeding was done by pipetting 0.1 ml spore suspension of *Penicillium italicum*. The inoculated flasks were incubated at $30° \pm 1°C$. At the end of each incubation period, the fungal colonies were thoroughly washed and were subsequently filtered on previously dried and weighed Whatman filter paper No. 42. The filter papers containing the fungal mat were subjected to 65°C in an electric oven for two days after which they were cooled and weighed. The treatment had four replications.

Sterile potato dextrose agar medium (25 ml) was poured into each of the four Petri dishes and allowed to solidify. In one set of experiments 0.25 ml oil emulsion was used and in another set 0.5 ml was used. Each petri dish was inoculated with 3 mm disk of inoculum, removed from a 7-day old culture of the decay pathogen grown on potato dextrose medium. After inoculation, petri dishes were incubated at $30° \pm 1°C$. Colony diameter was measured 3 days after inoculation and the growth was recorded.

Results and Discussion

At 30°C control fruits were susceptible to infection within four days and the decay amounted to 20 per cent (Table 1). The infected fruits were entirely covered wtih a blend of blue moulds and were unfit for human con-

TABLE 1. POST-HARVEST TREATMENT OF ORANGES WITH ESSENTIAL OILS

Treatment	Concn. (%)	Per cent decay				
		4th day	8th day	12th day	18th day	28ht day
Control	—	20	30	40	50	60
Geraniol	0.1	0	5	5	5	5
Mentha oil	0.1	0	5	10	10	10
Sweet basil oil	0.1	20	25	30	40	40

TABLE 2. EFFECT OF OIL EMULSIONS ON GROWTH OF CITRUS FRUIT PATHOGEN

Oil treatment	Av diameter of colony (mm) growth on indicated days							
	Concn. 0.25 ml				Concn. 0.5 ml			
	3rd	7th	12th	15th	3rd	7th	12th	15th
Control	6	60	75	100	6	60	75	100
Camphor	6	50	70	90	4	45	80	87
Citral	5	50	64	70	4	50	55	68
Citronellol	4	5	5	10	4	5	5	7
Eucalyptol oil	5	60	80	98	5	58	60	90
Geraniol	0	0	0	0	0	0	0	0
Lemongrass oil	0	0	0	3	0	0	0	2
Mentha oil	0	0	0	0	0	0	0	0
Peppermint oil	4	6	8	15	4	6	8	10
Sweet basil oil	2	4	4	6	2	2	2	2

sumption. At the end of 28 days, severe damage upto 60 per cent was noticed. On the other hand fruits treated with geraniol were almost completely free from infection ($\geqslant$ 95 per cent) as shown in Table 1, thus establishing their efficacy in holding a check over storage decay. Geraniol treatment showed less than 5 per cent decay even after 3 weeks at 30°C. The other treatments in descending order of effectiveness are mentha oil and sweet basil oil.

Geraniol and mentha oil were found to have completely retarded the growth of *P. italicum*, and are obviously the best fruit preservatives (Table 2). A concentration of 0.25 ml of oil emulsion was found to be effective against decay pathogen. The next essential oil in descending order of effectiveness is lemongrass where no growth was observed until 12th day but a little growth was observed on 15th day. Sweet basil oil and citronellol were also observed to have significant check on growth of the micro-organism but cannot be considered fully

TABLE 3. EFFECT OF OILS AND ISOLATE EMULSIONS ON GROWTH OF CITRUS FRUIT PATHOGEN

Oil treatment	Av dry wt (mg) of *P. italicum* growth on indicated days							
	0.25 ml concn				0.5 ml concn			
	3rd	7th	12th	15th	3rd	7th	12th	15th
Control	50.6	80.2	140.0	156.2	50.6	80.2	140.0	156.2
Camphor	30.5	90.2	132.6	140.0	26.2	80.6	110.6	136.2
Citral	40.0	100.6	150.2	154.4	20.2	98.2	154.6	160.8
Citronellol	6.0	8.0	10.2	10.8	4.0	6.2	6.4	8.2
Eucalyptol oil	30.0	100.1	156.2	152.5	20.8	90.8	140.3	150.6
Lemongrass oil	4.2	4.2	5.8	6	3	4.2	4.6	4.8
Peppermint oil	20.0	90.0	112.8	130.2	15.6	80.2	112.6	120.8
Sweet basil oil	3.0	4.2	6.2	6.8	4.2	5.6	4.2	4.8

No growth was observed in geraniol and mentha oil treatments

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effective. Peppermint oil, eucalyptol oil and camphor were not effective. Rind break down had not increased appreciably by any treatment. No burning or other phytotoxic effect was observed. Table 3 clearly indicates the effect of oil and oil isolates on the growth of *Penicillium italicum*. It is clear that mentha oil and geraniol completely checked the growth of this micro-organism. Sweet basil and lemongrass were also observed to be effective to a great extent. Next in descending order of effectiveness is citronellol, peppermint and camphor. Citral and eucalyptol have been observed to be ineffective.

The mechanism involved in the application of essential oils as fruit preservatives is not yet well established. The lipid solubility and surface activity of essential oils are considered possible reasons for their effectiveness.

Organoleptic tests was carried out on 28th day of the treatment as per Indian Standard Specification (No. IS: 6273 (Part II) 1971). Each person testing the sample clearly indicated that the sample was liked very much by him and further commented that the colour was almost the same as that of the original fresh fruit. The flavour and texture of the fruit were similar to that of the original.

The economics of any treatment is dependent upon the availability of the material, amount used and its cost. Diphenyl, thiabendazole, benlate, benomyl and sodium orthophenyl phenate are extensively used in the United States but not easily available indigenously. The oils of mentha, lemongrass, etc., are available in India. The effective dosage of essential oil for treatment are comparable to those of chemicals used in the United States and other countries. The cost is also comparable or rather cheaper in comparison with those chemicals extensively used. Geraniol is isolated from palmarosa oil and costs about Rs. 130/kg. Mentha oil costs about Rs. 75/kg, lemongrass oil Rs. 60/kg, eucalyptol oil Rs. 20/kg and citronellol Rs. 36/kg. The sweet basil oil, which is derived from *Ocimum basilicum*, costs Rs. 60/kg.

The present investigation demonstrates the potentiality of some of the essential oils as effective preservatives for oranges.

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Development of Quick Cooking Peas

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A process has been developed for the production of quick cooking dehydrated peas from dry commercial peas. It involves hydrating the peas by soaking in 0.25% sodium bicarbonate solution for 5 hr, pricking the soaked peas for 10 min followed by precooking for 4 min at 1.05 kg/sq. cm gauge pressure, and dehydrating the precooked peas in a standard tray dryer at 55 to 60°C for 2 to 2.5 hr to reduce the moisture content to about 8%. The processed peas reconstitute in boiling water in 5 to 6 min, thns reducing the cooking time by about 80%. The product keeps well for over one year at ambient temperature. As a preliminary to the production of quick-cooking peas on a large scale, peas pricking machine and experimental bean cooker for scaling up operations have been considered.

Legumes are primary source of dietary protein in India and many other areas of the world as well.[1] Amongst these peas are singularly the most popular legume throughout the world. Dried legumes as a class suffer from poor cooking quality. Since the trend is toward more and more convenience foods, it would follow that the future for an expanded market for dry peas depends on obtaining a quick-cooking bean.

Esselen and Davis[2] developed a dehydrated baked bean that could be reconstituted by rehydration for 90 min in cold water. The product was then ready to eat when heated. The 90 min hydration, may appear too long a preparation time to qualify the beans as quick cooking. Feldberg *et al*[3] developed a "quick-cooking" pea bean which satisfied the armed services' criterion of a combined rehydration and cook time of 5-10 min. In this process, the beans, soaked for at least 8 hr, and then cooked for 20 min at 118°C and frozen to minimize bursting, were dried in a cabinet dryer for 90 min at 77°C dry bulb (31°C wet bulb) or 75 min at 88°C dry bulb (35°C wet bulb). The process also proved satisfactory for beans of Great Northern type. Unfortunately, the need for a freezing step makes the process uneconomical. Their efforts to control bursting by sugar dip treatment prior to dehydration failed.

A method developed by Dorsey *et al*[4] for dehydrating blanched pea and red kidney beans again involves freezing of beans between cooking and drying to control butterflying. None of the processes reported by Hallmark[5], Nielsen[6] and Steinkraus *et al*[7] have been applied successfully with either quick-cooking or precooked dehydrated beans. However, Rockland *et al*[8] have developed a process for preparing quick-cooking Lima beans which with minor modifications has been found suitable for processing other dry beans.

Taking into account the advantages and disadvantages of these processes referred to above, efforts have been made to develop improved techniques for the production of relatively quick cooking dehydrated peas at comparatively low cost. The process involves hydration of dry peas by soaking in water with a little addition of sodium bicarbonate, precooking in pressure cooker and dehydrating in air dryer at 60°C. The process, also satisfactory for a wide variety of beans, resulted in reduction in cooking time to the extent of 80 per cent.

Materials and Methods

Dry peas (depodded whole peas) light green in colour were purchased from the local market and hand picked free of extraneous matter. The moisture content of the dry peas varied from 8 to 12 per cent. Laboratory grade sodium bicarbonate was used. For packaging of dried product, polycell (700 gauge) and polyethylene bags (700 gauge) were used.

pH measurement: pH of sodium bicarbonate solution was determined using Beckmann pH meter.

Moisture content: The moisture in peas (ground to 30-40 mesh) was determined using Cenco Infra red moisture balance operated at 95 volts for 15 min till constant reading was obtained[9]. These results were verified to be in agreement (±0.2 per cent) with those obtained by the AOAC vacuum oven method[10].

Cooking time: Cooking time for peas at various stages of processing was determined by using indigenously fabricated experimental bean cooker described later. The cooking time of the peas is taken as the time

taken for 50 per cent of the peas to be penetrated. The same bean cooker is used for determining the degree of cooking of the precooked dehydrated peas.

Butterflying percentage: This was determined in pre-cooked peas by the method described by Steinkraus *et al.*[7].

Rehydration ratio was determined by dividing the hydrated weight after soaking and/or cooking by the original starting weight of dehydrated peas.

Storage: Samples were stored at room temperature ($26\pm2°C$) and at 45 per cent RH. Quality of the stored product was assessed at regular intervals.

Sensory evaluation: This was carried out in cooked and uncooked samples by a taste panel consisting of 12 members from the scientific staff. Nine point hedonic scale was used for appearance, flavour, texture and over-all acceptance.

Process: Dry peas (200-g batch) cleaned of extraneous matter were subjected under different conditions, to hydration, pricking, precooking and dehydration and optimum conditions for each operation were established.

Hydration: Dry peas were dipped in water and 0.1 to 2 per cent sodium bicarbonate solution (to fix the colour) for 1 to 16 hr and degree of cooking after every hour was determined. Pea to sodium bicarbonate solution ratio on cooking time and final pH was also studied.

Effect of temperature on hydration medium: Temperature of hydration was increased from room temperature to 60°C and water uptake of peas per 100 g of dry peas was determined.

Peas pricking: Peas after hydration were drained and washed with tap water. Washed peas were then poured into a drum of peas pricking machine fabricated in the laboratory for this purpose. The drum of the machine is fitted with pointed pins from inside which, during the rotation, pricks the peas poured into the drum. The optimum time of pricking was determined by operating the machine for 1 to 10 min. Pricking helps in removing the internal gas of peas during precooking[12-15]. The amount of water removed during pricking step was insignificant.

Precooking: Pricked peas were either cooked in boiling water at $100\pm2°C$ for 6 min in a stainless steel vessel, or steamed in a pan or a vessel (placed on wire mesh) for 6 min or pressure cooked for 1 to 6 min in a stainless steel woven container of a laboratory pressure cooker at 1.05 kg/sq. cm pressure. Percentage of butter-flied peas in each type of cooking were determined. Pressure cooking helps in eliminating hard shell peas, whose skins are relatively impervious to moisture and also in inactivating enzymes, which through their activity during hydration, might lead to instability in the precooked, dehydrated product.[7] Precautions were taken to limit unnecessary exposure of peas to air, in order to prevent butterflying before drying.

Drying: Peas were pre-treated by soaking for 5 hr at 26°C in water or 0.25 per cent sodium bicarbonate solution followed by pressure cooking at 110°C for 4 min, and dried in a standard laboratory tray dryer at 55°C in the first stage of drying to 40 per cent moisture level to eliminate butterflying and finishing at 60°C to get a final moisture content of 8 per cent. The air was used at a velocity of 70 to 100 m per min. Tray loading of 20 kg/sq m was maintained during drying. The dried product was immediately packed in flexible pouches.

The quick cooking peas thus prepared were stored at ambient temperature and evaluated for quality using sensory tests and rehydration properties. Storage stability with respect to packaging material was also investigated.

Peas pricking machine: The peas pricking machine designed and fabricated (Fig. 1) has (*i*) pricking pins, (*ii*) spherical vessel for holding peas, (*iii*) brackets for holding the vessel, (*iv*) motor for drive arrangement, (*v*) gear assembly for speed reduction, and (*vi*) base plate, as main components of the machine and its mounting.

Peas to be pricked (punctured, perforated or slitted) are poured into the aluminium sphere through an opening. The sphere is fixed on brackets by a shaft. The shaft passes through a ball bearing housing. One end of the shaft is fixed with a pulley and driven by a motor with speed reduction gear assembly for speed in the range of 10 to 60 rpm.

After pouring the peas in the sphere, the opening is closed and the machine is started. Due to rotation of the sphere peas move up and fall down when they come in direct contact with the pricking pins (2 mm in diameter and 8 mm in length) on the inside surface of the sphere and thus get pricked. As the shape of the vessel is spheri-

1 PRICKING PINS
2 DRUM
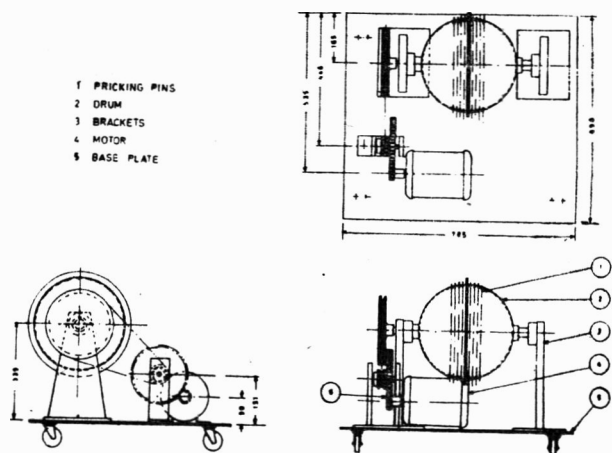3 BRACKETS
4 MOTOR
5 BASE PLATE

Fig 1. Peas pricking machine

cal and it rotates on its axis with peas inside, there is very little chance for peas to remain on these pins. On the contrary due to continuous rotation of spherical vessel, peas get pricked on its surface by the extended points of the pricking pins, and pricking is completed in a predetermined time. The speed of rotation is adjusted depending on the hardness of the peas. Pricked peas are removed from the opening by tilting the drum at a desired angle. The machine along with its accessories is fitted on a base plate. The base plate in turn is fixed on castor wheels for giving portability to the machine.

In general it was observed that for 1 kg of hydrated peas about 10 min were required for pricking. A standard 0.25 HP motor operated on single phase AC supply was found to be adequate for this purpose. Overall dimensions of the machine are shown in Fig. 1. The machine can be operated manually also in case of power failure. Possible designs for a commercial scale peas pricking machine have also been considered. The machine is simple in design and operation and low in cost. It gives scope for automation of operations.

Experimental bean cooker: The experimental bean cooker as shown in Fig. 2 consists of the following main components: (i) frame rest on which the whole body and parts of the cooker are supported, (ii) bottom plate on which the plate for holding peas will rest, (iii) peas holder plate—plate on which 100 peas could be arranged in saddles uniformly distributed on the plate, (iv) needle of 1.5 mm diameter which will penetrate the peas, (v) frame spacer for adjusting the height of frame, (vi) guide plate for plunger on which the rods holding plunger will rest, (vii) handle for increasing or decreasing the height of rod holding plunger, (viii) frame rod for holding the frame of the cooker, (ix) side plate for holding the moving plate and plunger, (x) moving plate with 100 spacings for plunger movement, (xi) plunger (90 g in weight) resting on the rod attached to the penetrating needle, and (xii) stationary plate through which

rod holding plunger and needle will move upwards or downwards.

A plate having 100 saddles of equal size and depth holds dehydrated peas. A vertical plunger weighing 90g and terminating at its lower end in 1.5 mm diameter stainless steel needle rest on each peas. Each rod holding the needle is perforated vertically and when a pea becomes sufficiently tender, the needle attached to the rod penetrates the peas and drops a short distance through the hole in the saddle.

During the run, the lower portion of the cooker holding the peas is lowered into water maintained at 100°C to cook the peas. At the end of each minute, one can record and count the number of peas which have been penetrated by their plungers. When these data are plotted they form an S-shaped curve and the cooking time for a sample is taken as the time required for 50 per cent of the peas to be penetrated.

The plungers are hollow and weighted with lead shot. Preliminary experiments showed that with the weight of a plunger adjusted to 90 g, it will just penetrate a pea when cooked to the right degree, judged by a taster. The cooking times measured with this apparatus are median cooking times for the 100 peas in a sample. The cooker is simple in design, operation, and low in cost. Cooking times of beans with different moisture contents could be determined easily and quickly using this cooker. The same cooker with variations in weight of plunger could be used for determining the cooking time of different beans. The overall dimensions of the experimental bean cooker are given in Fig. 2. Most of the parts of the cooker are made of brass or aluminium.

Results and Discussion

Hydration: It was found (Fig. 3a) that 5 hr dip treatment or soaking in 0.25 per cent sodium bicarbonate solution markedly stimulated rapid absorption of water in the first 3 hr as compared to dry peas soaked only in water. Total level of hydration was found to be more in peas soaked in sodium bicarbonate solution as compared to ordinary water. Cooking time for peas is reduced in direct proportion to soaking time (Table 1). Except for hard shell peas, extending the soaking time beyond 5 hr offers no advantage.

Effect of sodium bicarbonate: Sodium bicarbonate used during the hydration step[7,8] exerts the following useful effects.

1. It softens the pellicle or bran by its tenderizing effect.

2. It aids in the solubilization of proteins and starchy components.

3. It acts as buffer to maintain the pH.

4. It facilitates uniform penetration of the hydrating
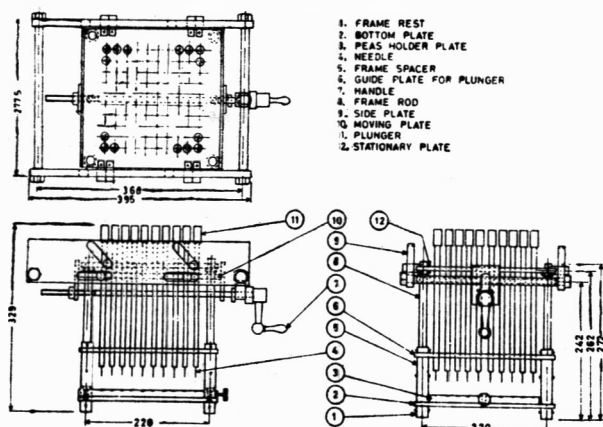
1. FRAME REST
2. BOTTOM PLATE
3. PEAS HOLDER PLATE
4. NEEDLE
5. FRAME SPACER
6. GUIDE PLATE FOR PLUNGER
7. HANDLE
8. FRAME ROD
9. SIDE PLATE
10. MOVING PLATE
11. PLUNGER
12. STATIONARY PLATE

Fig 2. Experimental bean cooker

medium into the centres of the peas, so that the final products have a uniform smooth texture.

5. It exerts the advantageous effect of extracting a great deal of highly pigmented material from the outer layers of the peas, whereby a desirable lightening of the colour of peas is attained.

6. Its presence in hydration medium contributes largely to the goal of attaining a product that is quick-

Fig 3b.   Hydration of dry peas at several temperatures

cooking i.e., one that can be prepared for the table by boiling in water for a period of time which is 80 per cent less than that required for cooking the untreated product.

The main criterion used for determining the optimum concentration of sodium bicarbonate and its ratio in hydration media was cooking time of peas. The tenderizing effect of sodium bicarbonate is shown in Table 2. Increasing the total concentration above 0.25 per cent did not offer extra advantage. Lowering the total level below this caused mottling of seed coats and increased cooking time of processed peas; hence 0.25 per cent concentration was selected as optimum. pH of the solution was measured which was 9 initially and dropped to 8.4 after 5 hr soaking at 26°C with 0.25 per cent sodium bicarbonate solution.

Peas to hydration medium ratio: Ratio of peas to hydration medium affected the cooking time of soaked peas, as shown in Table 3. Optimum ratio of peas to hydration medium was found to be 1:3 beyond which reduction in cooking time was not considerable. Raising the temperature of the hydration medium during soaking, as may be seen from Fig. 3b did not increase the water uptake capacity of peas to a greater extent after 5 hr soaking. Since, at 60°C, some cooking occurs making further processing difficult, room temperature soaking was considered to be satisfactory for further treatment; this was also so costwise.

Precooking: Optimum conditions for precooking step were determined based on cooking time and percentage butterflied peas. It was observed that for production of quick cooking peas, completion of precooking step should be carried out using pressure cooking
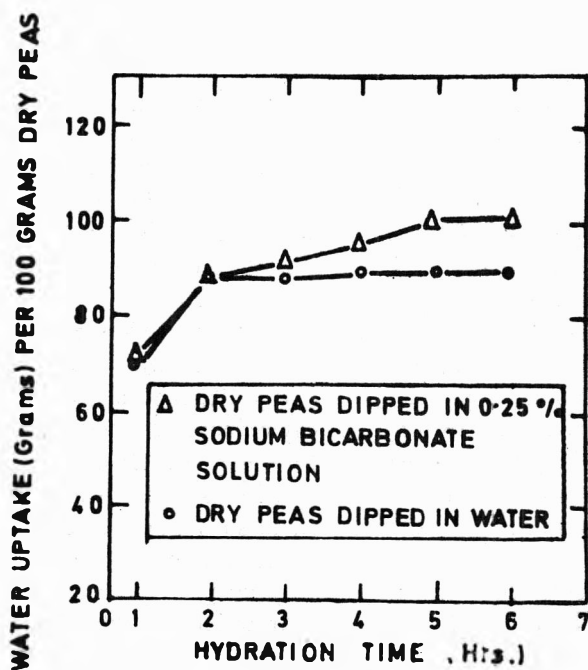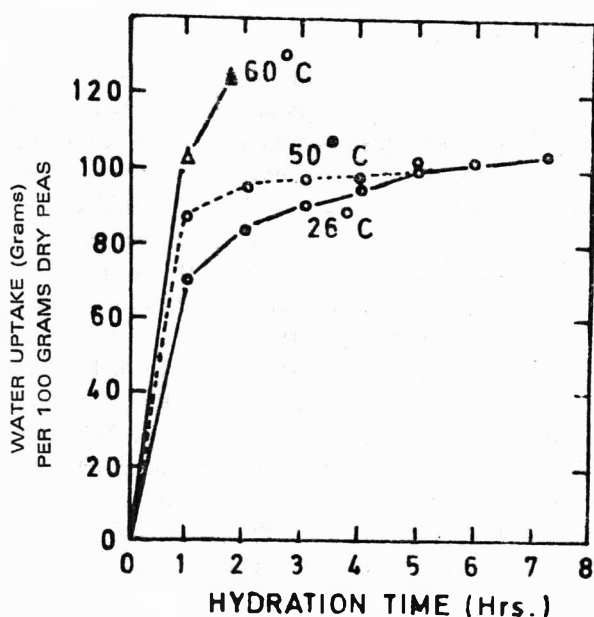

Fig 3a.   Hydration of dry peas at room temperature (26°C) with and without dipping in 0.25% sodium bicarbonate

TABLE 1.   EFFECT OF SOAKING ON COOKING TIME OF DRY PEAS

Soaking period (hr)	1	2	3	4	5	6	7	8	16
Cooking time (min) experimental cooker	45	33	25	20	18	15	15	15	14

TABLE 2.   EFFECT OF SODIUM BICARBONATE CONCENTRATION IN YDRATION MEDIUM ON COOKING TIME OF PEAS

$NaHCO_3$ concn (%)	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.50	1.0	2.0
Cooking time (min)	35	30	25	18	15	15	15	15	14	14

TABLE 3.   EFFECT OF HYDRATION MEDIUM RATIO ON COOKING TIME OF PEAS

Peas to hydration medium (ratio)	1:1	1:2	1:3	1:4	1:5
Cooking time (min)	35	25	17-18	15	15

TABLE 4. EFFECT OF PRECOOKING CONDITIONS

| | Precooking method | | |
	Boiling water	Steaming	Pressure cooking
Cooking time of processed peas (min.)	6	6	5
Butterflied peas (%)	40-45	30-35	5-10

TABLE 5. EFFECT OF PRESSURE COOKING ON COOKING CHARACTERISTICS OF PROCESSED PEAS

| | Precooking under pressure (min) | | | | | |
	1	2	3	4	5	6
Reconstitution time of processed peas (min)	11	9	8	5	5	5
Butterflied peas (%)	3	4	6	7	30	40

method. This resulted in less percentage of butterflying in peas and cooking time of processed peas was also found to be minimum (Table 4). Pressure cooking for 4 min resulted in quick cooking product with relatively less amount of butterflied peas (Table 5). Hence, optimum time of pressure cooking of peas was selected as 4 min.

Dehydration: Fig. 4a gives dehydration curves for precooked peas prepared by two different procedures. It was observed that 150 min of drying time was required to lower the moisture content of the 5-hr soaked peas in 0.25 per cent sodium bicarbonate solution, whereas 180 min were required to reduce the moisture content of the water-soaked peas to the same level. Thus, dehydration rate was greatly increased by using 0.25 per cent sodium bicarbonate solution as soaking medium.
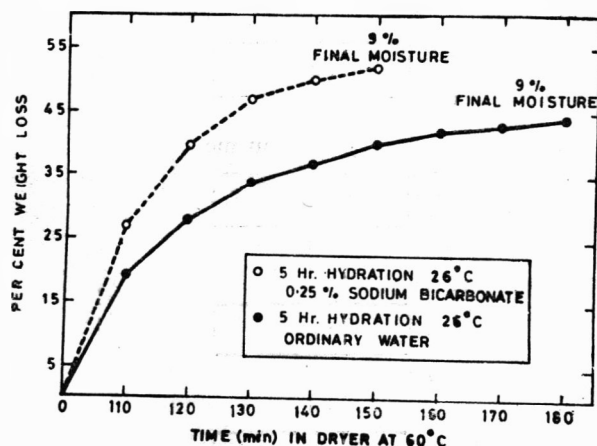

Fig 4a. Dehydration curves for dry peas processed by two different methods

Fig 4b. Rehydration curves for dry peas processed by two different methods

Figure 4b represents rehydration curves for these two types of processed peas. Sodium bicarbonate-soaked peas doubled their weight in boiling water in less than 3 min, whereas the water soaked peas required a little over 20 min to reach the same degree of hydration. Losses of solids during initial hydration were 0.8 and 4.5 per cent, respectively for the peas soaked in 0.25 per cent sodium bicarbonate and those soaked in water. Soaking in sodium bicarbonate solution has the advantage of giving higher yield.

Drying temperatures up to 60°C do not normally affect either flavour or colour. Colour intensity of the peas soaked in 0.25 per cent sodium bicarbonate was more than that of those soaked in water.

Degree of cooking of the precooked dehydrated peas: As the texture meter readings were indicative of peas' softness and did not reflect slight undercooking or lack of uniformity in cooking, determination of the cookability of peas was very important. Cooking times of the dehydrated peas processed by these two methods were compared with that of unprocessed dry peas using the bean cooker (Table 6). It was observed that peas which were soaked in 0.25 per cent sodium bicarbonate solution cooked in 5 min, as compared to more than 15 min taken by the peas soaked in water. Cooking time of 5 min as

TABLE 6. COMPARISON OF STANDARD AND QUICK COOKING PEAS

Variety of peas	Moisture content of peas (%)	Cooking time (min)
Dry peas (standard)	9.2	60
Quick cooking peas (H$_2$O soaked)	9.1	18
Quick cooking peas (NaHCO$_3$ soaked)	9.1	5

compared to 60 min required by ordinary dehydrated peas is a great advantage.

Sensory evaluation of the quick cooking peas: Results of sensory tests using 9 point hedonic scale on freshly prepared samples and samples stored for 12 and 15 months, respectively, indicated the scores to be 8, 8 and 7 in uncooked fresh states. Similar results were obtained when the samples were cooked and then served. Thus, the overall quality of the product was good on storage for up to 15 months at ambient temperatures.

Storage stability of the quick cooking peas: It is known that cooking times of peas or bean increases with the length of storage. Dawson *et al*[17] found that peas stored one year at 30°C, required longer cooking than those stored at 0°C. Morris and Wood[18] reported that peas with moisture content above 13 per cent deteriorate significantly in flavour and texture after 6 months at 30°C; in 12 months they become unpalatable. Peas below 10 per cent moisture maintained their quality for two years at 25°C, almost as well as control samples stored at 0°C.

Samples of the dehydrated quick cooking peas processed as explained earlier and stored in polyethylene bags (700 gauge) at room temperature (26±2°C), exposed to incident daylight and fluorescent light, had a cooking time of 6 min when stored for six months to one year. There was no detectable deterioration in the organoleptic quality of peas stored for 1 year.

Samples stored at 50°C and 50 per cent relative humidity for two months developed off flavours. These, however, had no detectable off flavours when rehydrated.

Cooking time of quick cooking peas processed by the above method with moisture contents of 8.2, 10.3, 12.2, 13.9 and 16.9 per cent and stored at 12, 25, and 30°C for 3 months is given in Fig. 5. It was observed that cooking time of the samples containing moisture contents in the range of 8.2 to 10.3 per cent and stored at 30°C remained 6 min, viz. constant, for a period of one year, but the samples containing moisture content more than 10 per cent and stored at 12 or 25°C showed increase in their cooking time as the time elapsed. It was concluded that quick cooking peas are stable at room temperature for a period of one year when the moisture content of peas are maintained in the range of 8 to 10 per cent.

Packaging: Both polyethylene and polycell bags of 700 gauge used for packaging of quick cooking dehydrated peas were found to be very good for storage up to one year. After one year storage, moisture absorption by the peas to a certain extent did occur resulting in increase of cooking time. Aluminium laminate pouches (2.4/1.0/0.5/1.5 ml) (Paper/polyethylene/aluminium/polyethylene) were found to be best for storage of quick cooking, dehydrated peas up to two years.
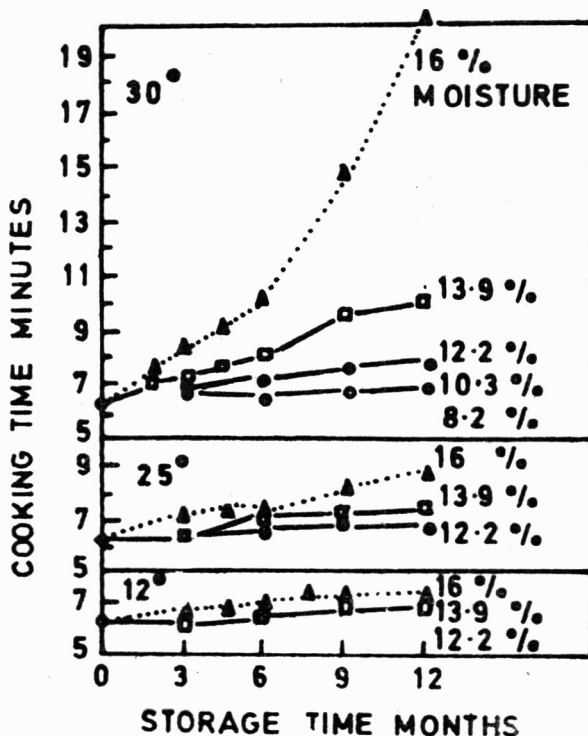

Fig 5.   Cooking time of quick cooking peas at various moisture contents and temperatures
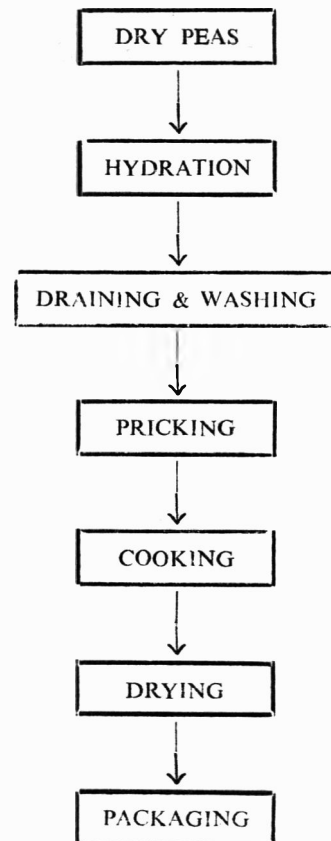

Fig 6.   Flow diagram of the process for quick cooking peas

Based on these studies, a flow diagram of the process for large-scale processing of quick cooking peas was arrived at, as shown in Fig. 6.

Applicability of the process for different beans: The method was found to be applicable to red gram, green gram, Bengal gram, field beans and fresh peas or beans. Whole grain as well as the husked, split grains (called *dals*) could be used for processing. The basic processing conditions as determined above could be applied with suitable variations to different beans for reducing their cooking time. As these beans form important constituents in the army rations, their use for troops at high altitudes will be advantageous as the product could be cooked quickly and easily. Similarly quick cooking peas or beans will be of great help in preparing convenience foods. The product processed by this method when reconstituted, more nearly resumed the shape of the original material.

The analysis of B group vitamins was not performed in this study. The sensitivity of thiamine and riboflavin to the action of alkali has been long recognised[12]. However, on dehydration of peas, no further degradation of B group vitamins may occur due to low water activity[19]. It appears that alkali treatment may not have adverse effect on ascorbic acid in peas as shown by similar studies of Sweeney and Martin[20] by boiling green beans in cooking media up to a pH of 8.4.

Economics: As the process involves additional operations of hydration and pre-cooking, it is to be expected that the cost of production will be higher than for ordinary dehydration. But the gain in reduction of cooking time will result in fuel savings. The process could be used for various other beans as well.

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Distribution of Oil in Different Anatomical Parts of Some Cucurbit Kernels

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The different parts (endosperm, germ and cotyledons) of kernels of cucurbit species viz., *Citrulus vulgaris* (watermelon,) *Cucumis melo* (muskmelon,) *Cucurbita moschata* (pumpkin,) *Cucumis sativus* (cucumber,) and two conventional oilseed crops namely, *Brassica campestris* var. *dichotoma* (mustard,) and *Sesame indicum* (sesame,) were dissected manually and their physical proportions and oil content studied. The watermelon and pumpkin kernels which had the highest 1000 kernel weight (42-91 g) also had the highest endosperm ratio (12-13%) and higher oil content (50.8-51.8%). Although, mustard and sesame seeds are much smaller in size and weight, these had a much higher propotion of germ (9.4-11.2%) than the cucurbit kernels (3-4%. The so called endosperm (seed coat + endosperm) in sesame seeds is exceptionally rich in oil content (44.3%).

The kernels of watermelon, muskmelon, pumpkin and cucumber have long been marketed in India and are used in confectionery, snacks and as an important home remedy for cure of general debility.

Recently, some cucurbit seeds, have been investigated and found to compare very favourably with conventional oilseed crops in their oil content[1]. If suitably exploited, some of these could form potential sources of oils and fats[2]. Their oils are also edible[3,4]. The distribution of oil in the different anatomical parts of these cucurbit kernels, in comparison with that in certain established oilseeds, is reported in this paper.

Materials and Methods

Seeds of the four cucurbit species namely, *Citrullus vulgaris* (watermelon, *tarbooz*), *Cucumis melo* (muskmelon, *kharbooza*), *Cucurbita moschata* (pumpkin, *kaddu*) and *Cucumis sativus* (cucumber, *kheera*) were obtained from the Division of Horticulture and the two oilseeds, namely *Brassica campestris* var *dichotoma* (mustard, *sarson*) and *Sesamum indicum* (sesame, *til*) from the Division of Genetics, I.A.R.I., New Delhi.

The seeds were cleaned of foriegn matter and bad seeds and kept in glass-stoppered bottles for further use. To obtain kernels, the seeds were decorticated by hand. Accurately weighed quantities of dried kernels of all the six materials were placed between folds of wet filter paper in separate petri-plates for about two hours, to soften the seedcoat. The kernels were manually dissected using fine forceps into endosperm, cotyledons and germ and the seeds into seedcoat (which includes the endosperm), cotyledons and germ. The separate parts were dried to constant weight at low temperature (60°C) to prevent any loss of oil and the percentage of each part

calculated. The oil content of each separated part, as well as the whole kernel and seed, was determined by the cold percolation method.[5]

Results and Discussion

While the variations in the proportions of germ and cotyledons in different species of cucurbits is only moderate, it ranges from 300 to 400 per cent for the endosperm (Table 1), which is high for watermelon and pumpkin kernels and low for those of musk melon and cucumber. Species with the highest 1000-kernel weight also show a high endosperm proportion.

The two oilseed species mustard and sesame differ markedly from the cucurbit species in the proportion of their anatomical parts. The endosperm in these seeds is

Table 1. Proportion of various anatomical parts of cucurbit and oilseed species

Seeds	1000 kernel* wt (g)	Proportion (%) of		
		Endosperm	Germ[b]	Cotyledons
Citrullus vulgaris	41.5	13.0	3.0	84.0
Cucumis melo	19.7	3.5	2.8	93.7
Cucurbita moschata	90.7	12.3	2.7	85.0
Cucumis sativus	25.3	4.3	4.1	91.6
Brassica campestris var. *dichotoma*	5.6*	16.4[a]	9.4	74.2
Sesamum indicum	3.1*	35.4[a]	11.2	53.4

*Whole seed weight
[a]Includes seedcoat also.
[b]Includes plumule and radicle.

Seeds	Oil content of whole kernel (%)	Oil content (%)		
		Endos-perm	Germ[b]	Cotyle-dons
Watermelon	50.8	0.8	42.5	54.4
Muskmelon	49.3	0.3	38.5	53.4
Pumpkin	51.8	0.9	43.3	54.3
Cucumber	45.7	0.2	34.4	49.8
Mustard	49.6*	10.6[a]	42.3	54.0
Sesame	53.1*	44.3[a]	63.8	65.2

*Whole seed.

[a]Includes seedcoat.

[b]Includes plumule and radicle.

adhering to the seedcoat and forms only a fragmentary part of it. In cucurbit seeds the endosperm layer surrounds the embryo and together constitute the kernel and these constituents are easily separable.

The two oilseeds are much smaller in size and weight than cucurbit kernels; the germ percentage is much higher, and that of the cotyledons considerably lower. Mustard and sesame seeds differ considerably from each other in their proportion of endosperm and cotyledon.

Table 2 shows that the percentage of oil in the four cucurbit kernels are of the same order as for mustard and sesame seeds, all six range between 46 and 53 per cent. Thus the cucurbit seeds, if properly exploited, could add to the national vegetable oil production. The cucurbit oils are known to be of good quality and quite suitable for edible purposes[1,4].

The oil content in the endosperm of the different cucurbits analysed is generally very low (less than 1 per cent) and its contribution to the total oil present in the kernel is of the order of only 0.25 per cent. Kernels of watermelon and pumpkin, which show a higher pro-

portion of endosperm (Table 1), also show a higher content of oil in the endosperm. The percentage of oil in the germ, though lower than in the cotyledons, lies between 34 and 44 per cent. However, germ oil contributes only 2.0-2.5 per cent to the total oil pool, the bulk of which is derived from the cotyledons.

The distribution pattern of oil in the two oilseeds is similar to that in the cucurbit species in respect of germ and cotyledons. It markedly differs in the endosperm, which in the two oilseeds include the seedcoat, and is rich in oil, especially in sesame.

In commercial practice, sesame seeds are not dehulled before crushing. Removal of the seedcoat from sesame seed, as for the production of edible protein, would lead to some oil loss.

The distribution pattern of oil content noted in various anatomical parts of cucurbit and conventional oilseeds is in sharp contrast with that in cereal[6,7], and pulse grains[8], where the percentage of oil in the germ is much higher than in the endosperm or cotyledons.

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Augmentation of Warfarin Toxicity by Vitamin A Acetate to Roof Rats *(Rattus rattus)*

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Resistance of rodents to warfarin and other anticoagulants has lead to an intensive research on improving the existing rodenticides and synthesis of newer compounds. Currently vitamin K antagonists, antibiotics and vitamin D are being exploited to combat the resistance problem among rodent populations. It is observed that vitamin A acetate at 0.2% in combination with warfarin at 0.005% baits increased the mortality (100%) in roof rats (*Rattus rattus*) as against warfarin control (40%). The onset of symptoms was faster and more severe in animals ingesting the baits containing both vitamin A acetate and warfarin than with warfarin alone. The result indicated that vitamin A acetate can potentiate warfarin poisoning in rats resulting in higher mortalities even in lower concentrations.

In the last three decades, anticoagulants such as coumarin and indandione derivatives have played a prominent role in rodent control. These compounds are vitamin K antagonists causing haemorrhage and death in animals. However, the widely used[1-4] warfarin has created a major concern on the development of resistance among rodent populations. This serious threat resulted in the development of alternative rodenticides such as RH-787, (N-3-pyridylmethy)-N-p-nitrophenyl urea[5], UK-786, 5-(N-piperidino)-10, 11-dihydro 5-H-dibenzo cycloheptane[6], Difenacoum, 3-(3-p-diphenyl 1-1,2,3,4, tetra hydronaphth-1-yl)-4-hydroxycoumarin[7] and calciferol[8,9] (Vitamin D_2). Attempts have been made to combat the warfarin resistant rats by incorporating vitamin D (Calciferol) in warfarin baits[3]. Rowe and Redfern[10] used vitamin A acetate with warfarin to control house mice populations. The concentration of vitamin A acetate used by them was very high resulting in poor acceptability of baits. Not much is known on the efficiency of vitamin A in potentiating the toxicity of warfarin in wild rat populations. Attempts are made here to test the possibility of increasing warfarin toxicity to roof rats (*Rattus rattus*) by incorporating vitamin A acetate at 0.2 per cent in baits containing warfarin at 0.005, 0.0125 and 0.025 per cent levels.

Materials and Methods

Tests in cages: Roof rats of both sexes weighing 100-150 g were individually caged after a 2-day conditioning period. They were fed with standard bait B (SBB) containing rice flour-68.5; wheat flour-18.5; groundnut cake flour-46; common salt-1.0; groundnut oil-7.4 g/100 g dry weight[11] and water prior to the test. Technical warfarin (Pest Control (India) Pvt. Ltd.,

Bombay) at 0.005, 0.0125 and 0.025 per cent was mixed with SBB and used. Vitamin A acetate at 0.2 per cent (Roche Products Pvt. Ltd., Bombay) was incorporated into warfarin baits and tested. The lower concentrations of vitamin A acetate were not effective. Hence 0.2 per cent was chosen for evaluation. Poison baits and water were supplied for a period of ten days to animals *ad libitum*. Daily records of bait intake, appearance of sickness and mortality were made. The animals that survived the test period were maintained on standard bait B for further period of ten days for observation. The data collected were analysed statistically.

Tests in rattery: The effective combination observed in the cage test (warfarin, 0.005 per cent + vitamin A acetate, 0.2 per cent) was evaluated against roof rat populations housed in the rattery. Healthy animals were conditioned for three days and the poison baits were laid at two food points for 10 days. Daily records of bait consumption and mortality were maintained. The survivals were observed for 13 days in the cages with surplus food and water.

Results and Discussion

Cage tests: Vitamin A acetate alone did not result in any sickness or mortality in rats during the test period. Warfarin at 0.005 per cent (the lowest concentration used) exerted only 40 per cent mortality while the same concentration of warfarin in combination with 0.2 per cent vitamin A acetate resulted in death of all test animals.

The results of 't' test indicate (Table 1) that the mean intake of warfarin exerting lethality in combination with vitamin A acetate was significantly lower as com-

TABLE 1. RESULTS OF TOXICITY TESTS OF WARFARIN AND VITAMIN A MIXTURES AGAINST CAGED ROOF RATS

Warfarin + vitamin A acetate (%) in bait	Warfarin consumption (mg/kg body wt) Mean ± S.D.	't'—value (dif)	Vitamin A consumption (mg/kg body wt) Mean	Mortality (%)	Mortality period (days)
0.005	24.56 ± 3.74^d	12.46***	—	40	5-10
0.005 + 0.2	7.84 ± 2.01^a	(18)	313.60	100	3-10
0.0125	34.82 ± 3.02^e	8.64***	—	90	4-9
0.0125 + 0.2	18.12 ± 5.32^b	(18)	289.92	100	3-12
0.025	100.82 ± 14.48^f	5.14***	—	100	4-8
0.025 + 0.2	69.70 ± 12.53^c	(18)	557.60	100	3-10
0 + 0.2	—	—	1116.73	0	—

abc—Significantly different from each other (P <0.05)
def —Significantly different from each other (P <0.05)
***—P<0.001
SD—Standard deviation
Figures in the parentheses indicate degrees of freedom.

pared to warfarin control. This was very much obvious in baits containing the lowest concentration of warfarin (0.005 per cent) and vitamin A acetate. There was no significant difference in mortality period. Comparatively animals ingesting the mixtures profusely bled externally. Symptoms of sickness and haemorrhage appeared earlier. There was a gross incordination in hind limbs resulting up in paralysis preceding death. The limbs were often edematous. Bleeding in nasal and anal region was profuse.

Rattery test: The response of roof rat populations was similar to that of individually caged rats. Warfarin at 0.005 per cent killed only 30 per cent of the population but in combination with vitamin A acetate resulted in 100 per cent kill (Table 2). The severity of haemorrhage and sickness was higher in rats consuming vitamin A fortified warfarin baits. Vitamin A alone was well tolerated by animals.

The above results indicate that vitamin A acetate influences the toxicity of warfarin to animals. It is well known[12] that coumarin compounds are potentiated by pyrazole compounds like phenyl butazone and oxyphenbutazone. These compounds reduce prothrombin level leading to haemorrhagic conditions thus potentiating the effect of anticoagulants. It is also known that vitamin A causes deficiency of vitamin K[13].

In this study perhaps vitamin A 'predisposed' the animals to become more susceptible to low doses of anticoagulants. There could be three possibilities: (i) vitamin A in excess might interfere with possible absorption and utilization of vitamin K for normal prothrombin formation; (ii) warfarin also has synergistic effect in prolonging prothrombin activity; and (iii) excess of vitamin A induces a secondary deficiency of vitamin K[14].

Our observations suggest that reinforcing warfarin baits with vitamin A acetate results in the reduction of lethal dose of warfarin, early appearance of sickness, pronounced haemorrhage together with increased mortality at lower intake of warfarin in roof rats. Vitamin A acetate at 0.2 per cent did not affect or reduce the palatability of baits which is an advantageous factor.

The above findings suggest the possibility of using vitamin A acetate as a potentiating agent for a few other anticoagulants also. Formulation of proprietory baits containing vitamin A and warfarin may be useful for effective rodent control. The combination could be effective in controlling anticoagulant resistant populations. Such combinations could be used as prophylactic treatments to prevent the occurrence of resistance among animal populations to low doses of anticoagulants.

TABLE 2. TOXICITY OF WARFARIN AND VITAMIN A ACETATE MIXTURE TO ROOF RAT POPULATIONS IN THE RATTERY

Warfarin + vitamin A acetate (%)	Bait consumption (g)	Warfarin consumption (mg)	Vitamin A consumption (mg)	Mortality (%)	Mortality period (days)
0.005 + 0	300	15	0	30	7-9
0 + 0.2	283	0	566	0	—
0.005 + 0.2	380	19	760	100	3-7

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Evalution of Spices and Oleoresins. II. Pungency of Capsicum by Scoville Heat Units—A Standardized Procedure

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The official objective methods for the quantitative estimation of capsaicin, the pungency factor of capsicum, are time consuming. Further, because of lack of correlation of these values with the subjective pungency, the international trade still prefers the subjective Scoville test. However, the existing procedures for determining the Scoville heat units suffer from a number of defects such as lack of experimental details, lack of definition of panel and poor reproducibility. This paper describes a standardized procedure for determining pungency of capsicum in Scoville heat units, taking into account experimental and other psychological errors, nature and limits of panel variations. The subjective data are expressed in a statistically acceptable manner. A definition of panel sensitivity is proposed to provide parameters to compare results by panels of different sensitivity. With the proposed procedure very highly significant linear regression is obtained between the Scoville heat units and the capsaicin content of the samples.

Capsicum, (chillies) are valued throughout the world principally for pungency, colour and by some for aroma also. Pungency is due to capsaicin and few related compounds present in minor proportions. Capsaicin content of capsicum is usually low, of the order of 0.01 to 0.1 per cent in paprika, (*Capsicum annum*, L) and 0.05 to 0.6 per cent in chillies, (*Capsicum frutescens*, L, and *Capsicum minimum* Roxb). These small amounts permit only colorimetric or spectrometric procedures in the quantitative estimation of pungent compounds[1]. In spite of a number of attempts, these estimations have proved to be unsatisfactory, because they are time consuming and have poor reproducibility[2,3]. Chromatographic column purification and spectrometry[3] or more recently gas chromatography[4] have been suggested as improved methods. These are also time consuming or involve costly modern equipment and have not been found suitable for routine use in quality control, parti-cularly in the developing countries where the spices are grown. However, a few recent objective procedures through the use of thin layer chromotography[5-7] and particularly the simpler and more widely adoptable paper chromotography[8] appear promising. This last method has been recommended for routine work[9]. Because of the lack of established correlation between objective and subjective evaluation of pungency, the international trade still prefers the subjective test in estimating pungency[10].

The sensory feeling of 'bite' or pungency at extreme dilutions (recognition threshold), is the basis of these subjective tests and the reciprocal of the dilution is expressed as Scoville heat units (SHU)[11]. The literature values for the limit of detection of the pungency of pure capsaicin has varied by a 100 per cent, that given by Suzuki *et al*[12] being 15×10^6 and by the Panel on Crude Drugs in U.K.[3] being 30×10^6. Though theoretically

simple several difficulties arise in carrying out subjective tests for pungency. Apart from the difficulties common to most sensory tests[13], lack of pure reference standards, build up of pungency during test sessions and lack of agreed procedures for conducting the tests and recording of the results, have resulted in poor reproducibility and great variations, and resultant sceptisism about the validity of the test itself. Newman[14] has earlier pointed out that the defects of the British Pharmacopea[15] procedure are, the use of a simplified extraction method, assumption of capsaicin value of sample to be 0.14 per cent and use of a single dilution to prove or disprove the pungency of a sample.

Fritzsche Brothers, Inc, in USA, published a method[16] for oleoresins with model dilution table but still worked it as a 'go-no-go', test, in which the value given by three out of five experienced judges is taken as the SHU, for determination of the threshold for pungency of a sample. It is surprising that this method also uses a single test samples as in the original British Pharmacopea. Possibly this procedure was designed by the company as a quick quality control procedure for regular supply within certain specified limits of pungency. But it is an improvement in the sense that a further dilution is recommended if the first is found to be very pungent. Another drawback is that this test is not done from higher to lower dilution as required in a threshold test. A more serious error is from non-definition of difference to be maintained between successive dilutions in terms of active pungent principle. It can easily be shown (Table 1) that the capsaicin content of fixed dilution differences, varies greatly with samples of different

capsaicin content. The validity of taking the value where three out of five judges agree is also questionable statistically. The same procedure is adopted by the Essential Oil Association of America as their standard[17]. The International Standards Organisation has recently adopted a procedure which follows the method of testing a series from lower concentration upwards but the SHU for the sample is again based on the highest value recorded by at least three out of a minimum of five panelists[18]. Inspite of these attempts at codifying the procedure the subjective method continues to be regarded as poorly reproducible.

Our study was therefore planned to find the difficulties in conducting Scoville tests, which affect reproducibility and validity of the results and recommend a procedure where possible sources of error are eliminated, provide a ready dilution table, the result given in a statistically acceptable manner and which enables a correlation with the objectively determined value for capsaicin. A definition of the panel is also given so that a comparison of values from different panels can be done. When our study was completed some years ago the method adopted by the Official Analytical Method of the American Spice Trade Association was published[10]. This method has discussed many of the above points, without however, detailing measures to overcome the sources of variation. It has pointed out the importance of palate clearing for avoidance of carry over effect between samples and conducting the test as regular threshold test. Also, a dilution table covering a wide range of Scoville values 1:100 to $1:15 \times 10^5$ is given which is very useful. But this method also uses a small number of judgements

TABLE 1. CAPSAICIN EQUIVALENTS IN $\mu g/100$ ml OF FIXED DILUTION DIFFERENCES FOR TWO OLEORESIN SAMPLES

Fixed dilution differences (in 1000 s)	Sample 1 (capsaicin content 5%)		Sample 2 (capsaicin content 2.5%)	
	Threshold dilution series (in 1000 s)	Capsaicin equivalent of width between successive dilution ($\mu g/100$ ml.)	Threshold dilution series (in 1000 s)	Capsaicin equivalent of width between successive dilution ($\mu g/100$ ml.)
120	840 ⎫ 720 ⎭ ⎫ 600 ⎭	0.99 1.39	440 ⎫ 320 ⎭ ⎫ 200 ⎭	2.13 4.69
85	865 ⎫ 780 ⎭ ⎫ 695 ⎭	0.63 0.78	405 ⎫ 320 ⎭ ⎫ 235 ⎭	1.64 2.83
45	765 ⎫ 720 ⎭ ⎫ 675 ⎭	0.40 0.47	365 ⎫ 320 ⎭ ⎫ 275 ⎭	0.96 1.28

and takes the SHU as that agreed to by 3 out of 5 judgements. Though the method mentions the use of experienced tasters, the procedure for screening, selection and training of panelists is not given. There is also no recommendation on the concentration difference in the dilution series. These could have been the main reasons for the recently reported[4] non-reproducibility of Scoville values when tested under the ASTA methods.

This paper, presents such results, which appear still valid and relevant. The comprehensive study relating to possible sources of errors, nature and limits of variations are given and a method which would give reproducible results that are statistically acceptable and provide parameters for comparing results from panels of different sensitivity is detailed. With such a standardized procedure it is shown that correlation with capsaicin content and SHU is very highly significant.

Materials and Methods

In this work, oleoresin locally made and also obtained from the international market representing varied capsaicin contents were used. Their capsaicin content as determined by paper chromotography[8] were used for subsequent regression analysis between subjective and objective values.

Subjective tests were done as threshold test[19] with clear instructions regarding bias factors influencing judgements and explanation of the evaluation procedure to the panel. Difficulties of panel members in doing the tests were discussed during preliminary and subsequent group meetings. Besides the problems common to subjective tests, special requirements in pungency evaluation, to eliminate aroma interference, build up of pungency and palate clearing between samples was followed. The use of neutral food like puffed rice and allowing a time lapse of 10-20 sec between successive samples was found efficient for clearing the palate of build up. Care was taken in sample presentation to avoid positional bias, by varying the number of blanks in the series of dilutions tested and using different dilution series in repeat tests.

The various steps studied are detailed under results and discussion and the final standardized procedure is described at the end. The procedure has now been adopted by the Indian Standards Institution[20].

Results and Discussion

1. *Screening and training of judges:* A total of 16 potential panelists were tested through five preliminary sessions. The range of dilutions tested were from 65,000 to 2,50,000, using two oleoresins of different capsaicin content. The individual judges' threshold for the samples varied from, 10,000-60,000 in repeat tests. By panel discussion, the stimulus, positional and other bias factors were avoided. Further repeat tests with a single sample of oleoresin were done. The panel was then classified roughly into two groups (*i*) a low threshold group (high sensitivity) giving threshold values of 1,00,000 and above; (*ii*) a high threshold group (low sensitivity), giving values below 1,00,000, for the same sample.

Throughout the rest of this study, five from the first group of eleven panelists with low threshold were used.

2. *Selection of dilutions for test:* All the test samples were first tested by the panel leader, who was in the same sensitivity group, by diluting samples in geometric series with a common ratio of 1.5 to fix the gross threshold. The samples were then tested by the selected panel in a closer common ratio of 1.2 to identify the approximate threshold; for example, the geometric series for a sample with 2.5 per cent capsaicin was as follows, 547, 456, 380, 317, 264 thousands. The approximate threshold was around 330×10^3. These steps enable prevention of great deal of waste of time by giving samples which could be either too dilute or too strong depending on the capsaicin content of the sample.

To facilitate determination of the width of difference in the arithmetic series of dilution to be maintained at the 'just noticeable difference (jnd) value, paired comparison directional difference tests were carried out separately with the samples, around the approximate threshold. The 'jnd' was found to be 0.11 to 0.13 of the approximate threshold (Table 2,). In the final tests using arithmetic series around the approximate threshold the width of dilution difference in successive samples was maintained at one tenth of the approximate threshold. From the intensity data on the successive samples in the different series, this concentration difference was found satisfactory.

Provision of different series of dilutions covering higher and lower concentration around anticipated threshold allows for individual variations in sensitivity. Ready reference for these dilutions are available through tables in the procedure adopted by the Indian Standards Institution[20].

3. *Variations in panel values and procedure for expression of results:* Table 2 gives the results of SHU determined according to the recommended method for four samples by five trained panelists in 4 repeat evaluations.

Table 3 gives the results expressed according to single dilution and serial dilution procedures using the data in Table 2. When the results are expressed using single dilution as test samples, different results on different occasions are obtained even by the trained homogenous panel. The situation will be worse when a non-homogenous group is used. Thus a single value cannot be given. Also, the percentage of panel observations cover-

TABLE 2.   SCOVILLE HEAT UNITS (THRESHOLD JNDIN THOUSANDS) OF DIFFERENT OLEORESINS—VARIATIONS IN REPEAT EVALUATIONS

Panelist	Oleoresin samples with capsaicin content (%)			Capsaicin (PC) (natural, 100%)
	F-Hi (2.35%)	F-DO (4.50%)	GL (4.90%)	
1	330	840	900	19,000
	330	840	900	17,600
	360	900	—	18,200
	—	—	—	18,200
2	300	840	641	16,000
	330	780	641	16,000
	360	810	641	16,700
	330	—	700	17,400
3	330	700	641	18,000
	360	700	700	17,600
	300	780	641	17,400
	300	810	700	16,700
4	330	840	900	17,600
	330	840	900	16,000
	390	810	—	16,700
	390	810	—	16,700
5	330	840	700	16,000
	360	780	800	16,000
	360	810	—	17,600
	—	—	—	16,700
Overall range	300-390	700-900	641-900	16,000-19,000
One sigma range	340 ± 27	808 ± 50	743 ± 111	$17,105\pm885$
'Jnd' near threshold	45	86	96	1,800

Note:—The unequal number of observations are due to absence of panelists
The 'jnd' values are obtained by paired directional difference test

ed, decreases from 100 to 60 per cent as the SHU increases. As it is well known, even with trained panelists day to day variations are found for the same sample as seen from data in Table 2. Expressing the result as one sigma range of the mean is therefore better, reflecting normal panel variations. The per cent observations covered by this range varies from 70 to 82 per cent. However including the values of panelists who have identified at higher dilutions the percentage varies from 80 to 100 per cent. It is therefore recommended that the SHU of a sample be given as the panel mean ± standard deviation.

4. *Panel consistency and definition:* The range of individual panelists variation in repeat threshold evaluations calculated for the data in Table 2 are given as capsaicin equivalents (μg/100 ml) in Table 4. These individual values are compared against the capsaicin

equivalent of the 'jnd' of pungency. The variation of a panelist in repeat evaluations of a particular sample should not exceed the 'jnd' to prove his/her consistency. Thus, 80 per cent of all the twenty values fall within 'jnd' which indicates the extent of individual panelists' consistency.

Similarly, the threshold of individuals in repeat evaluations of a particular sample should not fall outside one sigma range to prove the panel homogeneity. Thus as seen from values given in Table 2, 70 per cent of these values fall within one sigma range and 86 per cent fall above the lower limit which indicates the degree of homogeneity of the panel in this study. Having established a reasonable degree of panel consistency and homogeneity an acceptable panel definition through the panel's sensitivity in terms of SHU expressed as one sigma range about the mean threshold for either pure capsaicin or

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TABLE 3.   EXPRESSION OF SCOVILLE HEAT UNITS BY DIFFERENT PROCEDURES

Sample detail	Single dilution*		Serial dilution**		
	SHU† estimated	% observations corresponding and exceeding	SHU† One sigma range	% observations exceeding the lower limit	% observations between one sigma range
F-Hi	300	100	313-367	83	72
	330	90			
	360	80			
F-DO	700	100	758-858	88	82
	780	90			
	810	80			
	840	80			
GL	641	100	632-854	100	71
	700	70			
	800	60			
PC	16,000	100	16,220	80	70
	16,700	80	to		
	17,400	60	17,990		
	17,600	60			

*Fritzsche brothers' method
**Presently recommended method
*SHU—Scoville heat units (in thousands)

an oleoresin sample of known capsaicin content can be given. For example, the panel used in this study is defined as one giving $17 \times 10^6 \pm 0.88 \times 10^6$ SHU for pure natural capsaicin (8—methyl-N-vanilyl non-6-enamide, mol wt. 305) or value of $800 \times 10^3 \pm 50 \times 10^3$ SHU for an oleoresin sample of 4.5 per cent capsaicin. Results of panel of different sensitivity e.g. 30×10^6 SHU for pure natural capsaicin can then be calculated by proportionment. The SHU given by our panel for pure synthetic capsaicin (vanilyl-N-nonanomide, mol. wt. 293) was $8 \times 10^6 \pm 0.7 \times 10^6$. This can also be used for defining the panel sensitivity.

5. *Regression:* The regression of capsaicin per cent

TABLE 4.   RANGE OF INDIVIDUAL PANELIST'S VARIATIONS IN REPEAT THRESHOLD EVALUATIONS EXPRESSED AS CAPSAICIN (μg/ 100 ml.)

Panelist	F-Hi	F-DO	GL	PC
1	0.59	0.36	0.00	0.42
2	1.30	0.41	0.64	0.50
3	1.30	0.87	0.64	0.43
4	1.09	0.20	0.00	0.57
5	0.59	0.41	0.88	0.57
jnd	1.05	0.67	0.91	0.77

(y) on the SHU (mean threshold) (X_1) for all the 4 samples was found to be linear and the regression and correlation coefficients were very highly significant $(P<0.001)$. The regression equation is given below.

$$Y = 0.243430 + 0.005830X_1, \qquad (r=1.00)$$

where, X_1 is SHU in thousands.

The per cent deviation of the actual capsaicin per cent (y) of the experimental samples from the predicted capsaicin per cent (Y) ranged from 0 to 13 per cent from the equation.

The regression equation can be suitably modified for any other panel of different sensitivity; for example the SHU by one panel for oleoresin F-Hi of capsaicin content 2.35 per cent was 340×10^3 while another panel of low sensitivity gave a SHU of 182×10^3 for the same sample. The regression could be modified for the second panel as

$$Y = 0.243430 + 0.010891 \, X_1$$

where the coefficient of X_1 is increased in the ratio 340:182. For a panel of higher sensitivity the coefficient of X_1 needs to be decreased accordingly. Thus, results by panels of different sensitivity can be compared.

Standardized procedure

Based on the results obtained, the standardized procedure for conducting pungency tests is given below:

1. *Preparation of sample and preliminary testing:* Oleoresins and alcoholic extracts of capsicum (chilli) powder are diluted to obtain a stock solution corresponding to a dilution of 1:10,000 and 1:100 respectively in sugar solution. Glycerol monostearate (1 percent in alcohol) is used with oleoresins, as an emulsifier.

Prepare further dilutions from the stock solution in geometric progression with a common ratio, (say 1.5). Use this for preliminary testing by the panel leader to fix the gross threshold and roughly select the range of dilutions to be used. Prepare geometric series again with closer common ratio (say 1.2), around the value determined by the panel leader for preliminary testing by the panel. Use 10-12 potential panelists in two or three session where they test 4-7 dilutions (5 ml each) in sequential order starting from highest dilution with varying number (1 to 3) of blanks at the beginning of the series. Code all the samples and instruct the panelist to avoid stimulus bias due to aroma and allow sufficient time lapse (10-20 sec) between samples. Additionally use a neutral food like puffed rice to avoid build up of pungency. Testing can be stopped soon after pungency is felt as the next sample could be highly pungent. Use the score card given in Table 5. Use these sessions to: (*a*) educate the panel about the sensation perceived; (*b*) group the panelists as of higher or lower sensitivity by their threshold values based on the group mean; (*c*) fix the approximate threshold of the sample corresponding to the selected panel.

2. *Final evaluations in arithmetic series:* Prepare the arithmetic series of dilutions around the approximate threshold identified in preliminary testing maintaining 'one' 'jnd' width difference between solutions which is normally found to be one tenth of the threshold Scoville value. Use a minimum of 5 panelists of homogeneous sensitivity in 3-4 repeat tests to get 15-20 judgements.

3. *Treatment of data:* Decode the test series in terms of SHU for threshold value by each panelist, take the SHU corresponding to 'X'. Intermediate scale values as ?-X, X-1, when given for successive samples in the series, the mean of the SHU corresponding to these two dilutions is taken. As variations in panelists' sensitivity is likely to occur either because of psychological or physiological conditions, delete values which are exceeded by 2σ limits, from the group mean. Express the SHU of sample with the remaining values as the mean $\pm$ one σ.

4. *Panel definition:* Define the panel by the average SHU $\pm \sigma$ for pure capsaicin (natural or synthetic, specified) or an oleoresin of known capsaicin content, tested under conditions described earlier.

TABLE 5. SCORE CARD FOR THRESHOLD TEST FOR PUNGENCY

NAME:...................................   DATE:.......................   TIME:.......................

Instructions:
- Taste the samples in the sequential order given
- Swallow the whole quantity of the test sample, slowly.
- Wait for few seconds to recognize pungency if any (aroma of chilli should not be confused for pungency).
- Estimate the pungency of the samples using the intensity scale given below.
- Take some puffed rice and wait for 10-20 sec before testing the next sample.

Intensity scale:

None or sugar solution	.. 0	
Different from sugar solution.	.. ?	(pungency not identifiable—Stimulus threshold)
Threshold	.. X	(pungency identifiable—Recognition threshold)
Weak	.. 1	
Medium	.. 2	
Strong	.. 3	

(Intermediate scale values are expressed as, ?-X, X-1, 1-2 etc.)

Series I		Series II	
Code No.	Intensity	Code No.	Intensity

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ANTIBACTERIAL ACTIVITY OF VOLATILE COMPONENTS OF ONION
(*ALLIUM CEPA*)

The antibacterial effect of onion volatiles and that of lachrymators isolated from onion volatiles was tested. The microorganisms used for these investigations included *Proteus vulgaris*, *Salmonella typhimurium* and *Bacillus cereus* BIS-59. It was observed that 200 ppm of either onion volatiles or isolated lachrymators were effective in inhibiting the growth of microorganisms.

Besides the use of crushed onion as flavouring agent, its anti-microbial properties have long been recognised[1]. Vapours escaping from freshly cut onion have been reported to have bactericidal properties.[2] The pungent principles of onion have been presumed to have both antifungal and antibacterial properties[3,4]. Virtanen and Matikkala[5] identified the active components of onion juice as thiosulphinates and studied their antibacterial effect on various microorganisms. However, precise information on the compounds which exert antimicrobial influence is scanty in the literature. Earlier work from this laboratory had reported the isolation of lachrymatory factors characterised as thioalkanal S-oxide from onion juice[6]. It was of interest to examine whether these sulphur containing compounds also exert inhibitory influence on bacterial growth. The present report describes observations on the antibacterial activity of these compounds.

The microorganisms used for these investigations include *Proteus vulgaris* an active food spoiler, *Salmonella typhimurium* a food pathogen and *Bacillus cereus* BIS-59 which was isolated in this laboratory from blanched, irradiated shrimps[7]. TGY medium containing Bacto-Tryptone 3g, Bacto-Dextrose 1 g and Bacto-Yeast Extract 1 g, in 1000 ml tap water, was used for culturing each of the above microorganisms. Onion volatiles (OV) were isolated from onion juice according to methods described earlier[6]. The two major lachrymatory factors (LF I & LF II) having Rf values of 0.11 and 0.33, respectively were isolated from OV by preparative TLC using a glass plate with a thick layer (0.5 mm) of Silica Gel G (E Merck) and petroleum ether: diethyl ether (60:40 v/v) as developing solvent[6]. Quantitative estimation of lachrymators was carried out by monitoring the pink colour resulting on incubation of the volatiles with glycine-formaldehyde reagent[8]. In the presumptive tests, concentrations ranging from 50 to 200 ppm of the isolates were tested by zones of inhibition of growth on TGY agar after 24 hr. For confirmatory tests, the cultures of each organism, taken in the logarithmic phase, were inoculated at a concentration of approximately 10^6 cells/ml in TGY broth containing from 0 to 300 ppm onion volatiles, LF I and LF II, and cell growth was followed up to 7 days at 37°C.

The presumptive tests revealed that the OV fraction as well as the LF fractions were inhibitory. The zones of inhibition varied for each of the organisms, being maximum for *B. cereus* BIS-59 and minimum for *P. vulgaris*. The results of confirmatory tests showed that at 100 ppm concentration of the compounds there was only marginal increase in the lag phase. However, at concentrations of 200 ppm and above distinct effects could be observed as shown in Fig. 1. All three fractions, viz. OV, LF I and LF II exhibited varied degrees of inhibition on the microorganisms examined. The potency of the three fractions was of similar order of magnitude. Since OV contained only about 40 per cent LF, the other constituents like carbonyls and sulfur compounds[6] also seemed to posses antibacterial activity. The effects of OV, LF I and LF II on *S. typhimurium* were almost identical; at a concentration of 200 ppm, each fraction showed a distinct bacteriostatic effect, the
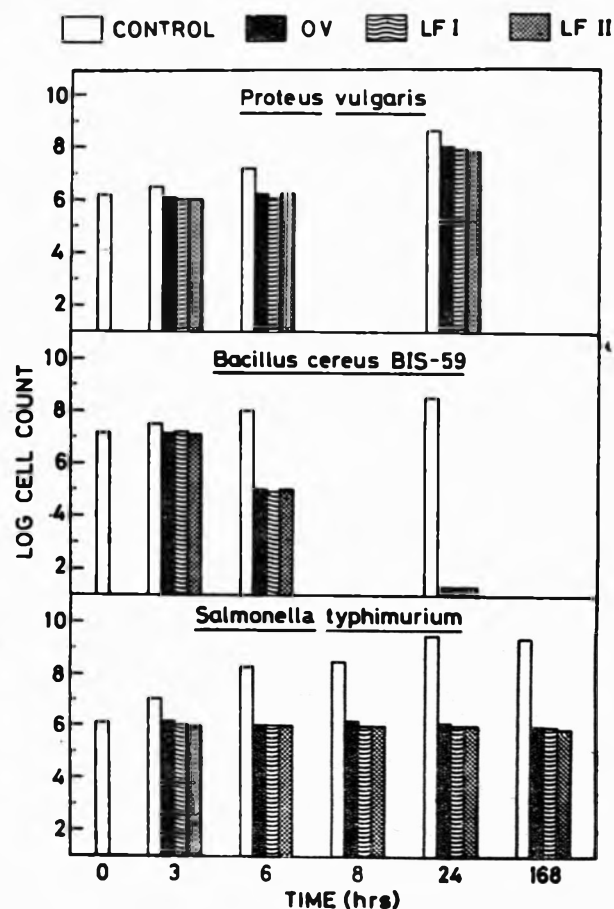

Fig 1. Effect of 200 ppm onion volatiles (OV) and lachrymatory fractions (I & II) on the growth of micro-organisms

growth of this organism being totally inhibited. However, there was no cell lethality in any of the treated samples as was evident from the findings that aliquots of treated cell suspensions proliferated normally when inoculated in fresh TGY medium. When the treated samples were kept on a shaker, bacterial growth was comparable to that of untreated cells, indicating that aeration apparently nullifies the antibacterial effects of OV, LF I and LF II. However, aeration of pure suspensions of OV, LF I and LF II, was found to decompose these compounds and make them ineffective as microbial inhibitiors. In the case of *P. vulgaris* although no bacteriostatic effect was evident using any of the three fractions, each fraction (200 ppm) was able to considerably extend the lag phase of this organism, after which the organism grew well. Even at a higher concentration (300 ppm) of onion extract, there was a significant extension of the lag phase, after which growth was normal. The studies with *B. cereus* BIS-59 indicated that each of the onion fractions was lethal to this organism. Microscopic examination of treated suspensions at regular intervals during incubation, revealed intact cells, indicating that cell inactivation was not due to lysis.

From the above finding it may be concluded that both OV and LF are potent inhibitors of microbial growth. The profound bacteriostatic action of OV, LF I and LF II on *S. typhimurium* offers scope for preventive measures against this pathogen.

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MINOR SEED OILS. XII. PHYSICO-CHEMICAL CHARACTERISTICS AND COMPONFNT ACIDS OF FIVE VEGETABLE OILS

In the search for new industrial oils and others containing acids of novel structure, five seed oils from *Rumex acetosa*, (N. O. Polygonaceae), *Celosia argentea* (N. O. Amaranthaceae), *Atriplex hortensis* (N. O. Chenopodiaceae), *Citrullus vulgaris* (N. O. Cucurbitaceae) and *Dodouaea viscosa* (N. O. Saphindaceae) have been examined by RPC for their component fatty acids. The seed oil content ranged from 10 to 27%. The oils had iodine values from 93.5 to 128.8, and all were rich in linoleic acid content (45.4-64.7%), except for *Rumex acetosa*, which contained about 35% each of oleic and linoleic acids.

Rumex acetosa, Linn. (N. O. Polygonaceae) (Canarese: *Palak*) is an erect, perennial herb found in the Western Himalayas. The plant is strongly antiscorbutic and is used in bronchial diseases. It is also reported to be used as a blood purifier and in homeopathy for the treatment of skin diseases and convulsions. Leaves, which are used as refrigerant and diuretic contain flavone glycosides and are eaten as salad or cooked like spinach.[1]

Celosia argentea, Linn. (N. O. Amarantaceae) (Canarese: *Annesoppu*) is an erect, glabrous annual and is a common weed in cultivated fields, hedges, river banks and open places throughout India and Ceylon. The seeds are considered efficaceous in diarrhoea and useful in blood diseases, mouth sores and diseases of the eye.[2]

Atriplex hortensis, Linn. (N. O. Chenopodiaceae) (Canarese: *Chakkota soppu*): The *Atriplex* genus of herbs and shrubs are native to temperate and subtropical regions, many being desert weeds. *A. hortensis*, an annual plant with succulent branches and leaves, is useful as a substitute for spinach and its crimson form makes it a handsome ornamental annual.[3] The flour of the seeds is reported to be valuable against vitamin A deficiency.[4]

Citrullus vulgaris, Schrad (N. O. Cucurbitaceae) (Canarese: *Kallangadi-Hannu;* English : water melon) is a trailing or climbing hispid annual with large ovoid fruits, dark green or mottled green in colour. The seeds of the watermelon are used as food being sometimes ground and baked into bread. They are also parched and eaten with other grains. The seeds from different localities contain varying percentages (20-40) of oil.[5]

Dodonaea viscosa, Linn. (N. O. Sapindaceae) (Canarese: *Bandarike*) is a genus of shrubs, rarely trees, mostly Australian, of which *D. viscosa* is widely distributed in India. It has been used as a sand binder and also for reclaiming marshy lands. The leaves are used in swellings and burns, and as a febrifuge and sudorific in gout and rheumatism. The bark is employed in astrin-

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS AND EXTRACTION DATA

Seed species	Oil content %	Unsap. matter %	Iodine value* Obs.	Iodine value* Calc.	Sap. eqvt.* Obs.	Sap. eqvt.* Calc.	Refr index of oil at 30°C	Protein in seeds %
Rumex acetosa	12.0	0.8	93.5	92.7	274.8	275.6	1.4680	13.3
Celosia argentea	10.0	1.3	103.3	102.0	275.8	275.7	1.4701	15.0
Atriplex hortensis	13.2	1.1	126.2	124.6	278.2	279.1	1.4651	19.9
Citrullus vulgaris	27.1	1.1	126.6	127.7	278.3	277.8	1.4682	50.5
Dodonaea viscosa	15.7	0.9	128.8	126.7	280.1	279.6	1.4681	23.5

*Values measured on mixed acids free from unsaponifiable matter and calculated from the composition determined in this investigation.

TABLE 2. CHROMATOGRAPHY RESULTS (MOLE %) ON PARAFFIN COLUMNS

Seed oil	Treatment of mixed acids*	Load mg	Recovery %	Eluting solvent (% aq. acetone) 62	67	73	78	83
Rumex acetosa	H.A.	17.8	99.1	0.5	25.9	72.3	0.4	0.9
	M.A.	21.6	86.5	34.6	60.2	3.3	0.8	1.1
	O.A.	85.2	—	2.1	24.6	3.2	0.4	0.3
Celosia argentea	H.A.	22.5	88.4	0.6	24.9	71.9	1.9	0.7
	M.A.	19.5	99.3	45.2	48.5	4.7	1.2	0.4
	O.A.	80.5	—	1.0	23.8	3.8	1.1	0.6
Atriplex hortensis	H.A.	13.1	100.0	0.4	10.7	85.7	1.8	1.4
	M.A.	14.2	99.9	56.9	37.0	3.3	1.8	1.0
	O.A.	82.5	—	1.2	13.6	1.5	0.5	0.4
Citrullus vulgaris	H.A.	19.9	98.4	1.0	13.5	84.0	0.6	0.9
	M.A.	16.2	100.0	65.1	25.3	8.4	0.5	0.7
	O.A.	100.2	—	0.6	15.1	6.4	0.3	0.1
Dodonaea viscosa	H.A.	18.7	97.8	0.5	10.4	86.6	1.8	0.7
	M.A.	18.5	93.8	59.7	32.0	4.9	2.3	1.1
	O.A.	91.7	—	1.6	12.6	2.4	1.4	0.5

*H.A.—Hydrogenated acids;   M.A.—Mixed acids without any treatment;   O.A.—Oxidised acids

TABLE 3. COMPONENT FATTY ACIDS (wt. %)

Seed oil	Acids 14:0*	16:0	18:0	20:0	22:0	18:1	18:2
Rumex acetosa	0.4	24.1	3.4	0.9	1.4	35.1	34.7
Celosia argentea	0.5	23.2	4.8	1.4	0.5	24.2	45.4
Atriplex hortensis	1.0	9.8	3.4	2.0	1.2	26.6	56.0
Citrullus vulgaris	0.8	12.5	8.6	0.6	0.8	12.0	64.7
Dodonaea viscosa	0.5	9.5	4.9	2.6	1.3	21.8	59.4

*These figures indicate the number of carbon atoms and the double bonds in the acids.

gent baths and fomentations. The seeds are said to be edible and the fruits were once used as a substitute for "truehops" in making yeast and beer.[6]

The seeds were collected around Dharwar and the oil soxhlet extracted from the crushed seeds with light petroleum. The fatty oil, unsaponifiable matter and the mixed acids were obtained as described earlier.[7]

Analytical direct TLC of the mixed acids and the mixed methyl esters on silica gel G plates with pertoleum ether: ether:formic acid (70:30:1) (by vol) and petroleum ether: ether: (70:30) (by vol) as developing solvents, revealed the presence only of the usual acids in all the oil samples. The mixed fatty acids as such, after hydrogenation, and after oxidation[8], were each examined by reversed phase partition column chromatography as described earlier[9]. The presence of oleic and linoleic acids were confirmed by preparing their corresponding hydroxy and bromo derivatives[10] from the acids isolated from the appropriate chromatographic fractions.

The seeds examined in this investigation are fairly rich in oil (Table 1). Four contain about 10-15 per cent oil, and the last, *Citrullus vulgaris* seeds, 27.1 per cent. The seed protein content also follows the same pattern, *Citrullus vulgaris* having a higher content (50.5 per cent) than the other four (13.3–23.5 per cent).

The seed oils have high iodine values and are rich in unsaturated fatty acids (Tables 2 and 3). Linoleic (45.4–64.7 per cent) is the major component acid in all the seed oils, except in *Rumex acetosa* which contains almost equal amounts of oleic and linoleic (35.1 and 34.7 per cent) acids. Palmitic acid is the major saturated constituent (9.5–24.1 per cent) with stearic acid next (3.4–8.6 per cent). Small percentages of myristic (0.4–1.0), arachidic (0.6–2.6) and behenic (0.5–1.4) were also found. These results are in conformity with the literature values for the respective seed oils.[11]

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STUDIES ON CHEMICAL CONSTITUENTS AND THEIR INFLUENCE ON COOKABILITY IN PIGEON PEA

Estimation of eight chemical constituents in 22 cultivars of pigeon pea revealed significant differences among cultivars for most of the constituents studied. None of these constituents was found associated with cooking period, except phytic acid content which showed positive association of appreciable magnitude. The varieties requiring minimum time for cooking were S-7, EB. 38-70, JA-3, NP (WR) 15 and UPAS-120.

Among the pulses, Pigeon pea (*Cajanus cajan* L) is consumed in appreciable quantity in the cooked form by the Indian population. Since cooking improves nutritive value of pulses, it would be interesting and useful to investigate the influence of chemical constituents on cooking time in pigeon pea. Cookability has been reported to be dependent on the composition of seed coat and cell wall in case of peas and beans[1]. Recent studies on whole and dehulled pigeon pea, however, suggested that the composition of endosperm may also have a considerable influence on the cookability. This paper deals with the relation between the contents of various chemical constituents and cooking time in pigeon pea varieties.

Twenty two pigeon pea varieties were grown during 1973-74 at the experimental farm of the J. N. Agriculture University, Jabalpur. Representative seed samples were drawn from each of these and the following parameters were determined as follows.

Weight of seed coat: Seed material (100 g) of each variety was weighed, and seed coat of each grain was removed with the help of pin and scalepel and weighed.

Starch content: This was estimated by the procedure of Hassid and Abraham[2].

Total sugars: Estimated by using Hane's ferricynide method according to Browne and Zerban[3].

Determination of cooking period: The cooking period was determined according to Singh *et al*[4] with the modification that the seeds were boiled in distilled water, until they were softend to uniform mass, when pressed between the thumb and fore finger.

Protein content: This was determined by standard microkjeldhal method using 6.25 for conversion from nitrogen to protein.

Digestibility of carbohydrate: The sample (2g) was suspended in 49 ml of buffer pH 4.6 and 1 ml of takadiastase (10 mg/ ml) was added. The incubation was carried for 1 hr at 37°C. The aliquot (5 ml) was taken at the end of the incubation period and reducing sugars liberated was determined as described earlier.

Phytic acid: This was estimated by modified method of Holt as described by Rosenbaum *et al*[5].

Mineral content: The extract was prepared by the method described by David[6,7].

Phosphorous was determined by the method described by Jackson.[8]

Mg and Ca determinations were carried out using the atomic absorption sepectrophotometer at the wave length of 2852. 1R and 4226.7R respectively.

Although variations (Table 1) were observed between varieties for the various parameters studied such as weight of seedcoat, starch, reducing and non-reducing sugars, protein, calcium, phosphorous, magnesium contents and digestibility of carbohydrate, no correlation was observed between the content of these constituents and cooking time with the exception of phytic acid for which positive correlation of appreciable magnitude (r=0.306) was recorded. Similar associations were reported by

TABLE 1. ANALYSIS OF QUALITY COMPONENTS IN PIGEON PEA

Variety	Seed coat wt* (g)	Starch %	Sugar Reducing (%)	Sugar Non-reducing (%)	Cooking time (min) Dehulled seed	Cooking time (min) Whole seed	Sugar released (mg glu/ 100 g material)	Protein (%) Whole seed	Protein (%) Dhal	Minerals (mg) in 100 g seed P	Ca	Mg	Phytic acid %
T.7	14.2	50.1	0.5	7.8	44.0	54	54.0	20.4	20.3	290	180	158	4.7
UPAS 120	15.3	44.6	1.2	9.2	21.0	45	59.7	21.9	21.2	250	185	169	5.5
J.A.1	15.1	50.2	1.3	8.3	38.0	56	74.1	19.4	19.3	325	276	172	5.5
AS. 44	13.9	40.2	1.2	9.1	23.5	67	90.1	19.6	19.2	295	185	16	7.0
Khargone-2	15.7	45.3	1.2	8.9	32.5	60	57.4	21.3	20.8	230	176	159	6.9
JA. 3	14.3	50.3	1.2	8.5	21.0	45	62.9	20.7	20.1	265	180	168	6.1
No. 1258	15.3	39.0	1.1	9.4	32.5	53	60.4	21.7	19.6	270	185	166	3.2
Gadarwara white	12.6	50.2	0.9	8.3	29.5	60	54.6	20.4	19.0	315	195	162	3.4
R. 3	12.2	43.4	1.2	9.3	31.0	55	60.9	21.4	20.0	350	195	168	4.3
NPWR. 15	14.2	50.8	1.2	9.1	21.5	60	88.1	20.8	18.4	235	185	166	2.8
M-9-19	15.2	55.9	1.1	8.7	26.5	50	76.2	20.3	20.8	235	181	161	4.3
Gwalior-3	14.0	51.9	0.9	8.1	30.0	60	67.1	19.5	19.5	300	185	159	5.5
Baigani	12.8	44.9	1.2	8.1	33.5	53	81.4	20.4	20.4	280	195	162	5.5
EC.2815	12.6	45.3	1.2	8.2	24.5	65	74.3	19.5	19.9	325	180	147	6.1
S.29	14.6	49.1	1.0	8.4	29.0	50	68.8	20.2	19.2	275	181	171	5.1
Prabhat	17.8	51.5	1.1	9.0	43.5	55	70.3	18.7	19.4	240	179	163	3.4
PM-1	13.4	47.6	1.1	8.4	28.0	53	72.5	19.4	19.4	300	176	177	2.8
No. 148	12.9	47.0	1.1	8.6	33.0	53	69.5	22.5	22.3	275	185	152	5.5
EB 38-70	14.8	47.7	1.0	8.3	20.5	50	81.6	20.8	21.1	315	179	159	4.3
S-7	17.2	48.0	0.9	8.6	20.0	45	63.4	20.1	20.3	325	180	162	3.4
B-51-7	13.7	48.8	1.1	8.0	24.0	46	71.9	22.3	22.1	350	185	161	4.3
BS-1	17.1	48.8	1.1	7.8	26.0	45	71.9	19.7	19.2	275	180	160	3.4
Mean	14.4	47.7	1.1	8.5	28.8	53.7	69.6	20.5	20.0	288.1	183.0	163.0	4.7

*From 100 g of seed

Rosenbaum et al[5], Rosenbaum and Baker[9] and Crean and Haisman[10] in peas. Hence varieties containing low phytic acid are likely to take less time for cooking and may have consumer preference over others. However further investigations are necessary before any definite conclusion is drawn on the relationship between phytic acid content and cooking time in pigeon pea.

Authors are thankful to Dr. D. P. Motiramani, Director Research Services, J.N.K.V.V., and Dr. S. P. Singh, Head of the Department of Plant Breeding for providing facilities to carry out the work.

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ADDENDUM

Colorimetric Estimation of Sucrose in Ice Cream by P.C. Pantulu and Others, this Journal, 1976, 13 (3), 157

Read under Fig. 2:   Colour development at 70°C for 40 min.

BOOK REVIEWS

Commercial Processing of Poultry, Food Technology Review No. 31: By G. H. Weiss, Noyes Data Corporation, Park Ridge, New Jersey, USA, 1976; pp. 253; Price: $ 50.00.

This book is one among a series of Food Technology Reviews on commercial processing of poultry published by Noyes Data Corporation. This book summarises the technological progress made in the commercial handling and processing of poultry based on U.S. patents.

Apart from the introduction, company index, inventor index and U.S. patent number index which help in providing easy access to the information contained in the book, the book is divided into 8 chapters under the headings: (1) Preservation; (2) chilling and freezing; (3) enhancing palatability; (4) stuffed products; (5) molded and compressed rolls and loaves; (6) butter-coated products; (7) special cooking procedures and products; and (8) poultry concentrates and flavours.

The first three chapters deal with various means of treatment and handling raw poultry. The first chapter illustrates various processes for increasing shelf life of poultry which includes treatment with antibiotics, chemicals, bacteriostatic agent in packaging material and heat drying of duck carcasses. The second chapter deals with different methods of minimizing dehydration, chilling and freezing techniques. As the palatability depends upon tenderness, flavour and appearance, the third chapter deals with different processes with methods for uniformly distributing the tenderizing or flavouring agents throughout the flesh of the birds, which includes antemortem injection, postmortem injection, addition of flavouring agents, soaking in enzyme solution and pigmentation process.

The next four chapters deal with consumer products such as stuffed poultry, rolls and loaves, butter-coated products and some special products like non-disintegrating chunks, stuffable boneless poultry rolls, sausage, smoked turkey roll, dough-encased sliced poultry load, use of residual deboned meat and fabricated poultry skin for wrapping poultry loaves and rolls. Chapter seven gives some special cooking procedures and products like drying chicken under pressure, cooking fowl in polyphosphate solution, microwave cooking, etc.

The last chapter in the book deals with the preparation of poultry concentrates and synthetic flavouring agents.

The processes are amply illustrated with figures, flowsheets and apparatus diagrams, thus providing more practical commercial process information than is available from any other source.

The printing and the get up of the book are good. The book is written in simple language and will be a valuable asset to scientists, students, food technologist and persons dealing with poultry processing and product development.

B. PANDA

Principles of Dairy Processing: by James N. Warner, Wiley Eastern Limited, AB 8 Safdarjung Enclave, New Delhi-110 016, pp. 317; Price: Rs. 20.

In his preface to the book the author states: "Milk production and milk processing were both discussed in my earlier book, Dairying in India. That text was used by students, milk producers, and dairy managers throughout India. Since then two major developments have taken place which indicate there is need for a new book dealing alone with dairy processing. A great number of new dairy-processing plants have come into existence in the past twenty years. Accompanying this important change has been a profound development of education and research facilities in dairy processing as distinct from those involving the milking animal and milk production.

The practical sciences of dairy processing are emphasized in the present book. It is intended both as a textbook for students of milk processing, of dairy technology, and as a handbook for managers and operators of dairy-processing plants."

The book admirably fulfills these purposes. Chapter 1 sets the scene with a quick summary of India's dairy processing industry in just two pages. There are four long chapters, 40 to 50 pages each, on dairy physics, engineering, methods, and processing. The two chapters on dairy chemistry and dairy microbiology are of shorter length, and there are besides a number of short chapters, each about 10 pages long, on dairy sanitation, fluid milk and various milk products like concentrated milk, dried milk, cream and butter, ghee, ice-cream, cultured milk, cheese and casein. This is followed by a list of 206 references to both research papers and textbooks, and a good subject index.

The text is a blend of practice with enough theory to support the former, and the balance between the two is admirably maintained. As an example of this practical approach, the chapter on dairy mathematics contains innumerable problems and methods for their calculation.

At its low price no one even remotely connected with the teaching, study or practice of dairying in India should be without this altogether admirable book.

K. T. ACHAYA

Modern Dairy Products: by Lincoln M. Lampert, U.K. Food Trade Press Ltd., London, Third Edition 1975, pp: 437; price: £ 13.50 (© 1975—Chemical Publishing Co., New York; also published by Eurasia 1974, pp 418; price Rs. 25.50p—original $ 17.00)

The book, originally an American one, is a welcome addition to the basic technical literature on Dairy Science and Technology. The objectives of the author, a former supervisor, Dairy Laboratory, California Department of Agriculture, are to present reliable information in a non-technical manner on the composition, nutritive value, manufacture, chemistry and bacteriology of milk and dairy products, especially useful to persons concerned in the various aspects of the industry as, the executive, milk sanitarian, regulatory official, home economist, dietetitian and instructors in the concerned technical institutions. These are achieved to an admirable extent by combining a lot of useful information, packed in a concise and lucid manner in the twenty one chapters of the book.

Each chapter comprises of an introduction of historical and compositional aspect and the description of pertinent details of the subject material.

The first introductory chapter deals with the national characteristics of production of milk in major dairy countries, general composition and milk as food. The following special reference is made to India as a country "where the populace is underfed, there are many cattle, but other than buffalo milk, little milk is available. The Hindu culture deems the cow as a sacred animal, not to be killed. The cows are undernourished and would yield little milk. The feed these animals consume deprives the younger cows of the food they could use to produce milk, which the people would use were it available". This is a stock criticism of many westerners about India who have no first hand information but base their judgement on half-truths. The problem of numbers has to be tackled on a different basis than the westerners' approach, as it involves other social factors. Many of the western critics have yet to realize the tremondous progress made in India in scientific diarying during the last few decades both regarding production and manufacture of milk and dairy products. The various dairy projects sponsored by the state, cooperative and private enterprises and many aided by International agencies, and convening the XIX International Dairy Congress in India for the first time in an Asian country, bear testimony for the changing attitude and placing this country as a leader in dairying in South East Asia. Those advanced dairy countries took much longer time to achieve what this country has achieved the past few decades, which is considered a phenomenal success in the field of dairy science and technology.

The five chapters from the second deal with the compositional aspects of milk, as fat, proteins, lactose, minerals and vitamins. These are admirably covered with a special stress on the nutritional aspect of the constituents which would be useful to the layman. The role of vitamins is lucidly described.

The chapter on microbiology and sanitation covering nearly forty pages is a good example of conciseness with clarity covering very useful technical information in a popular way. Information on antibiotics, pesticides and radio activity is an adequate introduction to a highly technical subject. The chapter on grades and classes of milk pertains chiefly to American standards and would be a guideline to others; similar is the one on flavour defects. The nine chapters from eleven to nineteen deal with processes and products. Both vat pasteurization and H.T.S.T. pasteurization are treated very briefly; more details with flow diagrams and figures of equipment in lieu of others would have been of greater use from the practical point of the technicians using this book.

The inclusion of human milk, goat milk does not go well in the chapter of fluid milk products; a special chapter for these and milk of other mammals could have found place at the beginning of the book and included in the first chapter itself.

The chapters on other milk products, especially on butter and cheese are excellent in their exposition. The one on testing of milk bear the experience of the author who has realized the needs of the analyses and practical aspects of an important subject having economic and legal implications in the industry.

The chapter on imitation milk products is brief and lucid.

The references at the end of each chapter are taken mostly from American literature though similar additional information is available in the journals of other countries. The index at the end of the book is useful, though incomplete as would be pointed out in the following comments.

As a reviewer, I will be failing in my duty if I do not point out the surprisingly large number of errors which have crept in many of them are probably due to improper proof-reading or hasty printing, unusual to an American printing. The errors quoted below are almost

exhaustive and their mention is made only with the view that these would be rectified in the next edition.

p. 24, Yadow should be Yadav; p. 38, ration should be ratio; p. 48, Ref. 11, Shahari, K. M. should be Shahani K. M.; p. 92, para 3, daily should be dairy; p. 126, rise should be rinse; p. 143, (Table)—is air should be in air; p. 146, one 'be' should be removed; p. 148, Ref 18 & 19 Murphy G. K., should be Murthy G. K., ; p. 166, world should be work; p. 167, value should be valve; p. 230, baceria should be bacteria; p. 234, pasturized should be pasteurized; p. 235, persons should be person; p. 242, stablizer should be sterlizer; p. 247, ref. 3, Craham should be Graham, Patton G. should be Patton S. (in all references); p. 250, 1, 23 & 24 repetition; p. 276, mg, mgs used indiscriminately for plural; p. 293, Index 2 is missing; p. 327, Table 19:2 Vit. c: 84 should be 94; p. 331, Table, figures are uplifted; p. 368, 19:8 should be 19:18; p. 371, Ref. 1 Tuckey, S. should be Tuckey S. L. and Words, G. T. should be Woods G. T.; p. 371, Ref. 9 49-89 should be 49:89; p. 391, deterents should be detergents; p. 398, 20:7 should be 20:10; p. 407, Harland-Ashford should be Harland-Ashworth; p. 428, Index, Breed Couont should be Breed Count; Index is incomplete, imitation milk under I is omitted; Coffee Whitener is omitted.

In conclusion the author is to be commended for his sincere efforts in reprint this book which will be valuable as a handy manual for students and teachers of the undergraduate classes as well as those connected with the industry especially in the production and processing of milk and dairy products. Though there is some confusion in pricing (UK £ 13.50, USA $ 17.6), Rs. 25.50 is most reasonable considering the versatality of the information made available and is within the reach of students and dairy personnel.

M. Bhimasena Rao

Processed Meat Technology: by Endel Karmas, Noyes Data Corporation, Park Ridge, New Jersey, USA, 1976, pp.: 368.

This book is one of a continuing series of reviews in Food Technology and is a compilation of information culled from patent literature covering the period 1960-76.

Thr foreword gives the reasons that prompted the publishers to rely on patent literature rather than on periodicals. The often confusing legalistic phraseology of patents has been avoided in order to present the different processes in easily understood language.

The subject matter is broadly classified into two parts. Part I deals with processing ingredients and methods and includes curing methods and ingredients, increasing water binding and yield, improved curing formulations, integral meats, smoking, thermal processing and miscellaneous methods. A few patents are also given dealing with dietetic curing compositions. Part II deals with processed meat products and includes bacon, ham, beef, patty type products, dehydrated snacks and other novel products. In most cases, before the actual patent is reproduced a short introductory para is given listing the aims and objectives of the method/process. This should help to understand the latter better. At the end company, inventor and patent numbers are again listed for convenience.

The book is written with the avowed object of giving reliable information about the direction in which work (mainly) in the USA is proceeding. This should obviate duplication of work by other researchers apart from avoiding legal complications later due to infringement of patents.

As can be expected, the book is written from the patent angle only and no inference can be drawn as to the safety of the ingredients/methods from the FDA point of view.

The book is expected to be of particular interest to workers, in this country where meat technology is, comparatively, still in its infancy.

The printing and the get up of the book are excellent although some readers may find the type size too small. All in all, the book should prove to be a good addition to any technical library.

T. R. Sharma

The Quality and Detection of Frauds in Citrus Juices: by International Union of Food Science and Technology; available from Dr. Royo Iranzo, "Intstituto de Agro-quimicay Technologia de Alimentos", Jaime Roig Street, 11, Valencia-10 (Spain). pp. 167, price: US £ 12.8.

This book includes papers presented at the International Conference on the "Detection of Frauds in Citrus Juices" held at Valencia under the aegis of International Union of Food Science and Technology. There are two general articles and seven research papers.

The first general paper by Veldhuis on "Quality Parameters in Citrus Juices: Quality Control and Grade Inspection of Citrus Juices in the United States" gives a bird's-eye-view of the production of citrus fruits in the most important citrus producing countries of the world,

USDA grade standards for various citrus juices and concentrates and stresses the need for quantitative analyses of limonia, naringin, furfural, etc. and chemical procedures for flavour evaluation as parameters of citrus juice quality.

The second general paper by Royo Iranzo on "Methods for the detection of frauds in citrus juice" indicates the need for a uniform criteria in citrus juices to check adulteration. With this in view, the necessity of reference tables of standard values for the most characteristic components of citrus juices is indicated. Towards this end, results obtained by various authors are compiled. Some observations have been made on the present status of adulteration in citrus juices and the possibility of their detection with a view to overcome the same.

The first research paper by Attaway, Carter, Fellers' Moore and Ting on "Acidity and Brix to acid ratio, key determinants of orange juice quality" is an evaluation of commercial samples of frozen orange juice concentrates from Florida during 1972–73. The reconstituted samples had an acidity lower than 0.98% and a Brix/acid ratio of more than 13. Experimental samples of Valencia orange juice with Brix/acid ratio of 13.3 to 15.5 were given the highest scores by flavour panel.

The second research paper by Berry, Nagy and Tatum on "Methods for determining bitterness, thermal history and flavour quality of citrus products" outlines rapid TLC method for the determination of naringin and limonin. A method sensitive to 5 pp? of furfural provides basis for an index of thermal history of citrus products. Thin layer chromatography combined with GLC have been indicated for determining compounds responsible for off-flavour in stored citrus juices.

The third research paper by Caro and Munoz-Delgado relates to the effects of cold storage on the main varieties of Spanish oranges and mandarins on the juice quality. Salustiana, Valencia Late and Verna varieties were superior to others with regard to storage quality.

The fourth research paper by Moreno, Caroana Prestamo is on "The specific electrical conductivity as a possible quality parameter and fraude detector in citrus juices". The specific electrical conductivity in citrus juices and citrus juice-based beverages is correlated with the usual parameters such as soluble solids, acidity, maturity index of citrus fruits and vitamin C. The possible application of electrical conductivity as a quality parameter and as an aid in the detection of adulteration in citrus products is explored.

The fifth research paper by Matsumoto and Obara on "The changes on amino nitrogen content of fruit juice powders during manufacture using different drying techniques" indicates variations in the amino nitrogen content as determined by amino acid auto analyzer. Amino nitrogen content of fruit juice powders has been found unsuitable as a parameter in the detection of their adulteration.

The sixth research paper by Royo Iranzo and Pauletti on "Detection of adulteration in citrus juice XX Chemical composition of Tangerine juice (*Citrus reticulata*, Blanco)" applies the usual parameters in the detection of adulteration in juices from citrus species Tangerine, Clementine and Satsuma.

The last research paper is by Romojaro, Lopez-Andreu, Leon and Llorente. From a comparison of several commercial samples of lemon juice, from different parts of the world, the usual parameters, viz. soluble solids, density, acidity, vitamin C and sugars have been found unsuitable as acids in the determination of purity of lemon juice. Chromatographic investigation of amino acids present and the formol value are better characteristics.

One general paper and four research papers are in English and the rest are in Spanish. The get up of the book is good. It is a good reference book for those involved in the analysis and quality control work on citrus juices and their beverages.

S. RANGANNA

International Development Research Centre Launches Two Projects to Help Grain Production and Milling in Africa

1. Rwanda

The need for research into improving present food crop production in Rwanda is made imperative by the limited amount of new arable land available to small farmers. Population density in areas presently under cultivation, and an extremely hilly countryside necessitate using land at higher elevations for food production.

A grant of $ 197,000 will permit the Institute of Agronomic Sciences of Rwanda (ISAR), the research organisation of the Ministry of Agriculture and Animal Husbandry, to develop a three-year selection, breeding and collection program for cereal grains and oilseeds that will perform well in high altitude areas.

Improved varieties of sorghum developed in the Lebanon are already being tested in Rwanda, and ISAR scientists will also be linked to the worldwide sorghum network through the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Hyderabad, India, and to the collection and breeding program in Ethiopia. Similarly, in their work on triticale—the wheat-rye hybrid on which research has mainly taken place in Mexico and Manitoba—the Rwandan team will keep in touch with breeding programs in Kenya and Ethiopia.

In oilseed research they intend to test sunflower varieties from the Soviet Union and Kenya for yield, disease resistance and wide adaptability in Rwanda's particular conditions and to test rapeseed developed by Canadian and other scientists on a number of small farms in different areas of Rwanda.

2. Botswana

The people of Botswana, a semi-arid country with a population of about 700,000 in southern Africa, consume about 80,000 tons of cereal grains a year. The grain is mostly sorghum and maize. Although the climatic conditions of Botswana are better suited to the production of sorghum than maize, there has been a shift by consumers in favour of maize, nearly all of which has to be imported from South Africa.

A major reason for this shift has been that industrially produced maize meal has become increasingly available, while the traditional method of milling sorghum is lengthy and involves a soaking process. The consequent high moisture content of the sorghum meal means that the flour ferments or goes rancid in a short time—and the sorghum meal therefore has to be consumed quickly.

The Government of Botswana is trying to increase sorghum production, in order to attain self-sufficiency in grains. To encourage this, it wants to establish flour mills at the village level that can make available a sorghum meal that can last a reasonable length of time and can compare in price with the imported maize meal. Government officials had heard with interest of the successful experiments at the pilot flour mill set up at Maiduguri in northeast Nigeria with IDRC support. In particular, they were interested to learn that Nigerian technicians were now using a dry process to remove the preicarp (or husk) of the sorgum grain before grinding.

The IDRC grant of £ 125,000 will enable the Botswana Agricultural Marketing Board to set up a pilot sorghum mill at the village of Pitsane, in a grain producing area 50 km from Lobatse. A mechanical decorticator, successfully tested in Nigeria, will be the basis of the dry milling system. Consumer acceptance trials on the sorghum products will be carried out in the area, while samples will be sent regularly for analysis to other projects in the network.

The International Exhibition for the Food and Allied Industries

Significant trends and developments in Europe's food industries provide a four-day programme crowded with stimulating topics for discussion at the Food Industries Conference in London next year (November 15-18, 1977). The event is to be organised in conjunction with the 10th International Exhibition for the Food and Allied Industries (FIE-Foodpack), which takes place at the Olympia exhibition centre.

An all-day session on technical advances in food processing and preservation foreshadows dramatic changes in Europe's diet, as well as its shopping habits. Delegates, will hear of research now going on in Britain to extract proteins from grass for human consumption; and of new uses for milk proteins in food products ranging from sausage meat to cakes and biscuits. Other speakers will discuss the microbiological aspects of food preservation; enzyme technology; sterilization and irradiation; and new ways of extending the shelf-life of dairy products.

A full day is devoted to food packaging and will pinpoint new developments in glass packing and new aseptic techniques for cans and plastics. Containers of the future and cans versus glass are also subjects to be discussed. Further papers will deal with toxicity and the compatibility of foods with containers; testing chemicals used in additives and containers; and legislation for food additives and colourings.

The fourth and final day examines food product coding and the operation and progress of a unified system for Europe.

In the meantime, unified product coding for Europe remains a controversial topic among UK food manufacturers and retailers, as well as companies concerned with the production of packs and labels. Conference speakers from a cross-section of international industry are being chosen to represent various viewpoints on this subject.

At the FIE-Foodpack exhibition visitors will be able to examine product coding systems and all associated equipment for labelling, weighing, scaning and data processing. Further information on the exhibition and conference can be obtained from the organisers, BPS Exhibitions Ltd., 4 Seaford Court, 220-222 Great Portland Street, London WIN 5HH. (Tel: 01-388 2117; Telex 21237).

Science Publishing in India: Problems and Prospects— PID Silver Jubilee Seminar

The Publications and Information Directorate (PIS), New Delhi, organised a two-day seminar on Science Publishing in India: Problems & Prospects, on 18 and 19 November 1976. The seminar, organised as a part of PID's Silver Jubilee Celebration, was attended by about 250 delegates—Science editors, writers, publishers, university professors, scientists, information specialists and others—representing different organizations from all over the country.

Inaugurating the seminar, Prof. B.P.Pal. President, Indian National Science Academy, expressed his satisfaction at the organization of the first national seminar of the kind on "a problem which has been rather neglected for a long time". He said, "Any decision that we take now should be calculated to advance the cause of Indian science and should be completely sustainable by total indigenous efforts at least in the near future if not immediately". He hoped that this seminar would not just give a state-of-art report but would suggest concrete remedies for the problem that face us.

The seminar was held in three technical sessions on: (i) Role of authors, referees and editors: and standards of publications (chairman: Shri B. N. Sastri, former Chief Editor, PID); (ii) case studies (Shri S. Parthasarathy, scientist-in-charge, INSDOC); and (iii) problems and plans for the future (Shri A. Krishnamurthi, former chief editor, PID). Altogether 58 papers were presented. The sessions were made lively by the many discussions following the presentation of papers.

The technical sessions were interpersed with guest lectures. Dr. Jagjit Singh, the Kalinga Prize winner, spoke on his experiences of breaking into print as a science writer. Later in the day Dr. Jagjit Singh spoke on 'Limits to Growth' wherein, with his characteristic wit, he explained the tyranny of the exponent in all growth processes, depletion of natural resources, etc. Shri Samuel Israel, Director, National Book Trust of India, gave a lucid account of growth and development of the indigenous science book publishing industry. Shri Ram D. Taneja, Chief Editor & Director (Publications), Indian Standards Institution, spoke on the need for standardization in publishing and the efforts made in India.

Recommendations of the Seminar

1. The Seminar recommends that a compendium of expertise in science & technology available in the country should be prepared. The initiative for this should be taken by the major publishing agencies like the Publications & Information Directorate. who can seek the collaboration of the learned academies, professional associations, university departments, and R & D laboratories. The compendium will be useful in selection of referees for Journals, in selection of readers by book publishers, and in selection of experts to scrutinize research proposals by different funding agencies. The compendium should list the experts with their areas of specialization as specific as possible and must be updated periodically.

2. The Seminar recognises that the traditional system of refereeing, in spite of its inherent delaying character, is indispensable for the growth of science and technology. However it is felt that there is a need for evolving a rapid, but fool proof system of evaluation through the combined efforts of the major science publishing agencies in the country. It is the consensus of the Seminar that by and large the referees' role in the process of communication of information remains unrecognised. It is, therefore, felt necessary that their role should be recognised in an appropriate manner; one way would be to give a list of active referees, at frequent intervals, in the journal itself at an appropriate place.

3. The Seminar recognises the need for an active role by the funding agencies in controlling the quality of communications emanating from research supported by them. They should take adequate steps to curb the tendency of research workers to communicate substandard papers for publication.

4. The Seminar notes the trend in certain quarters for repetitive and fragmentary reporting of research results. It therefore urges both the generators of information and editors of scientific periodicals to take effective measures to discourage this tendency. One specific way of doing this could be for the editors to

demand a declaration from the authors that the results have not been sent for publication elsewhere.

5. The Seminar recognises the difficulties currently faced in the country in the matter of printing and production of scientific periodicals and books, especially those with high mathematical content. A concerted effort for switching over from the conventional methods to modern methods of printing in order to ensure speed, efficiency and possible reduction in cost, is called for. Science publishing agencies, both under the government and outside of it, should urge the appropriate government authorities to make available the necessary foreign exchange for the procurement of required equipment until such time as the indigenous manufacture of the equipment becomes possible.

6. Standards are to be evolved for typesetting mathematical and scientific texts, the initiative for which is to come from the Indian Standards Institution.

7. A national style manual for scientific and technical editors ad authors is to be prepared. The initiative for this may be taken by the Indian Standards Institution, the Indian Council of Agricultural Research, the Indian Council of Medical Research, the Publications & Information Directorate, the National Book Trust and other agencies.

8. Areas of science & technology where the research output is substantial in India and yet adequate number of journals are not being published in the country are to be identified and the lacunae filled by establishing journals in such areas.

9. The Seminar notes with concern the absence of appropriate training programmes for technical writers and editors in the country. To remedy the situation, it is suggested that universities and institutions of higher learning be urged to impart training in technical writing to doctoral and postgraduate students and even to provide training courses in technical writing and editing at the master's degree level; and organisations like PID should conduct regular training courses and workshops for authors, and technical writers and editors, for which the faculty and the trainees may be drawn from different publishing agencies.

10. Efforts should be made to create conditions which will induce Indian scientists to prefer Indian journals to foreign journals for reporting their results. It is realized that the surest way to achieve success in this direction is to upgrade the quality of Indian journals

11. An encyclopaedia of science with a slant to the Indian context may be prepared by the Publications & Information Directorate, using the abundant talent available in the country.

12. Taking note of the difficulties experienced in the compilation of encyclopaedic publications like *The Wealth of India* with regard to non-standard nomenclatural practices, taxonomic and structural anamolies existing in the source materials, it is felt that steps be taken by various agencies concerned with the publication and dissemination of source materials of this nature to observe standard practices in all respects. Authors of phytochemical investigations may be requested to provide a plant profile.

13. The Seminar suggests that the agencies responsible for bringing out scientific publications give concerted attention to the circulation aspect and take adequate steps for promoting circulation and securing advertisements as a means of reducing the gap between financial inputs and returns.

14. The Seminar takes note of the growing importance of the need for dissemination of scientific information in Indian languages. It is, therefore, recommended that both governmental and non-governmental agencies look into the problems relating to science publishing in Indian languages and take appropriate steps to make both printing facilities and funds available for the purpose.

15. The Seminar is concerned about the considerable delay involved in getting short communications/letters published in Indian scientific and technical periodicals, and urges that concrete steps be taken to strengthen the existing letters periodicals and, if necessary, to establish new ones.

16. In the opinion of the participants, the Seminar proved very useful and such meetings should be held at regular intervals in the future. It is proposed that a society of scientific and technical editors be formed. Shri Y. R. Chadda and Shri R. N. Sharma are authorized to work out the details concerning the formation of the society, coopting others in the task of formulating plans for the purpose. The society, when formed, can, among other things, organize future seminars and bring out a newsletter to keep science editors informed about current developments in all aspects of science publishing.

Third International Symposium on "Nutrition and Work" (Alimentation et Travail)

The theme of the Symposium is "Technological Influences on the Nutritional Value of Foods Intended for Human Consumption"

This will be a meeting place for the specialists from the disciplines of Nutrition, Food and Food Industry, Food Service and Agronomy. The Third International Symposium "Nutrition and Work" should provide a great opportunity to exchange scientific and practical infor-

mation between manufacturers, those responsible for food service, and the research workers of public and private sector.

The following themes will be treated:

—New technologies applied to protein sources.
—Influences of Technological treatments upon nutritional value of proteins.
—Technological processes related to the keeping of nutritional quality.
—New processes of food conservation.

—New foods. Basic products sprung from new technologies.

All correspondence may be address to:

Symposium International
Alimentation et Travail
Dept of Nutrition and Metabolic Diseases
University of Nancy
40, Rue Lionnois
54000 Nancy-France.

Southern Zone, Madras

Lecture Delivered on 22nd Nov 1976 on Frozen Fruits, Vegetables, Juices and Pulps by Shri O. P. Gera, Deputy Director (FRVP) South Zone, Madras.

The frozen fruits and vegetables, said the speaker are gaining much importance in international trade and to substantiate this he quoted extensive trade statistics. Production of frozen products in India is 2439 tonnes in 1974, this comprised mainly of ice candies/fruit beverage candies. Consumption of frozen foods (kg.) per capita in different countries is as follows.

	1961	1970	1975 (assumed)
U.S.A.	21.280	32.82	42.2
Great Britain	2.906	7.46	10.19
Sweden	3.485	15.79	23.09
Norway	2.05	6.8	11.49
West Germany	2.09	10.053	16.4
Denmark	2.34	10.12	16.57
The Netherlands	0.94	6.5	11.0
Italy	0.022	0.605	1.95

But in India the per capita consumption in 1971 is less than 5 g.

At present India, need not depend upon foreign countries for refrigeration machinery. About 100 crore rupees worth of frozen sea-foods and frog legs are exported every year from India. With the expertise and machinery available in the country the speaker questioned as to why it will not be possible for us to transport chilled/frozen apple juice from Kashmir, Himachal Pradesh and U.P. to Delhi, Nagpur, Ahmedabad, Bombay, Hyderabad, Madras and such other places? Pineapple juice and orange juice in the similar way can also be transported and marked in major cities of India. Currently this is being done in case of chilled milk which is transported 400 to 600 kilometers. Talking about the cost, the speaker said that pasteurized chilled juices can be purchased at about Rs. 1.50 p. per litre at growing centres, transported in chilled form @ 0.30–0.50 ps. per litre, stored at a cost of 0.50 to 80 ps. per litre. Finally the pure juice will reach the consumers in 180 ml bottles at about Re. 1 to 1.25 p. On account of high cost of tin containers excise and other factors, now the juice is sold at Rs. 7/- per can of 850 g and Rs. 3.50 per bottle of 280 ml (apple juice), and as such the consumers are reluctant to purchase.

Further the speaker stressed the need for utilising the surplus fruits and vegetables produced in glut seasons for freezing. During glut seasons, he explained that a unit may have 8-9 tonnes of peas, beans, carrots, cauliflower okra, mango, etc., which could be quick frozen for $1\frac{1}{4}$ to $1\frac{1}{2}$ hr at—40°C and this can be achieved by using indigenously prepared 3 to 5—tonne capacity cold air blast tunnel. The material thus frozen can be packed in polybags/cartons and stored at —15 to—18°C or 0°F. Vegetables may require blanching and juices and pulps require prepasteurization (preferable to freeze in 30 to 40 per cent sugar syrup), before quick freezing. Attempts are being made by MAFCO unit of Bombay in collaboration with TPI, London to freeze mangoes in different forms. Frozen mango pulp retains better flavour as compared to canned mango pulp.

The speaker lastly concluded by giving examples of the cost of processing, warehousing and selling of Florida frozen orange concentrate and the cost of processing apple juice in Germany.

Lecture Delivered by Mr. A. Saddanha, Regional Director, Central Warehousing Corporation, Madras on the "Role of Warehousing in India" on 29th November 1976.

The speaker after tracing the development of the warehousing in India since 1928, said that it was established after passing the Warehousing Act in the year 1962. The warehousing scheme was established with the sole object of providing scientific storage facilities for agricultural produce and to facilitate finance on easy terms to the farmers in this country. This storage period which lasts from 3 to 6 months or more will enable the farmers to sell their produce when the prices are higher. Currently there are 166 warehouses with a total capacity of 22.1 lakh tonnes; the capacities of hired godowns and plinth storage are 6,25,738 and 3,21,576 tonnes respectively. The CWC has plan to construct Base Depots at 14 centres with a capacity of 50,000 tonnes each and also plinths to the extent of 1,61,500 tonnes.

Warehousing, the speaker said was not able to make profit until 1965–66, but it recorded a profit of Rs. 1.70 crores in 1975–76.

Although there are 200 agricultural commodities accepted for storage, only foodgrains, fertilizers and pulses continue to be the major commodities stored in the central warehouses with sugar, gur, cement, cotton, jute, tea, rubber, chemicals, drugs, etc., occupying lesser importance. Besides providing the necessary storage facilities the Central Warehousing Corporation (CWC), helps to reduce waste and loss by following scientific storage methods, assists in orderly marketing by introduction of standard rate of specification, training personnel, assisting the government in their scheme of price support and price control.

The speaker further continued that the total annual loss of foodgrains in India is estimated at 15 million tonnes the value of which is Rs. 4,000 crores. The field loss is 28-30 per cent of total production, the loss in the threshing yard, transportation and in the process of storage works out to 10-12 per cent. The loss for other commodities like pulses is valued at Rs. 2.000 crores. The total loss works out to Rs. 6.000 crores.

In Tamilnadu, the speaker observed that efforts are being made to provide additional storage facilities by construction of plinths in some of the existing warehouse campuses and by March 1976 a capacity of nearly 90 lakh tonnes has been provided; schemes are being chalked out to construct an additional godowns of conventional type with a total storage capacity of about 1 lakh tonnes in different places in Tamilnadu. The CWC is also hiring godowns from government departments and private parties. The Food Corporation of India STC, MMTC, National Seeds Corporation, Indian Dairy Corporation and Co-operative Marketing Federation are using the CWC godowns.

The speaker pointed out that the CWC takes all precautions like preventive and prophylactic measures such as spraying, dusting, fumigation, etc., to keep the stocks pest free. It also undertakes pre-shipment fumigation of goods meant for export. CWC also arranges handling and transportation to and from the warehouse for the depositors on nominal charges.

The paid up share capital of the CWC, said the speaker has now stands at Rs. 30/- crores. CWC subscribes to the share capital of all state warehousing corporations to the extent of 50 per cent of the total share capital.

While concluding the speaker said that CWC playing a pioneering role in training personnel and this facility has been extended to other organisations like cooperative societies, state civil supplies corporation, etc. More than 1,700 officers and other personnel have so far got the benefit of this training.

Hyderabad Chapter

Dr. Dhyaneshwar B. Chawan, Ph.D. (USA), Project Leader, Research Center, Foods Division, Borden Inc., Syracuse, New York addressed the members of the Association on 24th Dec. 1976 at the Home Science College, Hyderabad. The topic was 'Retail Product Quality Audit—Its importance in Food Industry'. The speaker described at length with practical examples the development of new foods, and improvement and modification effected to the existing foods in his company.

The lecture was followed lively discussion.

This was co-sponsored by the Hyderabad Agri-Horti cultural Society.

Student Members

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Mr. A. P. Karigar, Fish Processing Technologist, New India Fisheries Limited, Sassoondock, Colaba, Bombay-400 005.

Mr. S. Ganesan, J. B. Boda Marine General Survey Agencies Pvt. Ltd., Analytical Division, 149 Broadway (5th Floor), Madras-600 001.

Dr. U. M. Ingle, Associate Dean & Principal, College of Agricultural Technology, Marathwada Agricultural University, Parbhani-431 401.

Shri J. K. Chavan, Biochemist, College of Agricultural Technology, Marathwada Agricultural University, Parbhani-431 401.

Mr. A. B. Afzalpurkar, Oils Division, Regional Research Laboratory, Hyderabad-500 009.

Association of Food Scientists and Technologists (India)

FOR KIND ATTENTION OF ALL MEMBERS

Dear Member,

If you have not already sent the details regarding your name and area of specialisation for inclusion in the proposed AFST Directory, please immediately fill up in this tear-off proforma and send it by return of post to *Shri M. R. Chandrasekhara, President, AFST, 53, 13th Cross Road, Malleswaram, Bangalore-560 003.*

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (INDIA)

M. R. Chandrasekhara
President

53, 13th Cross Road
Malleswaram, Bangalore-560 003
December 21, 1976.

SECOND CALL

This information is required for the preparation of the AFST Directory. Please fill in the form at the ealiest and mail it immediately to the address noted above. It is urgent that the information should be in our hands, if your name and specialization has to be included in the Directory.

Mark √ for items that apply to you.

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Affiliation to Region or Chapter

Professional Affiliation

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    Research: Government/Industrial

    Administrative: Government/Industrial

    Consultancy:

    Other fields (specify what they are):

P.T.O.

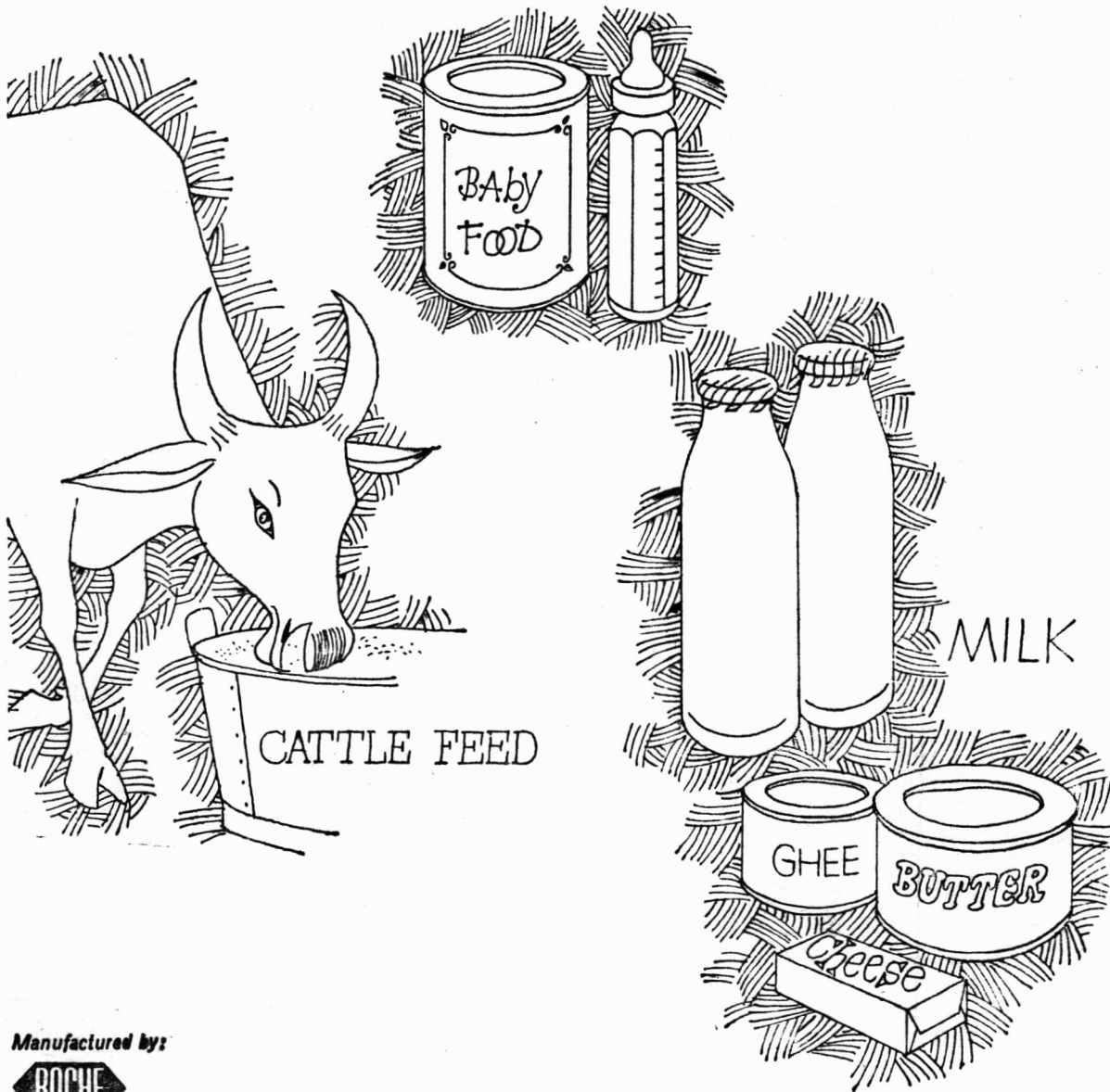Indicate not more than two in each group:

A. Field of Specialization
Agricultural Sciences
Bakery Technology
Biochemistry
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Engineering
Enzymes
Flour Milling
Food Standards
Food Technology
Infestation Control
Marketing
Nutrition
Operational Research
Packaging
Operational Research
Packaging
Plant, Design and Fabrication
Protein Technology
Quality Control
Sensory Evaluation
Transport of Foods

B. Commodity Specialization
Alcoholic Beverages
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1. Manuscripts of papers should be typewritten in double space on one side of the paper only. They should be submitted in **triplicate.** The manuscripts should be complete and in final form, since no alterations or additions are allowed at the proof stage. The paper submitted should not have been published or communicated anywhere.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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