ISSN-0022-1155

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS, INDIA

VOL. 24. NO. 6 NOV./DEC. 1987

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(INDIA)

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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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Research Papers

Research Notes

Nisin as an Aid for Thermal Preservation of Indian Dishes — *Upma* and *Kheer*

LOKENDRA SINGH, M. S. MOHAN AND R. SANKARAN*
Defence Food Research Laboratory, Mysore-570 011, India

Received 18 *November* 1986; *revised* 8 *April* 1987

Nisin, a nontoxic metabolite of *Streptococcus lactis* has much potential as a thermal aid for preservation of food. Studies carried out on two Indian dishes viz., medium acidic *Upma* (pH 5.0-5.2) and non-acidic *Kheer* (pH 6.2-6.4) showed its antimicrobial effect on *Bacillus stearothermophilus*. The acidity of the food decided the amount of nisin required for its preservation. Nisin at levels of 25 and 200 IU per g could sufficiently lower the thermal processing of *Upma* and *Kheer* respectively and enhance the storage life to more than 6 months.

The heat treatment employed in processing of different canned foods is targetted to eliminate all types of micro-organisms which are responsible for spoilage and toxin production. Most of these bacteria are spore forming and heat resistant and require prolonged heat treatment for destruction. To be on the safer side, canners normally apply much greater amount of heat which often results in impairment of texture, sensory and nutritional qualities[1]. These quality attributes of foods could be greatly enhanced by employing a heat sterilization aid which meets the requirements of being nontoxic, heat stable, economic and being active against all types of spores surviving normal heat processing[2].

Nisin is an active antibacterial metabolite of *Streptococcus lactis* and is the natural constituent of cow's milk. It is considered as a good and safe thermal sterilization aid and has been used for various foods in many developed countries[3-6]; however very little work has been done in our country on prepared foods[7-9]. Considering these facts, the study was carried out on two Indian dishes—*Upma* (medium acid) and *Kheer* (non-acid).

Materials and Methods

Upma was made in the conventional way. *Soji* (wheat semolina) was fried and kept aside. Oil was heated and green chillies and ginger cut to small pieces and mustard were fried and vegetables, required quantity of water and salt added and simmered until cooked. The *soji* was then added and kept for 5 min. *Kheer* was made with milk, rice and sugar in the proportion of 14 l milk, 840g rice and 2.25 kg sugar. Four hundred grams of each preparation were transferred to 1 lb jam size cans, stream exhausted and then thermal processed as required for the different experiments.

Bacillus stearothermophilus (ATCC-1518) was used as the test organism. The spore crop was prepared as described by Thangamani[10]. Nisin obtained from M/s. Aplin and Barrett Ltd., Yeovil, Somerset, England was used. Thermal processing of the canned foods was monitored by Digital F_o computer (Ellab, Denmark) and was reported as F_o value.

Inoculation pack studies: Thoroughly washed spores of *B. stearothermophilus* were suspended in sterilized distilled water and added to the foods to get a final spore concentration of 3×10^5 and 10×10^3 per g in *Upma* and *Kheer*, respectively. Nisin, dissolved in distilled water (pH 4.0) was added at 25, 50 and 75 IU/g to *Upma* and 200, 300 and 400 IU/g to *Kheer*. Samples without added nisin served as controls. Packed cans were heat processed at processing values of 2.0–4.0.

Analysis: The cans were observed for physical appearance, vacuum, pH, residual nisin and microbial load, just after the processing and at the end of incubation for 6 days at 55°C. The surviving spores were enumerated on *Bacillus stearothermophilus* medium[10]. Residual nisin was estimated by plate diffusion assay using *Micrococcus flavus* as the test organism[11]. Cans stored at ambient temperature were analysed for residual nisin, microbial load, pH, etc. upto 6 months.

*To whom all queries regarding the paper should be addressed.

TABLE 1. EFFECT OF NISIN ON BACILLUS STEAROTHERMOPHILUS DURING THERMAL PROCESSING OF *UPMA*

Initial nisin concn (IU/g)	*B. stearothermophilus* counts/g.					
	$F_o=2$		$F_o=3$		$F_o=4$	
	A	B	A	B	A	B
Control	1950	2380	1300	1700	1150	1260
25	1100	990	950	925	700	650
50	890	800	780	640	550	490
75	780	670	670	500	260	100

Initial counts of *B. stearothermophilus* was 3×10^5/g,
A—After processing,
B—After incubation at 55°C for 6 days

Results and Discussion

The pH of *Upma* ranged from 5.0 to 5.2 and that of *Kheer* from 6.2 to 6.4. The effect of nisin on *B. stearothermophilus* in *Upma* has been presented in Table 1. *Upma* cans initially contained 3×10^5 spores per g and heat processing reduced the counts to 1950, 1300 and 1150 per g at F_o values of 2.0, 3.0 and 4.0, respectively. Nisin incorporation increased the destruction of spores proportionately and the microbial load varied from 780 to 1100 ($F_o=2$), 670-950 ($F_o=3$) and 260-700 ($F_o=4$) per g. Thermally processed cans, when subjected to accelerated incubation (55°C, 6 days) showed a rise in population from 9.5 to 30.7 per cent in control. Nisin containing cans on the contrary showed a reduction in population at the rate of 2.6-10, 10.1-25.4 and 13.9-61.5 per cent with initial nisin concentrations of 25, 50 and 75 IU/g, respectively.

Because of the nonacidic nature, *kheer* cans were inoculated with lower number of spores (10×10^3/g) and higher concentration of nisin (200-400 IU/g). Observations made in Table 2 show that heating of *kheer* cans (control) caused the destruction of spores, resulting in spore concentrations of 1140 ($F_o=3$) and 880 ($F_o=4$) per g. The nisin supplementation at rate of 200 IU/g resulted in elimination of all the spores excepting few (<10/g) at F_o value of 3.0. The incubation of processed cans induced the multiplication of bacteria and the number reached 3000 ($F_o=3$) and 1770 ($F_o=4$) per g in control cans and to 45 per g in nisin (200 IU/g) containing cans after 6 days at 55°C. The higher nisin concentration (300-400 IU/g) during thermal processing resulted in sterilization of food and no count was observed in the course of study.

Fig. 1 and 2 show the residual level of nisin in the two

TABLE 2. EFFECT OF NISIN ON BACILLUS STEAROTHERMOPHILUS DURING THERMAL PROCESSING OF KHEER

Initial nisin concn. (IU/g)	*B. stearothermophilus* counts/g.			
	$F_o=3.0$		$F_o=4.0$	
	A	B	A	B
Control	1140	3000	880	1770
200	10	45	Nil	Nil
300	Nil	Nil	Nil	Nil
400	Nil	Nil	Nil	Nil

Initial counts of *B. stearothermophilus*—10×10^3,
A—After processing,
B—After incubation at 55°C for 6 days.

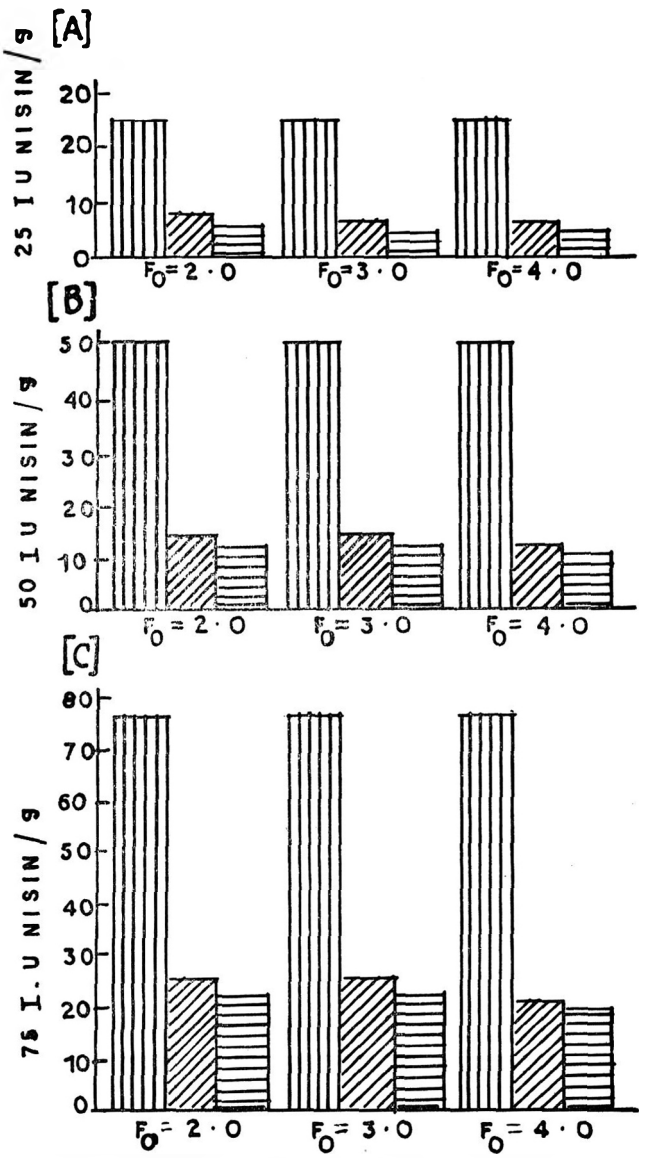

Fig. 1 Changes in nisin level on processing of *upma*

—Inital concn; —After processing; —After 6 days incubation (55°C)

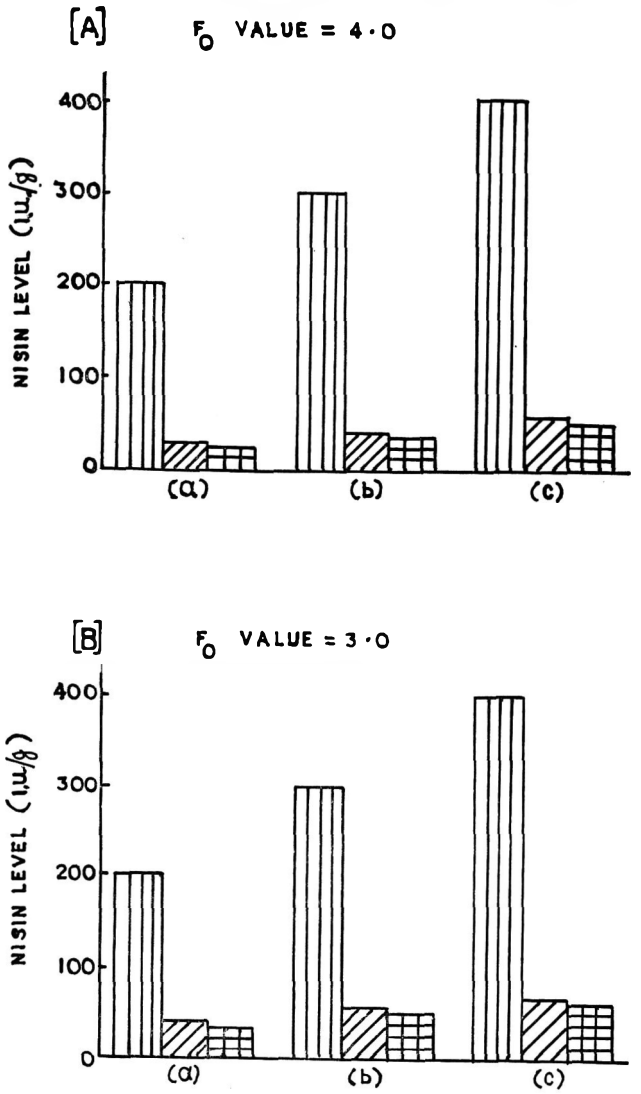

Fig. 2. Changes in nisin level on processing of *kheer*

—Inital concn; —After processing; —After 5 days incubation (55°C)

(a) —200 I.U./g; *(b)* —300 I.U./g; *(c)* —100 I.U/g

foods as a result of thermal processing and subsequent incubation. In case of *upma* nisin degradation increased with increase in processing value (Fig. 1). The residual nisin levels in cans have been 7.0, 5.0 and 4.0 IU/g (initial nisin 25 IU/g), 14.0, 12.8 and 10.5 IU/g (initial nisin 50 IU/g) and 25.0, 23.0 and 20.0 IU/g (initial nisin 75 IU/g) after thermal processing at F_0 values of 2.0, 3.0 and 4.0 respectively. Incubation of cans resulted in further degradation of nisin, though not to such an extent. *Kheer* processing showed about 6 fold decrease in nisin level at F_0 value of 3.0 and 8 fold decrease at F_0 value of 4.0 (Fig. 2). Incubation of *kheer* cans also caused the destruction of some nisin.

Thermal processing efficiently inactivated the spores of *Bacillus stearothermophilus* and the effect has been proportional to the amount of heat applied (F_0 value). Nisin incorporation accelerated thermal destruction of the spores, the extent of destruction being dependent on the amount added. Nisin appeared to increase the heat sensitivity of spores. The exact mechanism of its action however is not clearly understood so far[12].

Nisin, being proteinaceous[13] in nature, undergoes degradation during heat treatment. The degradation was observed to be lesser in *upma* than in *kheer* and could be attributed to the acidic nature of *upma* as it is known that nisin is stable in acidic conditions.

Since it is the residual nisin which is most important for the storage stability of the product, from the present data it can be concluded that nisin at level of 50 IU per g with thermal processing value of $F_0 = 2$ and 300 IU per g with thermal processing value of $F_0 = 3$ is required for *upma* and *kheer* respectively for attaining satisfactory shelf life. Its use should be promoted on commercial scale especially for foods which are acidic in nature and have delicate flavour and texture. With the increasing trend of using flexible packages for thermal processing the necessity of using such a thermal sterilization aid need not be overemphasized.

Acknowledgement

We are deeply indebted to Dr T. R. Sharma, Director Defence Food Research Laboratory for the constant encouragement and Shri K. C. Eapen for helpful suggestions.

References

1. Heinemann, B. Voris, L. and Stumbo, C. R., Use of Nisin in processing food products. *Fd Technol.*, 1965, **19**, 160.
2. *Lowering the process value of thermostabilised canned foods with a view to enhance the organoleptic qualities and consumer acceptability.* Technical report, RD-P1-80/DFRL-150, DFRL, Mysore, India, 1986.
3. Poretta, A., Casolani, A. and Cassara A., The use of nisin in the preparation of tomato juice. *Ind. Conserve*, 1968, **1**, 13
4. Poretta, A., Giannone, L. and Casolani, A., The use of nisin in the processing of peas. *Ind. conserve*, 1966, **2**, 89.
5. Nekhotenova, T. I., The possibility of modifying the sterilisation process of green peas by adding nisin. *Konserv. i. Oveshchesushil, Prom.*, 1961, **16** (11), 21.
6. Heinemann, B., Stumbo, C. R. and Scurlock, A., *The Use of Nisin as a Heat Sterilisation Aid in Canned Mushrooms.* Technical information No. 36723, M/s. Aplin & Barrett Ltd., Towbridge, Wiltshire, England. Producers Creamery Company, Springfield, Missouri, 1963.
7. Eapen, K. C., Thangamani, Mohan, M. S., Sankaran, R. and Vijayaraghavan, P. K., Enhancement of organoleptic qualities and energy conservation in heat processed foods. II Indian convention. Fd Sci. Technol. CFTRI, India, 1981.

8. Eapen, K. C., Thangamani, Mohan M. S., Sankaran, R. and Vijayaraghavan, P. K., Effect of nisin on the spores of some thermophilic bacteria. Paper presented at Int. Fd Conf., (*Ahara*) Bangalore, India, 1982.
9. Mohan, M. S., Singh, L. and Sankaran, R., Effect of nisin on lowering the processing requirements of *upma* and *channa* curry. V ICFOST, N. Delhi, India, 1985.
10. Thangamani, *Effect of Different Salt and Sugar Concentrations and pH on the Survival of Spores of* Bacillus stearothermophilus, 1978, M.Sc. Thesis, University of Mysore.
11. Tramer, J. and Fowler G. C., Estimation of nisin in foods. *J. Sci. Fd Agric.*, 1964, **15**, 522.
12. Eapen, K. C., Sankaran, R. and Vijayaraghavan, P. K., The present status on the use of nisin in processed foods. *J. Fd Sci. Technol.*, 1983, **20**, 231.
13. Gross, E. and Morell, J. L., The structure of nisin. *J. Am. chem. Soc.*, 1971, **93**, 4634.

Study of Physical Properties, Milling Performance and Cooking Time of Minor Millets

B. L. MANDHYAN, S. K. SHARMA AND H. R. TYAGI
College of Agricultural Engineering,
Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur-482 004, India

Received 21 *July* 1986; *revised* 15 *June* 1987

Physical properties, milling and cooking quality of kodo (*Paspalum scrobiculatum* L.) kutki (*Panicum miliaceus* L.) and sawan (*Panicum miliare* L.) were studied. The study revealed that kutki is smallest and kodo is the largest in size. The milling efficiency was highest in sawan followed by kodo and kutki while the husk content was highest in kodo followed by kutki and sawan. The total cooking time taken by all the three minor millets was found to be 25 min.

Minor millets occupy a sizeable chunk of the cultivated land in India. The area under cultivation of minor millets was 3.393 million hectares in 1984–1985 and yielded 1.319 million tonnes of grain[1]. These crops are very hardy and remarkably drought-resistant and are grown in the shallow, gravelly and light uplands of Karnataka, Maharashtra, Tamil Nadu and Madhya Pradesh[2]. They provide staple food to the tribals of the above states living in the hills and forests. The crops are reported to have a marvellous storability and do not require much of tillage operations. They are consumed after hulling which is performed only by hand pounding. No improved machines are available for hulling/dehusking of these crops. There is no technology for preparing ready-to-eat snacks from them.

The present study was therefore undertaken to find out the physical properties, milling quality and cooking characteristics of these grains.

Materials and Methods

One variety each of kodo, kutki and sawan were procured from the Minor Millet Improvement Scheme, Zonal Agricultural Research Station, Rewa. Moisture content of cleaned grains was determined using Burrow digital moisture computer. Thousand grain weight was measured using an electronic balance.

Dimensions of ten grains from each millet were measured using a dial calliper; length/breadth ratio of the grains was also determined. The shape of the grains was expressed in terms of its sphericity calculated by the following expression[3].

$$\text{Sphericity} = \frac{\text{Geometric mean diameter}}{\text{Major diameter}}$$

in which, geometric mean diameter, $= (abc)^{\frac{1}{3}}$ mm

Where, a = Longest intercept; mm,

b = Longest intercept normal to 'a', mm;

c = Longest intercept normal to 'a' and 'b', mm.

Liquid (kerosene) displacement method was used for determining specific gravity. Bulk density was measured by weighing the sample of known volume. The angle of repose was measured by slump cone method. A cylinder of size 10 cm × 6m was filled upto top with sample and inverted on a plane surface. The paper was taken out gradually and cylinder was raised vertically; thus conical shape of the material was obtained. Angle of repose was calculated by using the relationship:

Angle of repose (°) $= \tan^{-1}(2H/B)$, Where H = Height, m; B = Base of the cone, m.

TABLE 1. PHYSICAL PROPERTIES OF SOME MINOR MILLETS

Millet	Variety	Moisture (% db)	Sphericity	Equivalent dia. mm	Length breadth ratio	Thousand grain wt. g.	Sp. gr.	Bulk density (g/ml)	Angle of repose (°)
Kodo	RPS-123	11.30	0.89	1.82	1.00	4.89	1.08	0.57	28°50'
Kutki	RPM-81	11.90	0.93	1.07	1.06	2.00	1.35	0.72	20°21'
Sawan	REF-51	11.00	0.90	1.59	1.07	4.03	1.17	0.55	24°25'

The milling (dehusking and polishing) was done with centrifugal dehusker and kisan rice polisher as these were similar to commercial conditions. The operation was done in three replications. The milling efficiency was calculated by the expression[4].

$$E=\left(1-\frac{Muh}{Mt}\right)\times\left(1-\frac{Mb}{Mt}\right)\times 100;\ \text{where,}$$

E=Milling efficiency %

Muh=Mass of grains after hulling, g

Mb=Mass of brokens including mealy waste after hulling, g

Mt=Total mass of grains before hulling which do not contain hulled and broken grains, g.

For cooking test milled grains were soaked in three replications with 25 g sample each in 100 ml test tubes. They were kept in a thermostatically controlled water bath where 100°C temperature was maintained. Soaking was done in excessive water by dipping the test tubes in water bath. After specific time interval (5 min), the material was removed, dabbed with filter paper and weighed. The initial moisture content of the milled grain was determined. The moisture content after soaking was calculated by the following relationship:

$Q_1\ (100-M_1)=Q_2\ (100-M_2)$;

Where, Q_1 =Initial weight of sample, g

M_1=Initial moisture content of the sample, % wb.

Q_2 =Final weight of sample after soaking, g

M_2=Final moisture content, % wb.

When the grain weight became constant, the soaked sample was placed in between two glass slides. If no hard core was observed when it was pressed, the sample was considered to be fully cooked. The moisture content (%, wet basis) was converted into dry basis using following relationship:

$$M=\frac{m}{100-m}\times 100$$

M=moisture content, % db

m=moisture content, % wb

Results and Discussion

As seen from Table 1, the average geometric mean diameter of kodo is higher than that of sawan and kutki. The sphericity of kutki is observed to be highest among the three minor millets and kodo is the least spherical. The knowledge of geometric mean diameter and sphericity will help in deciding the clearance between two abrasive surfaces for dehusking and polishing. It will also help in designing the cleaner and separator. The length breadth ratio is highest in sawan (1.07) indicaitng that sawan grain is the most slender of all. Thousand grain weight is the highest in kodo followed by sawan and kutki, but specific gravity is the highest in kutki (1.35). The bulk density of kutki (0.72g/ml) is the highest followed by kodo (0.57 g/ml). Sawan having more sphericity and length-breadth ratio entraps more space, thus reducing its bulk density. Gravimetric properties such as specific gravity, bulk density and thousand grain weight are necessary in design and analysis of separation, design of hoppers and blowers. The angle of repose of kodo (28°50') was observed to be the highest followed by sawan (24°25') and kutki (20°21'). The knowledge of angle of repose will help in predicting the motion of particles during milling and in determining the pressures of grain against machine walls so as to decide the thickness of metal sheet for dehusker and polisher.

Milling performance: Table 2 shows that the husk content of the kodo, kutki and sawan grain was18.95,

TABLE 2. MILLING PERFORMANCE OF SOME MINOR MILLETS

Millet	Variety	Husked grain (%)	Unhusked grain (%)	Brokens (%)	Husk (%)	Milling efficiency (%)
Kodo	RPS-123	56.25	6.39	17.91	18.95	76.43
Kutki	RPM-81	54-57	10.23	17.46	17.74	74.10
Sawan	REF-51	71.50	5.68	11.60	11.22	83.38

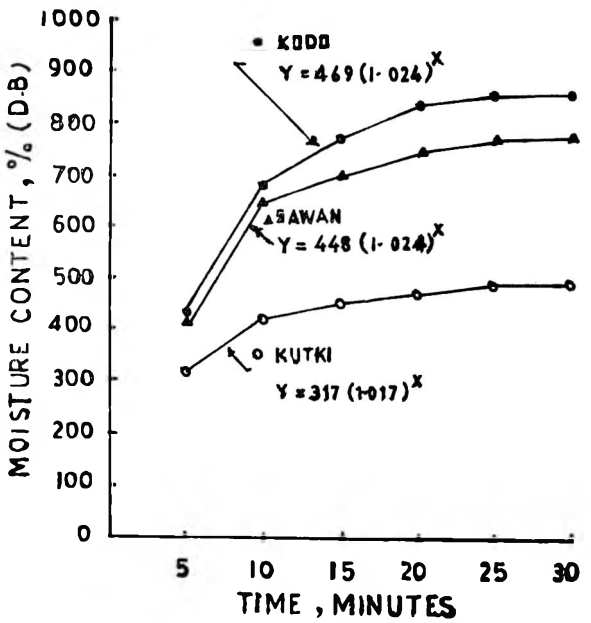

Fig. 1. Moisture absorption of minor millets during cooking

17.74 and 11.22 per cent respectively. The total recovery of husked sawan grain was observed to be 71.5 per cent after polishing while in kodo and kutki the recovery was 56.25 and 54.37 per cent respectively. The reason for lower recovery in kodo and kutki is due to the fact that some grains are powdered and get mixed with bran. Because of the higher husk content the machines has to be operated for more time and this gives more brokens as well as flour. Also sawan supplies more edible matter per unit weight than the other two.

Moisture absorption during cooking: The initial moisture content of kodo, kutki and sawan was 11.3 11.5 and 11.0 per cent (db) respectively. The samples were soaked for 30 min. and moisture content was determined at 5 min intervals. Fig. 1 shows the moisture content and soaking time. It can be seen that moisture absorption was rapid within the first 10 min and declined thereafter. After 25 min of soaking moisture absorption became constant. Exponential equations were developed by the use of which moisture absorption can be predicted at any time of soaking at 100°C (Fig. 1). It is further seen that the rate of moisture absorption varies with the grain but the total time taken to achieve a constant moisture absorption stage is the same for all three i.e. 25 min. of cooking. This shows that milling performance for same milling conditions do not affect the cooking characteristics of the minor millets.

References

1. Anon., All India crop estimate, 1984–85. *Agric. Situat. India*, 1985, **40**, 1069.

2. Jaiswal, P. L. (Ed.), *Hand Book of Agriculture*, Indian Council of Agriculture Research, New Delhi, 1980, 835.

3. Mohsenin, N. N., *Physical Properties of Plant and Animal Materials*, Gardon and Broach Science, New York, 1980, 58.

4. Sexena, R. P., Singh, B. P. N. and Agrawal, U. S., Milling behaviour of pea (*Pisum sativum* L.), grain for making dhal on cylinder-concave dehuller, *J. agric. Engng*, 1985, **22**, 101.

Effect of Addition of Defatted Soy Flour on Physico-chemical Characteristics and Acceptability of Green Gram *Barian**

MEENA TANDON AND GURMUKH SINGH
Dept. of Food Science and Technology,
G.B. Pant University of Agriculture and Technology, Pantnagar-263 145, U.P. India

Received 24 *July* 1986; *revised* 25 *February* 1987

The Effect of addition of defatted soy flour (5 to 30 per cent) on physico-chemical characteristics and acceptability of green gram (moong) (*Phaseolus aureus*) *barian (Wadian)* was investigated. There was increase in protein, ash and crude fibre contents and decrease in crude fat and total carbohydrates with the increase in the level of defatted soy flour in the product. The visual colour of *barian* became darker and the extent of non-enzymatic browning increased with increasing level of soy flour. Poor rehydration of soy fortified *barian* was on account of their hard texture. The consumer acceptability of *barian* decreased with the increase in the level of defatted soy flour. Addition of defatted soy flour up to 10.0 per cent did not make any significant difference to the overall acceptabilty.

Soybean has great potential in overcoming protein-calorie-malnutrition (PCM) on account of its high protein (38-42 per cent) and fat (18-20 per cent) contents. Defatted soy flour is a common form in which soybeans can be incorporated in various food preparations. Its use in bread, biscuits, chapati, snacks and textured products has been demonstrated[1-4]. No attempt had been made so far to incorporate defatted soy flour in moong *barian* (*wadian*) which are traditional savoury preparations of Punjab, Uttar Pradesh, Haryana and adjoining states. They are produced on a cottage scale and are used in vegetable curries. Extensive studies on their manufacture, composition, storage aspects and standards have been carried out[5]. The persent investigation was therefore undertaken to study the effect of addition of defatted soy flour on the quality characteristics and acceptability of moong *barian*.

Materials and Methods

Materials: Defatted soy flour was procured from M/S General Foods, Indore (M.P.) and was desolventised by keeping in an oven at 60°C for 2 hr. It was then given a heat treatment at 121°C (15 psi) for 20 min to inactivate antinutritional factors. Dehulled green gram dal pumpkin and spices were purchased from the local market.

Preparation of barian: For preparation of *barian*, green gram dal was soaked in water for 12 hr at room temperature (24-32°C). The hydrated dal was washed with water 3 times to remove frothings and ground in a waring blender with addition of small quantities of water until a thick paste was obtained. Defatted soy flour, mixture of spices and freshly comminuted pumpkin (after boiling) were also mixed during the grinding stage. The ground paste (*peethi*) was beaten by hand for 15 min. Small round lumps weighing about 50 g were made and then placed directly on an oil smeared enamelled tray at a distance of about 3 in from one another. The trays containing *barian* lumps were then sun dried for 4-6 days. After 1-2 days of exposure, the lumps were turned over by hand for uniform drying. The dried *barian* were packed in polyethylene bags and stored in air tight containers. Green gram dal and defatted soy flour were used in proportions of 100:0, 95:5, 90:10, 85:15, 80:20, 75:25 and 70:30 in the preparation of *barian*. Pumpkin and spice mixture[6] were added at 2 and 8 per cent respectively during the grinding.

Analytical methods: Powdered samples of *barian* were analysed for moisture, protein, crude fat, ash and crude fibre by standard methods[7]. Acidity and extent of non-enzymatic browning were estimated by the procedure described by Ranganna[8]. Colour of samples was expressed on the basis of Munsell colour charts. Hardness of *barian* was found out using a Hardness Tester (Kiva Seisakusho) and was expressed as crushing resistance (kg). For rehydration studies, a known weight (30-40g) of dry *barian* was hydrated in 3 times the weight of water at room temperature (24-32°C). Samples were drained after 30 min, 1, 2 and 4 hr and the wet weights

*Research paper No. 4014 through the Experiment Station, G.B. Pant University of Agriculture and Technology, Pantnagar (U.P.).

TABLE 1. PRODUCTION DETAILS AND PRODUCT CHARACTERISTICS OF GREEN GRAM BARIAN FORTIFIED WITH DEFATTED SOY FLOUR

Moong dal (%)	Defatted soy flour (%)	Water added during grinding (ml/kg)	Wt. of paste (kg)	Yield of *barian* (g/kg)	Dehydration ratio	Non-enzymatic browning[1]	Mean acceptability score*
100	0	240	2.45	830	2.95:1	0.07	6.80
95	5	220	2.40	845	2.84:1	0.10	6.50
90	10	200	2.30	846	2.72:1	0.12	6.15
85	15	185	2.25	852	2.64:1	0.14	4.80
80	20	175	2.20	860	2.60:1	0.17	4.25
75	25	160	2.10	875	2.40:1	0.17	4.00
70	30	150	2.05	880	2.33:1	0.19	3.80

1. Expressed as optical density

*Significant at 5% level of significance; critical difference at 5%—1.19

were recorded. Per cent water absorption was calculated at each stage. The acceptability of *barian* was determined by serving them as vegetable curry to 20 trained panelists. They were asked to evaluate the products for quality attributes namely colour, texture and flavour on a 9 point hedonic scale[9].

Results and Discussion

Table 1 gives production details of soy fortified green gram *barian*. The amount of water needed during grinding decreased as the proportion of defatted soy flour in *barian* increased. Lesser requirement of water during grinding appeared to be due to low water absorption by soy flour. The moisture content of wet paste (*peethi*) ranged between 61.50 and 63.62 per cent. The yield of *barian* ranged from 845 to 880 g per kg of raw material for those containing defatted soy flour as against 830 g per kg for the control. The dehydration ratio of the product lowered with the increase in the level of defatted soy flour.

The proximate composition of soy fortified green gram *barian* varied considerably. Moisture content of all the samples was less than 8.2 per cent. The contents of protein, ash and crude fibre increased whereas crude fat and carbohydrates decreased by increasing the level of defatted soy flour in *barian*. The control sample contained (in per cent) protein 18.23, fat 1.15, ash 4.10, crude fibre 0.13 and carbohydrates 68.22 whereas the sample containing 30 per cent soy flour had protein 27.55, fat 1.05, ash 4.31, crude fibre 0.78 and carbohydrates 58.90. *Barian* containing 30.0 per cent defatted soy flour had about 9.0 per cent higher protein content than those prepared from green gram dal alone. Increase in protein and ash content of soy fortified green gram *barian* is attributed to their higher content in soy flour than in green gram dal[6]. A gradual reduction in titratable acidity and increase in acid insoluble ash content was also noted when soy flour was added in increasing proportions in the preparations.

The colour of control sample was yellowish brown. Addition of defatted soy flour at 5.0 per cent level did not bring any change in colour whereas at 10.0 per cent level, the colour of *barian* became brownish yellow. Samples containing 15 to 30 per cent defatted soy flour had similar colour parameters and were typically dark brown. The extent of non-enzymatic browning in the samples also increased with the increase in the proportion of defatted soy flour (Table 1). Apparently, sugar-amino reactions increased upon incorporation of defatted soy flour in the preparations.

Soy fortified green gram *barian* exhibited greater hardness than the control. The extent of hardness increased with increasing level of defatted soy flour in the product. The crushing resistance of control sample was 2.2 kg while for those containing 30 per cent defatted soy flour it was 17.3 kg.

Rehydration studies on *barian* indicated that the control sample had the highest water absorption whereas that containing 30 per cent defatted soy flour had the lowest water absorption over the entire period of study (Fig. 1). There was gradual decrease in the water absorption of *barian* with the increase in the level of defatted soy flour. Fig 1 and hardness data revealed that samples having maximum hardness had the minimum water absorption in 4 hr. Apparently, hard texture of soy fortified *barian* lowered water imbibition by the product.

Studies on organoleptic evaluation of the products showed that average acceptability score as well as consumer response was maximum for the control sample

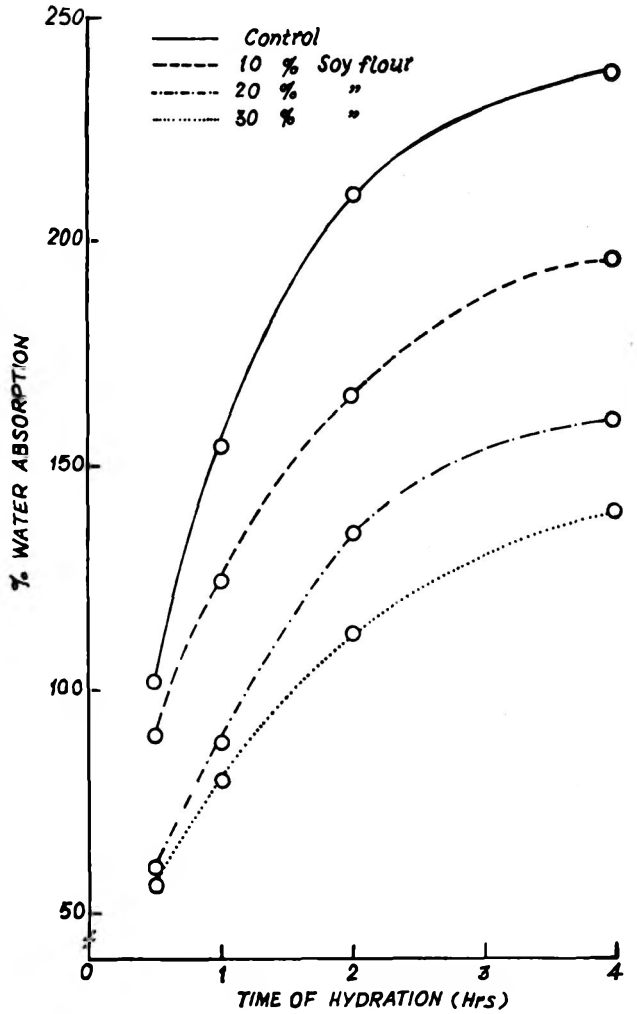

Fig. 1. Per cent water absorption by moong *barian* fortified with defatted soy flour. (Each point is mean of 3 values)

and decreased with the increase in the level of defatted soy flour in the product (Table 1). The control samples were liked to varying degrees by 85 per cent judges whereas those containing 5 and 10 per cent defatted soy flour were liked by 80 and 70 per cent panelists, respectively. Significant differences at 5 per cent level of significance were seen among various preparations. Samples containing 5 and 10 per cent defatted soy flour were similar to controls in acceptaiblity whereas those containing 15 per cent or higher were unacceptable. It is, therefore, concluded that addition of up to 10 per cent defatted soy flour in green gram *barian* does not cause any significant change in the acceptability of the product.

Acknowledgement

The authors are grateful to Director of Research, Dean, College of Agriculture and Head, Food Science & Technology of the University for providing the necessary facilities.

References

1. Rathod, K. L. and Williams, S. W., Acceptance of soybean enriched wheat flour for use in chapaties. *Indian J. Nutr. Dietet.*, 1973, **10**, 18.
2. Hoover, W. J., Use of soy products in cereal products. *J. Am. Oil chem. Soc.*, 1974, **51**, 186.
3. Sushma, K., Satinder, B. and Bhat, C. M., Nutritional composition and acceptability of soybean supplemented dishes. *J. Res. Punjab agric. Univ.*, 1979, **16**, 111.
4. Chauhan, G. S. and Bains, G. S., Effect of defatted soy flour on the physico-chemical characteristics of extruded rice products. *J. Fd Sci. Technol.*, 1985, **22**, 115.
5. Pruthi, J. S., Manan, J. K., Kalra, C. L. and Raina, B. L., Studies on physico-chemical composition and formulation of quality standards for traditional savoury foods. Part I: Black gram wadian and green gram wadian. *I.S.I. Bull.*, 1981, **33**, 56.
6. Tandon, M., *Use of Defatted Soy Flour in Urd and Moong Wadian.* 1985 M.Sc. Thesis G.B. Pant Univ. of Agric. & Tech. (U.P.).
7. *Official Methods of Analysis*, Association of Official analytical Chemists, Washington, 1975.
8. Ranganna, S. *Manual of Analysis of Fruit and Vegetable Products.* Tata McGraw Hills, New Delhi, 1979.
9. Larmond, E. *Laboratory Methods for Sensory Evaluation of Foods.* Canada Department of Agriculture. Publication 1637. Ottawa, 1977.

On Factors Affecting the Acidity of Soft Dough Biscuits

C. N. Vatsala and P. Haridas Rao
International School of Milling Technology & Baking Technology,
Central Food Technological Research Institute, Mysore-570 013, India

Received 18 *April* 1986; *revised* 16 *February* 1987

Studies on acidity of extracted fat, one of the criteria used for evaluating biscuit quality, showed that in addition to major ingredients like flour and fat, minor ingredients like corn syrup, milk powder and baking chemicals considerably influenced the acidity of soft dough biscuits. Incorporation of 3% corn syrup and 2% non fat dry milk solids increased the acidity to 0.49 and 0.48% respectively from 0.37% observed for control biscuits without these ingredients. On the other hand, addition of baking chemicals reduced the acidity of biscuits markedly from 0.86 to 0.42%. Sodium bicarbonate brought about maximum reduction in the acidity. Increasing the baking temperature from 175 to 260°C, increased the acidity from 0.35 to 0.73% while increasing the baking time from 12 to 21 min increased the acidity from 0.38 to 0.86%.

In India, biscuits occupy a prominent place among baked products. The present production of biscuits exceeds 4 lakh tonnes per annum valued at Rs. 400 crores[1] and its consumption is steadily increasing at the compound rate of 9.2 per cent. At present the export of biscuits to South East Asia, Middle East and African countries is valued at Rs. 2 crores[2]. There is considerable scope for further increasing the production and export which call for improvement in the quality of biscuits.

The quality of biscuits in India is governed by specifications of the Indian Standards Institution (ISI) which are optional and those of the Prevention of Food Adulteration Act (PFA) which are mandatory. Apart from laying the limits for moisture and acid insoluble ash, these specifications specify a maximum limit of 1.5 per cent for acidity of fat extracted from biscuits[3].

At present, several biscuit production units, particularly those of the small scale sector without adequate infrastructural facilities for quality control are confronted with the problem of maintaining the acidity of biscuits within the specified limit. It is well known that the acidity of biscuits which is an important factor in obtaining a product of desired shelf life is mainly influenced by acidity of major ingredients like flour and shortening. Minor ingredients as well as processing conditions might also contribute to acidity and such information is lacking. With this background, the results of studies on the acidity in biscuits as influenced by various factors are presented in this paper.

Materials and Methods

Materials: Commercial grade refined soft wheat flour (*maida*) procured from the local market was used.

Preparation of biscuits: Soft dough biscuits were prepared according to the recipe and the method described earlier by Haridas Rao, *et al.*[4] However, to study the effect of different minor ingredients such as glucose syrup, milk powder and baking chemicals on the acidity, biscuits were made with and without any one of the minor ingredients. The influence of processing conditions on the acidity was determined by varying (*i*) the resting period (0-5 hr) of the dough before moulding (*ii*) baking temperature (150-325°C) and (*iii*) baking time (12-21 min). Changes in acidity during storage at room temperature (27°C) were determined by storing biscuits wrapped in wax coated paper in an air-tight container.

Analysis: The acidity in biscuits was determined according to the standard ISI method[5] while standard AOAC method[6] was followed for flour and fat. In all the cases the acidity was expressed as per cent oleic acid. Moisture, total ash, gluten, sedimentation value and farinograph water absorption of *maida* were determined according to standard AACC methods[7].

Results and Discussion

Chemical characteristics of maida: The analytical data obtained on *maida* as expressed on 14 per cent moisture basis were: total ash, 0.63, per cent dry gluten, 8.7; per cent sedimentation value, 21 ml; farinograph water absorption, 56.5 per cent. The low values for

gluten, sedimentation value and water absorption indicated that the *maida* selected for the study was from soft wheat suitable for biscuit making[8].

Acidity of commercial biscuits: The fat acidity determined for 12 commercial samples of sweet biscuits varied from 0.40 to 0.88 per cent with an average value of 0.65 per cent and was within the maximum permissible limit of 1.5 per cent, as specified by PFA.

Baking conditions: The acidity of biscuits baked at different temperatures for an optimum period to yield a product of desired surface colour indicated that the acidity increased from 0.35 to 0.73 per cent when the baking temperature was raised from 150 to 325°C (Fig. 1). The acidity in biscuits baked at temperatures higher than 350°C may be due to the faster rate of hydrolysis of fat to free fatty acids as compared to that baked at a lower temperature of 150°C. Similar observation was made for oils wherein the acidity increased with increase in frying temperature[9].

The effect of baking time on acidity was similar to that of baking temperature (Fig. 1). The acidity increased from 0.38 to 0.75 per cent when baking time was increased from 12 to 21 min. The optimally baked biscuits had an acidity of 0.41 per cent while the unbaked biscuit dough had an acidity of 0.32 per cent indicating thereby an increase in acidity during baking. Higher acidity due to over-baking was confirmed by the observation that acidity was higher in the peripheral (0.55 per cent) than in the central portion (0.40 per cent) of biscuits which are subjected to more heat.

Resting the dough: Generally, biscuit doughs are rested for about half an hour for uniform hydration before shaping; a 2 hr. resting period led to negligible increase in acidity (Fig. 2). However, resting the dough for more than 2 hr slightly increased the acidity of biscuits. The acidity increased to 0.48 per cent from 0.42 per cent when the dough was rested for 5 hr.

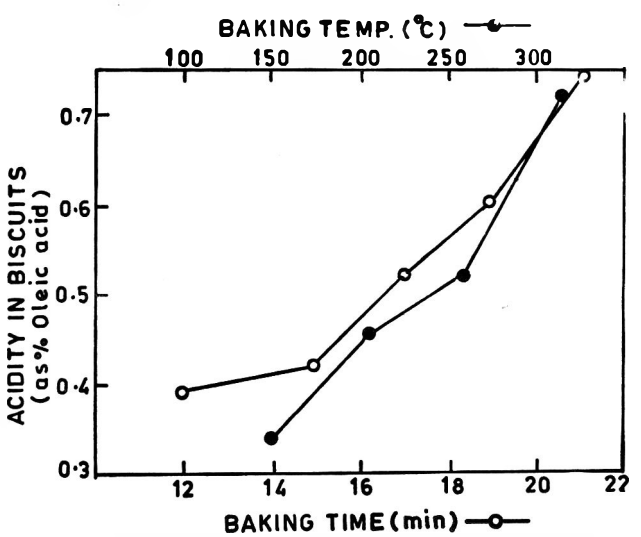

Fig. 1. Effect of baking time and baking temperature on the acidity of biscuits.

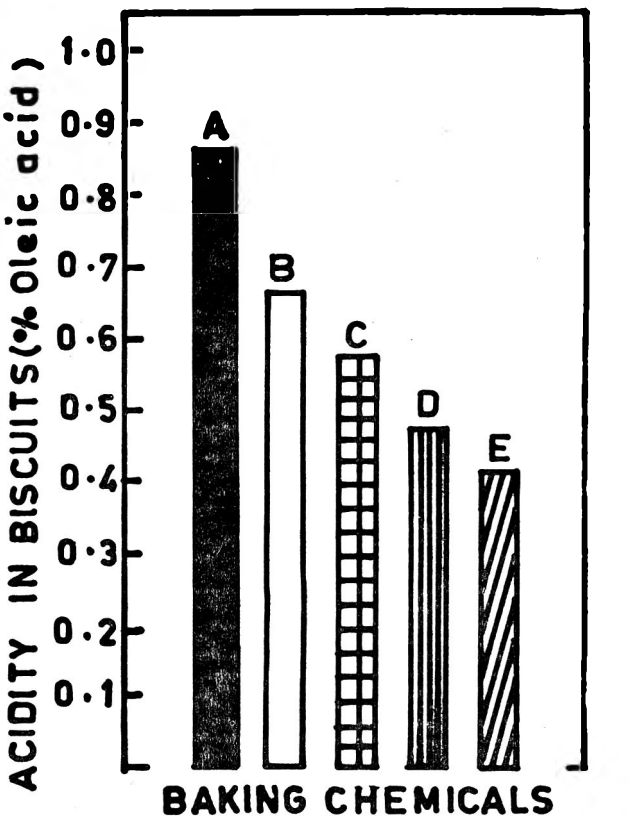

Fig. 2. Effect of different baking chemicals (used at 0.5% level) on the acidity of biscuits.

A: No baking chemical; B: Baking powder; C: Ammonium bicarbonate; D: Sodium bicarbonate; E: B + C + D.

Types of fat: The acidity of biscuits depended on the acidity of fat used (Table 1). Biscuits prepared with unrefined oil having an acidity of 1.7 per cent had the maximum acidity of 1.4, while those made with bakery shortening of acidity 0.06 per cent had only 0.42 per cent acidity.

TABLE 1. ACIDITY OF BICSUITS MADE WITH DIFFERENT TYPES OF FAT

Type of fat	Acidity (as % oleic acid)	
	Fat	Biscuits
Bakery shortening	0.06	0.42
Oil, refined	0.10	0.48
Oil, unrefined	1.70	2.40
Hydrogenated fat	0.10	0.49

Average of 3 determinations

TABLE 2. EFFECT OF MINOR INGREDIENTS ON THE ACIDITY OF BISCUITS

Ingredient		Acidity
Name	% used	(% oleic acid)
None	0	0.37
Corn syrup	1	0.42
	2	0.45
	3	0.49
	4	0.54
Non-fat dry milk	1	0.41
solids	2	0.44
	3	0.48

Average of 3 determinations

Baking chemicals: Addition of normally used baking chemicals like ammonium bicarbonate, sodium bicarbonate and baking powder lowered the acidity in biscuits (Fig. 2). However, maximum effect on the acidity was observed in case of sodium bicarbonate. This is due to the neutralisation of free fatty acids formed during baking by sodium carbonate[10]. The combined effect of the different chemicals was less than the sum of their individual effects. Incorporation of different baking chemicals at varying levels (0 to 1 per cent) showed that maximum effect in reducing the acidity was observed in the range of 0.2 to 0.75 per cent. However, when sodium bicarbonate was added along with acid calcium phosphate in amounts required to neutralise the residual sodium carbonate which imparts an unpleasant taste, no change in the acidity was observed.

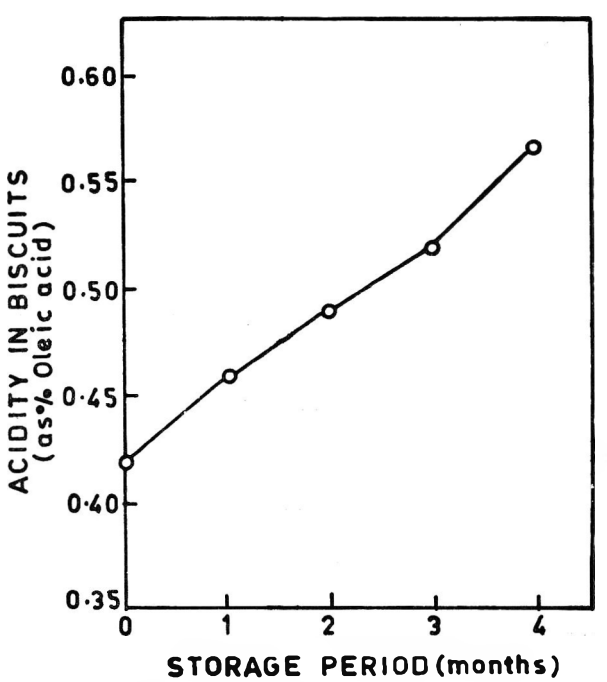

Fig. 3. Effect of storage on the acidity of biscuits.

Minor ingredients: Generally, in the preparation of soft dough biscuits, corn syrup is used to obtain the desired color and milk powder to improve the taste and nutritive value[11]. Inclusion of these ingredients were found to increase the acidity in biscuits depending on the levels used (Table 2). Increase in the acidity due to incorporation of these ingredients may be due to their acidic nature[11].

Effect of storage: Acidity in biscuits as expected, gradually increased from 0.42 to 0.57 per cent during storage for 4 months (Fig. 3). However, the increase would be naturally higher at higher temperature of storage[12]. An increase in the acidity by 0.32 per cent was observed when biscuits were stored at 37°C (instead of room temperature) for 4 months[13].

References

1. *A Ten Years Perspective Plan for Bakery Industry in India*, 1981–1990. *The Society of Indian Bakers* Published by the Institute of Economic and Market Research, New Delhi.
2. Rao, R. B., Changing patterns in production and consumption of bread and other bakery products in India and the scope of Indian wheats meeting the quality requirements of local milling and baking industry. *Indian Bak.*, 1976, **6**, 13.
3. *The Prevention of Food Adulteration Act*, 1954, 8th Edn., Eastern Book Co., Lucknow, 1985.
4. Haridas Rao, P. and Shurpalekar, S. R., Utilization of milo in bakery products. *J. Fd Sci. Technol.*, 1976, **13**, 293.
5. Specification for Biscuits. IS: 1011–1981, Indian Standards Institution, New Delhi.
6. *Methods of Analysis.*, Association of Official Analytical Chemists. Washington, 11th Edn., 1970.
7. *Approved Methods*, American Association of Cereal Chemists. Minnesota, 1969, Vols. 1 and 2.
8. Smith, W. H., *Biscuits, Crackers and Cookies*. Applied Science Publishers Ltd., Barking Essex, 1972. Vol. I.
9. Hussain, S. S., Morton, I. D., Absorption by food of frying oils. *Proceedings of 4th International Congress of Food Science and Technology*, Madrid, Spain, Sept. 1974, **1**, 322.
10. Bracco, U., Dieffenbacher, A. and Kolarovic, L., Frying performance of palm oil—liquid fractions. *J. Am. Oil Chem. Soc.*, 1981, **6**, 1981.
11. Manely, D. J. R., *Technology of Biscuits, Crackers and Cookies*. Ellis Horwood Ltd. Chichester, West Sussex, England, 1983.
12. Kumar, K. R., Unpublished, Central Food Technological Research Institute, Mysore, India.

Detection of Beef and Pork in Fresh and Heat Treated Meat Products Using Enzyme Immunoassay

E. K. KANG'ETHE AND K. J. LINDQVIST
Department of Public Health, Pharmacology and Toxicology, University of Nairobi
P.O. Box 29053, Kabete, Nairobi, Kenya

Received 19 *May* 1986; *revised* 6 *March* 1987

Raw and heat-treated pork and beef products were purchased from the outlets of two leading Kenyan manufacturing firms. Pure beef and pork sausages and pork sausages containing 1, 5 and 10% beef were prepared in the laboratory. Antigens from these products were extracted with phosphate buffered saline. The presence of beef and pork in these products was determined using absorbed goat antisera to cattle and pig thermostable muscle antigens in an enzyme immunoassay. The assay was able to detect beef in pork sausages at the level of 10% and 5% but not at 1%. Of the 44 commercial beef products labelled as containing only beef, 23 (52.3%) were shown to contain pork and 23 (50%) of the 46 pork products were shown to contain beef. Antisera to thermostable muscle antigens proved to be of great value in species identification not only of fresh unheated meats but also of heated (cooked, pasteurized and autoclaved) meat products in an enzyme immunoassay.

Food analysts have relied heavily on conventional analytical methods for the detection of adulteration of food products. The potential of immunoassays as methods of choice for replacing less specific and more costly methods has been recently appreciated. The application of immunoassay for the detection of adulteration or accidental contamination of one kind of food with another is, therefore, still only in its early stages.

Identification of the species of origin of raw, unprocessed meat represents a recurrent problem in the meat industry. Methods such as immunodiffusion[1,2] electrophoretic techniques[3,4] and enzyme linked immunosorbent assay[5,6] have been used for species identification of raw, unprocessed meat with comparable results.

Recent reports on substitution of sheep, horse and kangaroo meats in Australian beef exports[6] and horse in British beef imports[5] have shown that processed meat products may also contain meat from animals other than those specified by the manufacturer. Speciation of meat in processed products, especially those that have undergone some degree of heating, has not been successful using the immunoassays commonly employed for raw meats. Assays of heated products must be based on the detection of heat stable antigens which have retained their species specificity. Milgrom and Witebsky[7] described the presence of heat stable antigens in adrenals. Hayden[8] used antisera to such antigens to detect adulteration in thoroughly cooked beef sausages using immunodiffusion tests. In our recent reports[9,10], we have shown that antisera to thermostable muscle antigens (TMA) can be used to identify the species of origin of fresh, cooked and autoclaved meat extracts from even phylogenetically closely related species in immunodiffusion tests.

This report describes the use of an enzyme immunoassay for the detection of beef and pork in raw and heat-treated meat products using antisera to thermostable muscle antigens.

Materials and Methods

Processed beef and pork products such as sausages, beef burgers, cooked ham, luncheon meat and corned beef were purchased from the retail outlet of two leading Kenyan manufacturing companies. Ninety samples bearing different production and batch dates were obtained over a period of two months.

Extraction of antigens from the products: After removal of the casings and other wrappings, the products were homogenized in phosphate buffered saline (PBS, 0.15M, pH 7.2) in the ratio of 1:1 (W/V). The homogenate was centrifuged at 4000 × **g** for 30 min and the supernate filtered through a Whatman No. 3 filter paper to remove fat and coarse particles. The filtrate was stored at 4°C after the addition of sodium azide to 0.01 per cent final concentration.

Production of antisera: Antisera to cattle and pig TMA were produced in goats according to the method described by Kang'ethe *et al*[9].

TABLE 1. RECIPE FOR THE LABORATORY PREPARATION OF SAUSACES

Sausage ingredients	100% pork	1% beef	5% beef	10% beef	100% beef
Beef lean (g)	—	12	60	120	700
Beef fat (g)	—	—	—	—	200
Pork lean (g)	700	688	640	580	—
Pork fat (g)	200	200	200	200	—
Ice/water (g)	100	100	100	100	100
Milk powder (g)	50	50	50	50	50
Salt (g)	15	15	15	15	15
Seasonings (g)	5	5	5	5	5

Absorption of antisera: Each antiserum was tested for cross-reactions and specificity using Ouchterlony's double diffusion test as described by Crowle[11]. The antiserum was absorbed by copolymerized pooled serum from pig, warthog, bushpig, cattle and buffalo using the ethyl-chloroformate method of Avrameas and Ternynck[12]. The absorbed antiserum was further tested for specificity using an enzyme immunoassay. If required, further absorptions were carried out using appropriate cattle and pig TMA and fresh meat antigens (FMA) coupled to cyanogen bromide activated sepharose 4B.

Preparation of pork sausages containing various quantities of beef: Fresh beef and pork sausages containing 1, 5 and 10 per cent beef were made in 1 kg lots (Table 1).

Preparation of rabbit anti-goat IgG glucose oxidase conjugate: Rabbit anti-goat IgG were separated from rabbit anti-goat IgG serum by precipitation at 50 per cent saturation with ammonium sulphate and chromatographed on DEAE-cellulose. The IgG fraction was conjugated with glucose oxidase according to the periodate method of Wilson and Nakane[13], with slight modifications. Briefly, the procedure was as follows; Seventy six milligrams of glucose oxidase were dissolved in 9.2 ml of distilled water and oxidized by addition of 1.9 ml of 0.1M sodium metaperiodate solution. The mixture was stirred in the dark for 30 min at room temperature. It was dialysed against 100 ml of 1 mM acetate buffer pH 4.4 containing 0.1 ml ethylene glycol for 10 min, after which the dialysis was continued using 1 mM acetate buffer, pH 4.4 without ethylene glycol for 1 hr. The oxidized enzyme was mixed with rabbit anti-goat IgG in the molar ratio of 1:1, in 0.5M carbonate buffer pH 9.0 and left stirring for 2 hr at room temperature. The pH of the enzyme-IgG mixture was adjusted to between 7.5 and 7.8 and left at 4°C overnight. Twenty milligrams of glycine was added to the mixture which was left stirring for 1 hr at room temperature to block the remaining aldehyde groups. The pH was finally adjusted to 7.4-7.8 using 1 N HCl. After clarification by filtration through a 0.45 μm millipore filter, the conjugate was stored at –20°C after the addition of an equal volume of glycerol.

Enzyme immunoassay: Optimal dilutions of the antisera, conjugate and antigens were determined by checkerboard titrations. Appropriate antigen, antiserum, conjugate and substrate controls were included. Tests for specificity using each antiserum were also performed with homologous and heterologous antigens.

The antigen extracts of the meat products were diluted in the coating buffer (0.05 M carbonate-bicarbonate pH 9.6); 100 μl/well of the 1:400 dilution of the antigen were used to coat the microtitre plates (Dynatech flat bottomed plates M 129 A, batch 300266). The plates were incubated at room temperature overnight in a humid chamber, washed with PBS containing 0.05 per cent Tween 80 (PBS-Tween) and tapped dry on absorbent paper. 100 μl/well of absorbed goat anti-cattle TMA diluted 1:50 and goat anti-pig TMA diluted 1:50 were added and incubated for 1 hr at 37°C in a humid chamber. The serum diluent consisted of 75 g of KCl + 2.5 g disodium EDTA + 1 g benzoic acid + 1 ml Tween 80 + 5 per cent normal rabbit serum in 1000 ml of 0.05 M phosphate buffer pH 8.0; the pH of the diluent was adjusted to 7.5.

After incubation, the plates were washed, tapped dry and 100 μl/well of the rabbit-anti-goat IgG glucose oxidase conjugate, diluted 1:1000 in the conjugate diluent was added and incubated for 1 hr at 37°C in a humid chamber. The conjugate diluent was similar to the serum diluent except that 0.05 per cent of Tween 80 was used instead of 0.01 per cent. After incubation, the plates were washed, tapped dry on absorbent paper and 100 μl/well of the substrate added and incubated for 1 hr at room temperature. The absorbance was read at 410 nm using a micro-ELISA Mini-reader MR 590 (Dynatech, Santa Monica, California). For 1 plate the substrate solution was made as follows:

10 ml of 0.05 M ammonium acetate buffer pH 5.0

1 ml of a 20 per cent glucose solution

0.1 ml of peroxidase type II solution (1 mg/ml) in 0.05 M acetate buffer (pH 5.0)

0.1 ml of 2,2'azino-di-methyl benzthiazoline sulfonic acid (ABTS) (25 mg/ml in distilled water).

Those products that were negative for beef or pork were further tested starting with an antigen dilution of 1:2 in the coating buffer. For the differentiation of known beef and pork, the reaction was considered positive, when the absorbance exceeded 3 times that of the mean of the negative sample[14].

Results and Discussion

Forty six labelled samples of pork, and 44 labelled samples of beef were examined for the presence of pork and beef. Table 2 summarizes the results of EIA using absorbed goat anti-pig and -cattle TMA sera.

Nineteen of the 46 samples labelled pork were found to give positive reactions for beef. This represents 41.3 per cent of the pork samples. Included in these 19 samples are 8 samples that gave negative reactions for pork.

Twenty three of the 44 samples labelled beef gave positive reactions for pork. This represents 52.3 per cent of the total beef samples. Among these are 3 samples that were negative for beef. Three other samples were found to be negative for both beef and pork. These samples gave positive reactions that were on the borderline for beef and pork. If the cut off point were to be lowered to 2 times the absorbance of the pure pork sample, they would have been positive for both beef and pork.

Table 3 shows a comparison of the products from the two factories. Substitution of pork with beef was found in 50 per cent of the pork products from both factories. Substitution of beef with pork was found in 78.6 per cent of the beef products from factory A and only in 6.3 per cent of the beef products from factory B.

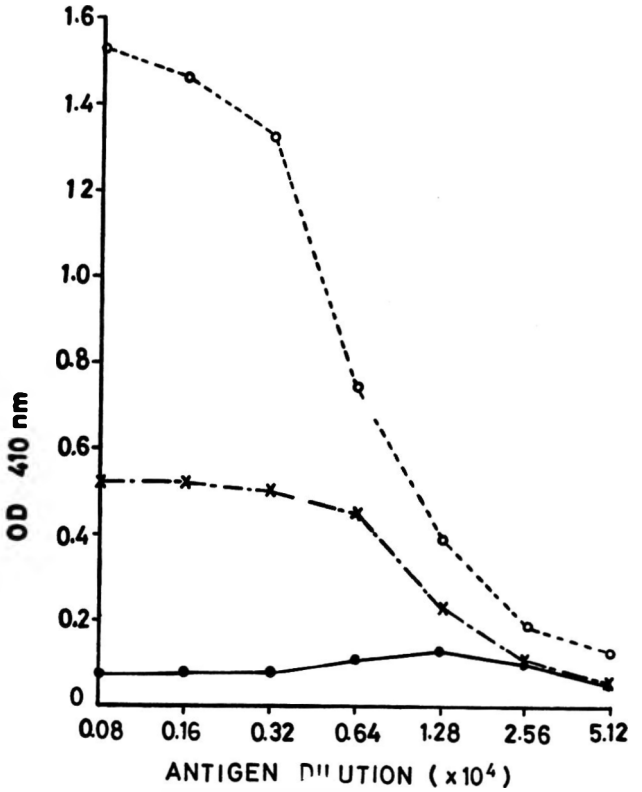

Fig. 1. Effect of milk powder on the detection of beef in pork products containing milk powder.

Pure beef O- - -O; Pure pork X- . -X; 5% milk powder ●—●

TABLE 2. DETECTION OF BEEF AND PORK MEAT IN PORK AND BEEF PRODUCTS BY ENZYME IMMUNOASSAY

Product label	Samples tested (No.)	Positive for pork (1)	Positive for beef (2)	Positive for (1)+(2)
Beef	44	23 (52.3%)	38 (86.4%)	23 (52.3%)
Pork	46	38 (82.6%)	23 (50%)	23 (50%)

TABLE 3. RESULTS OF ENZYME IMMUNOASSAY SHOWING FACTORY-WISE DETECTION OF BEEF AND PORK MEAT IN PORK AND BEEF PRODUCTS RESPECTIVELY

Factory	Antiserum used	Product label	Samples tested (No.)	Samples positive (No.)
A	Goat anti-cattle TMA	Pork	24	12 (50%)*
	Goat anti-pig TMA	Beef	28	22 (78.6%)+
B	Goat anti-cattle TMA	Pork	22	11 (50%)*
	Goat anti-pig TMA	Beef	16	1 (6.3%)+

*Substitution of pork with beef

+Substitution of beef with pork

This high rate of substitution of pork for beef and *vice-versa* in products from both factories cannot be referred to as adulteration, since the Kenya Bureau of Standards' Specification for the manufacture of beef and pork sausages allows inclusion of other unspecified meats. For beef products, the allowed minimum lean beef should be at least 32.5 per cent while the pork products should contain at least 52 per cent lean pork[15].

Fig. I shows the results of enzyme immunoassay (EIA) designed to examine the effect of 5 per cent milk powder on the detection of beef in pork sausages. This quantity of milk powder used in the sausage mix did not produce false positive reactions for beef in pork sausages. Apparently, milk powder does not contain antigens related to cattle TMA. It is also possible that the absorbed goat anti-cattle TMA serum has no antibody activity to bovine milk components. Species identification of meat can be achieved in meat products containing milk powder added as a binder using anti-TMA sera. Di Antiono *et al*[17]. were unable to differentiate between pork sausages containing bovine milk powder with those that contained bovine meat in immunodiffusion tests using anti-bovine sera supplied by a commercial firm. The

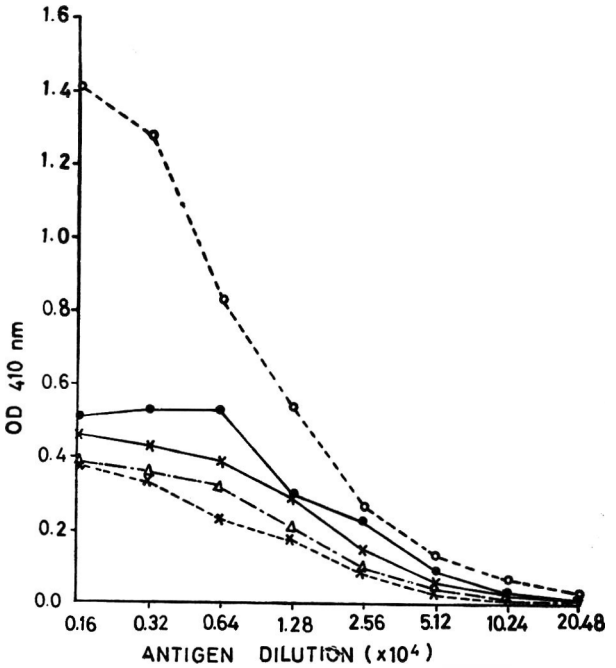

Fig. 2. Detection of beef in pork sausages using goat serum to cattle TMA in enzyme immunoassay.

Pure beef sausage O- - -O; Pure pork sausage △- . -△; 10% beef in pork sausage ●—●; 5% beef in pork sausage X—X; 1% beef in pork sausage X- - -X.

discrepancy between our results and those of Di Antonio *et al*[17]. may be due to the differences in the specificities of the antisera as well as in the methodologies.

Fig. 2 shows the results of EIA designed to detect the lowest level of beef in pork sausages. The method was able to detect 5 per cent beef in pork sausages ($P \leq 0.01$) but not 1 per cent. These results compare well with those reported earlier by Kang'ethe *et al*[5]. and Whittaker *et al*[6]. for fresh unheated meat samples. Lower levels of pork (1 per cent) in beef have been detected using a double antibody or 'sandwich' ELISA by Jones and Patterson[16]. The methods described previously[5,6,16] are suitable only for use in the examination of fresh unheated products.

In this study, we have shown that the presence of milk powder used as a binder in the sausage mix. does not impair the detection of beef in pork products. It has also been shown that the use of antisera to thermostable muscle antigen allows species identification of meat in raw unheated products (fresh sausages), heat treated or pasteurized products (luncheon meats) and also in commercially sterilized products (canned corned beef) in this type of an assay.

Acknowledgement

This project was sponsored by the Kenya National Council for Science and Technology, grant NCST/SEC/4400-419 and the Norwegian Agency for International Development (NORAD).

References

1. Swarts, K. S. and Wilks, C. R., An immunodiffusion method for the identification of the species of origin of meat samples. *Aust. vet. J.*, 1982, **59**, 21.

2. Dokerstein, K. H. and Greuel, E., The preparation of specific antisera for the identification of African antelopes species in agar-gel precipitation test. *Fleishwirtschaft* 1982, **62**, 1011.

3. Glesson, L. J., Slattery, W. J. and Sinclair, A. J., Species identification of meat sample. Unusual case. *Aust. vet. J.*, 1983, **60**, 27.

4. Slattery, W. J., and Sinclair, A. J., Differentiation of meat according to species by electrophoretic separation of muscle lactate dehydrogenase and esterase isoenzymes and isolectric focussing of soluble muscle proteins. *Aust. vet. J.*, 1983, **60**, 47.

5. Kang'ethe, E. K., Jones, S. J., and Patterson, R.L.S., Identification of the species of origin of fresh meat using an enzyme linked immunosorbent assay procedure. *Meat Sci.*, 1982, **7**, 229.

6. Whittaker, R. G., Spencer, T. L, and Copland, J. W., An enzyme linked immunosorbent assay for species identification of raw meat. *J. Sci. Fd Agric.*, 1983, **34**, 1143.

7. Milgrom, F. and Witebsky, E., Immunological studies of adrenal glands. 1. Immunization with adrenals of foreign species. *Immunol.*, 1982, **5**, 46.

8. Hayden, A. R., Use of antisera to heat-stable antigens of adrenals for species identification in thoroughly cooked beef sausages. *J. Fd Sci.*, 1981, **46**, 1810.

9. Kang'ethe, E. K., Lindqvist, K. J. and Gathuma, J. M., Immunological reactions of thermostable muscle antigens and their possible use in speciation of cooked and fresh animal meats. In. *Biochemical Identification of Meat Species.* by R.L.S., Patterson (Ed.) Elsevier Applied Science Publ. Ltd., London, 1984, 129.

10. Kang'ethe, E. K., Gathuma, J. M. and Lindqvist, K. J., Identification of the species of origin of fresh, cooked and canned meat and meat products using antisera to thermostable muscle antigens by Ouchterlony's double diffusion test. *J. Sci. Fd Agric.*, 1986, **37**, 157.

11. Crowle, A. J., In *Immunodiffusion.*, Academic Press, New York, 1973, 2nd Edn., 286.

12. Avrameas, S. and Ternynck, T. J., Biologically active water insoluble protein polymers. *J. biol. Chem.*, 1962, **242**, 1651.

13. Wilson, M. B. and Nakane, P. K., Recent development in the periodate method of conjugating horseradish peroxidase (HRPO) to antibodies. In *Immunofluorescence and related staining techniques.*, by W., Knapp, K. Holubar, and G. Wick, (Eds.) Elsevier/North-Holland Biomedical Press, Amsterdam. 1978. 215.

14. Savingy, De D. and Voller, A. J., Communication of ELISA data from laboratory to clinician. *J. Immunoassay.*, 1980, **1**, 105.

15. *Specification for Fresh or Cooked Pork and Beef Sausages, Pork and Beef Sausage Meat.* KS. 05-59, 1978. Kenya Bureau of Standards.

16. Jones, S. J. and Patterson, R.L.S., Double antibody ELISA for detection of trace amounts of pig meat in raw mixtures. *Meat Sci.*, 1985, **15**, 1.

17. Di Antonio, E., Anellini, P., Severini, M. and Vizzani, A., Detection of beef in the presence of powdered milk sausages declared to consist of pure pig meat. In *Biochemical Identification of Meat Species* by R.L.S., Patterson, Ed. Elsevier Applied Science Publ. Ltd., London, 1984, 86.

Species Differentiation in Raw and Heat Treated Meats Using Serological Methods (Agar Gel Precipitation Tests)

A. T. SHERIKAR, J. B. KHOT, B. M. JAYARAO AND S. R. PILLAI
Department of Food Hygiene & Veterinary Public Health,
Bombay Veterinary College, Parel, Bombay-400 012, India

Received 17 *October* 1986; *revised* 4 *June* 1987

Rabbit antisera to extracts of raw and partially heated (70°C) meats of buffalo, cattle, sheep, goat, pig and poultry were used in an immunodiffusion test to differentiate their raw, heated (70°C) and boiled (100°C) meats. The antisera reacted with raw and heated (70°C) meat extracts producing a spectrum of species specific precipitation bands which enabled their identification. Cross reactions were observed between related species but anti-poultry sera and antisera to heated pork extracts gave only species specific reactions. Antisera to raw and heated (70°C) meats showed weak reactions with minor variations when tested against extracts of heated (70°C) and raw meats. Boiled (100°C) meat extracts neither produced antibodies nor reacted with these antisera and hence could not be identified after pathopal concentration. Antisera to raw meats stored at –20°C for 9 years were also found to be competent.

Many techniques have been used to detect adulteration of meat of which the remarkable specificity of serological reactions was first demonstrated by Landsteiner[1]. The most commonly employed serological methods are the tube ring test[2] and agar gel precipitation test[3]. Tizard *et al*[4]. and Kurth and Shaw[5] found that the tube ring test could not be employed alone to prove the identity of the meat due to occurrence of false positive reactions and lesser sensitivity. The agar gel precipitation test can resolve the constituents of an antigenic mixture, compare these with reference antigens at the same time[6] and is sufficiently sensitive to detect most commercial degrees of adulteration. Further, this test has been found to be simple and convenient by several workers[7–12].

Serum proteins were used earlier but soluble meat extracts were observed to be superior for production of antisera in rabbits injected by the intramuscular route[7,13–15]. False positive reactions have also been reported with serum proteins because of the specificity of the antisera to serum or blood proteins rather than muscle proteins[16]. Very few reports are available on the detection of origin of heat treated meats and available antisera were unable to detect meats heated above 80°C[17,18]. In the present investigation, attempts have been made to study the application of the agar gel diffusion test by considering the spectra of the precipitation lines and further its application to meats heated to 70°C and 100°C using rabbit antisera to raw and heat treated meats.

Materials and Methods

Preparation of meat extracts for immunisation: Freshly collected lean meat samples from multiple sites of cattle, buffalo, sheep, goat, pig and poultry were first minced in a meat mincer and then homogenised to a paste by adding 75 ml of normal saline solution (NSS) containing 8.5 g NaCl/100 ml to 100 g of meat as described by Sherikar *et al*[9]. The extracts were filtered and centrifuged at 3000 rpm for 30 min. The supernatants were filtered through a seitz filter and the filtrates were tested for their sterility. The sterile meat extracts were distributed in 10 ml vials and thiomersal (1 :10,000) was added as a preservative. The protein contents were estimated by the biuret method[19]. In case of heated and boiled

meats, 100g of meat were heated to 70°C and boiled at 100°C, respectively for 20 min. The samples were cooled to room temperature, homogenised, filtered, centrifuged and the extracts were collected as described above. Boiled meat extracts were concentrated by addition of pathopal pellets (M/s. Ambika Enterprises, Bombay) at the rate of 5 g/20 ml before use. The extracts were allowed to stand for 45 min so that the pellets absorbed water and reduced the fluid to nearly 1/3 its original volume. The swollen pellets were reused after repeated washing with distilled water and then dried in the incubator at 37°C for 24 hr.

Immunisation schedule: Two rabbits each were immunised with respective antigens of raw meat extracts which contained 3.7-4.2 mg per cent protein. One millilitre of the antigen mixed with equal quantity of Freund's complete adjuvant was injected (deep) intramuscularly as described by Campbell *et al*[20]. at weekly intervals for 5 weeks. Test bleeding was done seven days after the last injection and agar gel diffusion test was carried out to monitor the immunoresponse. The serum was collected aseptically and distributed in 5 ml screw cap glass tubes. Thiomersal was added in the proportion of 1:10,000 and it was stored at -20°C.

In order to prepare antisera to heated (70°C) meats, water extracts of such meats containing 3.1 to 3.8 mg per cent protein were injected into the rabbits in the above manner whereas, extracts from boiled (100°C) meats containing 2.1 to 2.5 mg per cent protein were used.

Preparation of test meat extracts: Raw, heated and boiled meat extracts were prepared by weighing 20,50 and 100g respectively of lean meats, mixing with equal amounts of distilled water and heating to 70°C or boiling at 100°C for 20 min in case of heated and boiled meats. Heated and boiled meats were later cooled to room temperature. All the mixtures were homogenised, centrifuged (5000 rpm, 20 min) and the supernatants collected were used as test meat antigens, except boiled meat extracts which were concentrated as described earlier.

Immunodiffusion test: Immunodiffusion tests were carried out as described by Crowle[6]. Undiluted antiserum @ 5 μl was put in the central well and test meat extracts of raw, heated (70°C) and boiled (100°C) meats @ 5 μl were loaded in the peripheral wells. Antisera to raw meats raised 9 years ago and preserved at –20°C were also subjected to immunodiffusion test.

Results and Discussion

Use of antisera raised against raw meat extracts: Results of the study revealed that the rabbit antisera to buffalo, cattle, sheep, goat and pig raw meat extracts reacted with extracts of homologous species as well as showed cross reactivity amongst each other maintaining a spectrum of precipitation bands specific for each species (Table 1). However, both anti-poultry serum and its meat extracts produced only homologous reaction. Though the precipitation bands developed after 16 hr they became prominent at the end of 24 hr.

From Table 1, it is also evident that each species has more than one antigenic component. The pattern of these precipitin lines indicates the characteristics of the components, viz. their diffusion rates and their molecular weights. The component having a high rate of diffusion formed a precipitation line nearer the antiserum well bending towards the well. The diffusion components with lower diffusion rate formed precipitation lines near the antigen well bending towards the well. The components which had the same diffusion rate as that of antiserum, formed a straight line without exhibiting any bending effect. The corresponding concentration of these components determined the thickness or thinness of the precipitin lines formed, giving further information on the relative concentrations of all the components.

Van den Heever[21] also found that raw meats could be identified qualitatively based on the number of precipitin lines and their characters. Ramdass and Misra[12] could identify meats species on the basis of number of lines of identity and partial identity with antisera of different species. Since, poultry is not related phylogenetically with other species, cross reactions were not evidenced.

Extracts of heated (70°C) meats were tested against antisera to raw meat extracts, wherein precipitin lines were observed but the intensity of the bands were found to be weak. These meat extracts also showed cross reactivity with other species except poultry (Table 2). On comparison of the number and type of precipitin bands with raw meat extracts, it was observed that with the exception of buffalo all other species, showed minor variations. These variations reveal the succeptibility of native proteins to heat, probably resulting in decrease in the intensity of the bands, due to changes in the nature of the proteins[22]. Similar observations were recorded by Warnecke and Saffle[7] and Hashimoto and Yasui[15].

The antisera against raw meat extracts revealed no precipitin bands in case of boiled meat extracts. Absence of precipitin bands could be due to changes in the solubility of sarcoplasmic and myofibrillar proteins with increasing temperatures of heating above 70°C. At temperatures above 70°C uncoiling of the peptide helix along with loss of sulphydryl groups[23] could have resulted in the loss of antigenic properties due to which precipitin lines could not be observed. Ginsberg[23] also stated that meat samples which have been heated upto 80°C for 30 min will not react with sera prepared by use of unheated material.

The soluble properties and antigenic competence of test proteins of meat systems are altered considerably when products are highly heated rendering immunochemical methods ineffective when used to analyse suspect material or products heated above 80°C[11]. The results in the present study indicate that the test is adaptable to identify meats heated upto 70°C.

Use of previously raised antisera: The previously raised antisera against raw meat extracts of the animals under study were employed to observe their efficacy in immunodiffusion test. Reproducible results were observed indicating that the antisera can be preserved at -20°C for prolonged periods of time. However, Hayden[10] observed some non-specific cross reactivity between the rabbit antiserum to chicken troponin and extracts of beef sausage after storage of the antiserum for a period of 3 months, though that was specific for chicken species.

Use of antisera produced against heated meat extracts: Precipitation lines could not be obtained with raw meat antisera when tested against boiled meats. whereas reactions were found when they were tested against heated meat extracts. Based on these findings, it was envisaged to prepare antisera against extracts of meats heated to 70°C and 100°C. The antigens from boiled meat extracts used for the purpose of raising the antisera were concentrated three times in order to enhance the protein content of the extracts. The results of the study indicated that precipitin bands were observed in case of all the heated (70°C) meat extracts with the antisera

TABLE 1. SCHEMATIC REPRESENTATION OF REACTIONS BETWEEN RAW MUSCLE ANTIGENS AND ANTISERA TO RAW MEAT EXTRACTS IN OUCHTERLONY'S DOUBLE GEL DIFFUSION TEST

Antisera	Antigens					
	Buffalo	Cattle	Sheep	Goat	Pig	Poultry
Buffalo	———	- - - - -	- - - - -	- - - - -	- . - . -	0
Cattle	- - - - -	———	- - - - -	- - - - -	- . - . -	0
Sheep	- - - - -	- - - - -	———	- - - - -	- . - . -	0
Goat	- - - - -	- - - - -	- - - - -	———	- . - . -	0
Pig	- . - - -	- - - - -	- - - - -	- - - -	———	0
Poultry	0	0	0	0	0	———

——— : Reaction with homologous antigen.
- - - - - : Reaction with heterologous antigen showing line of identity
- . - . - : Reaction with heterologous antigen showing partial identity.
0 : No reaction

TABLE 2. SCHEMATIC REPRESENTATION OF REACTIONS BETWEEN HEATED MUSCLE ANTIGENS AND ANTISERA TO RAW MEAT EXTRACTS IN OUCHTERLONY'S DOUBLE GEL DIFFUSION TEST

Antisera	Antigens					
	Buffalo	Cattle	Sheep	Goat	Pig	Poultry
Buffalo	———	- - - - -	- - - - -	- - - - -	- . - . -	0
	———	- - - - -	- - - - -	- - - - -		
	———		- - - - -	- - - - -		
	———					
Cattle	- - - - -	———	- - - - -	- - - - -	- - - - -	0
	- - - - -	———	- - - - -	- - - - -		
		———				
		———				
Sheep	- - - - -	- - - - -	———	- - - - -	- . - . -	0
	- - - - -	- - - - -	———	- - - - -		
	- . - . -		———	- - - - -		
			———	- - - - -		
Goat	- - - - -	- - - - -	- - - - -	———	- . - . -	0
	- - - - -	- . - . -	- - - - -	———		
	- - - - -		- - - - -	———		
	- - - - -		- . - . -	———		
				———		
Pig	- - - - -	- - - - -	- - - - -	- - - - -	———	0
					———	
Poultry	0	0	0	0	0	———
						———
						———
						———

——— : Reaction with homologous antigen.
- - - - - : Reaction with heterologous antigen showing line of identity
- . - . - : Reaction with heterologous antigen showing partial identity.
0 : No reaction

to heated meats giving homologous reactions; however, cross reactions were also observed within the group except with antigen from poultry (Table 3). When the raw meat extracts of buffalo, cattle, sheep and goat were tested with antisera to extracts of heated meats, though precipitin bands were observed, they were of weak intensity and showed cross reactivity with other species except poultry (Table 4). But pig and poultry antisera gave only homologous reactions with heated and raw meat extracts.

Minor variations with respect to the number and type of bands were observed when the antisera were tested against raw meat extracts in comparison to the results obtained with heated meat extracts. These variations could be attributed to the type of antisera which consist of a mixture of antibodies against heated and native proteins[25]. Hashimoto and Yasui[15] stated that the species specificity is recognizable upto heating temperature of 70°C only in case of application of the antisera to which heated serum and myoglobin are used as antigens for their preparation.

Though antisera of buffalo, cattle, sheep and goat showed cross reactions with extracts of heated and raw meat of pig, anti-pig serum did not react with the above species. This could be attributed to the inability of the specific cross reacting fractions present in the pig antigen

to produce sufficient antibody titre to exhibit the presence of precipitin lines, since meats like pork are very sensitive to heat treatment[22].

No reactions were recorded with boiled meat extracts using the antisera to heated meat extracts.

Extracts of boiled (100°C) meats failed to produce antibodies in rabbits. Though antibodies may have been produced they were found to be very weak and non-specific[14,15]. Hayden[11] reported the loss of antigenic competence of meat on excessive heating.

A necessary condition for the detection of animal meats is the presence of native proteins in meats to denote their ability of antibody formation. So, the precipitin reaction appears only when there exist some corresponding native proteins in the extract of meats and strength of the reaction decreases with the amount of native proteins in the extracts. When all proteins in meat are denatured by heat, precipitin reaction does not take place any more.

When these denatured proteins are used as antigens, they do not develop antibodies in rabbits so that the precipitin test cannot be adopted to such meats which are severely heated.

From the present study, it was concluded that species

TABLE 3. SCHEMATIC REPRESENTATION OF REACTIONS BETWEEN HEATED MUSCLE ANTIGENS AND ANTISERA TO HEATED MEAT EXTRACTS IN OUCHTERLONY'S DOUBLE GEL DIFFUSION TEST

Antisera	Antigens					
	Buffalo	Cattle	Sheep	Goat	Pig	Poultry
Buffalo	————	– – – – –	– – – – –	– – – – –	– . – . –	0
	———	– – – – –	– – – – –	– – – – –		
	———	– – – – –	– . – . –	– . – . –		
	———					
Cattle	– – – – –	———	– – – – –	– – – – –	– . – . –	0
	– – – – –	———	– – – – –	– – – – –		
	– – – – –	———	– . – . –	– . – . –		
		———				
Sheep	– – – –	– – – – –	———	– – – – –	– . – . –	0
	– – – –	– – – – –	———	– – – – –		
	– . – . –	– – – – –	———	– – – – –		
			———	– – – – –		
Goat	– – – – –	– . – . –	– – – – –	———	– – – – –	0
	– . – . –	– . – . –	– – – – –	———	– . – . –	
	– . – . –		– – – – –	———		
			– – – – –	———		
			– – – – –	———		
				———		
Pig	0	0	0	0	———	0
					———	
					———	
Poultry	0	0	0	0	0	———
						———

——— : Reaction with homologous antigen.
– – – – – : Reaction with heterologous antigen showing line of identity
– . – . – : Reaction with heterologous antigen showing partial identity.
0 : No reaction

TABLE 4. SCHEMATIC REPRESENTATION OF REACTIONS BETWEEN RAW MUSLCE ANTIGENS AND ANTISERA TO HEATED MEAT EXTRACTS IN OUCHTERLONY'S DOUBLE GEL DIFFUSION TEST

Antisera	Antigens					
	Buffalo	Cattle	Sheep	Goat	Pig	Poultry
Buffalo	————	- - - - -	- - - - -	- - - - -	- . - . -	0
Cattle	- - - - -	————	- - - - -	- - - - -	- - - - -	0
Sheep	- - - - -	- - - - -	————	- - - - -	- - - - -	0
Goat	- - - - -	- - - - -	- - - - -	————	- . - . -	0
Pig	0	0	0	0	————	0
Poultry	0	0	0	0	0	————

———— : Reaction with homologous antigen.
- - - - - : Reaction with heterologous antigen showing line of identity
- . - . - : Reaction with heterologous antigen showing partial identity.
0 : No reaction

of origin of raw meats and meats heated upto 70°C could be identified using antisera to raw and heated (70°C) meat extracts. However, the spectrum of the precipitation bands, their position and intensity is required to be compared with standard photographs or reactions of known standard meat extracts for which technical expertise is necessary.

References

1. Landsteiner, K., *Specificity of Serological Reactions.* Dover Publ., New York, 1945.
2. Oudin, J., Immunological methods of analysis involving selective precipitation in solidifying media. *Comp. Rand. Acad. Sci.*, 1946, **222**, 115.
3. Ouchterlony, O., Antigen antibody reactions in gels. *Acta. Path Microbiol. Scand.*, 1949, **26**, 507.
4. Tizzard, I. R., Fish, N. A. and Caoili, F., False positive reactions in the immunopreciptation test for meat identification. *J. Fd Prot.*, 1982, **45**, 353.
5. Kurth, L. and Shaw, F. D., Identification of species of origin of meat by electrophoretic and immunological methods. *Fd Technol. Aust.*, 1983, **35**, 328.
6. Crowle, A. J., *Immunodiffusion*, Academic Press, New York, 1973.
7. Warnecke, M. O. and Saffle, R. L. Serological techniques—identification of species origin and comminuted meats, *Proceedings of Reciprocal Meat Conference*, 1968, 391.
8. Van den Heever, L. W. and Marias, S., Specific serological identification of ostrich meat and meat products. *J. S. Afr. vet. med. ass.*, 1975, **46**, 261.

9. Sherikar, A. T., Ajinkya, S. M., Khot, J. B. and Vaidya, A. M. Differentiation of meats by gel diffusion techniques. *Indian J. anim. Sci.*, 1979, **49**, 350.

10. Hayden, A. R., Detection of chicken flesh in beef sausages. *J. Fd Sci.*, 1977, **42**, 1189.

11. Hayden, A. R., Use of antisera to heat stable antigens of adrenals for species identification in thoroughly cooked beef sausages. *J. Fd Sci.*, 1981, **46**, 1810.

12. Ramdass, P. and Misra, D. S., Differentiation of meats of different species of animals by slide agar gel diffusion test. *Indian J. anim. Sci.*, 1983, **53**, 1361.

13. Lynch, G. R., The technique for protein test and its forensic value. *Analyst, Lond.*, 1928, **53**, 5.

14. Proom, H., The preparation of precipitating sera for the identification of animal species. *J. Path. Bact.*, 1943, **55**, 419.

15. Hashimoto, Y. and Yasui, T., Research on the detection of meat by serological test. *Fac. Agric., Hokkaido*, 1957, **50**, 171.

16. Gleeson, K. J., Slattery, W. J. and Sinclair, A. J., Species identification of meat samples: An unusual case. *Aust. vet. J.*, 1983, **60**, 127.

17. Karpas, A. B., Myers, W. L. and Segre, D., Serological identification of origin of sausage meats. *J. Fd Sci.*, 1970, **35**, 150.

18. Carnegie, P. R., Ilic, M. Z., Etheridge, M. O. and Stuart, S., Use of histidine dipeptides and myoglobin to monitor adulteration of cooked beef with meat from other species. *Aust. vet. J.* 1985, **62**, 272.

19. Dietterbrandt, M., Application of the Weichselbaum biuret reagent to the determination of spinal fluid protein. *Am. J. Clin. Path.* 1948, **18**, 439.

20. Campbell, D. H., Garvey, T. S., Cremer, W. E. and Sussdorf, D. H., *Methods in Immunology*, W. A. Bengomentic, New York, 2nd Edn. 1970.

21. Van den Heever, L. W., Serological identification of meat from different species by the agar gel diffusion method *J. S. Afr. vet. med. ass.*, 1962, **33**, 215.

22. Hultin, H. O., in *Principles of Food Sciences*. Part I. *Food Chemistry*, Marcel Dekkor Inc., New York, 1st Edn. 1976.

23. Lee, F. A., *Basic Food Chemistry*, AVI Publishing Co. Inc., West Port, Connecticut, 1975. Ch. 17.

24. Ginsberg, A., The differentiation of meats by precipitation test. *Vet. Res.*, 1948, **60**, 683.

25. Furth, J., Antigenic character of heated protein., *J. Immunol.*, 1925, **10**, 777.

Studies on SO_2 Treatment of Minced Goat Meat. 1. Effect on Storage Life and Organoleptic Qualities

K. S. REDDY* AND USHA V. MANDOKHOT

Department of Veterinary Public Health & Epidemiology, College of Veterinary Sciences, Haryana Agricultural University, Hissar-125 004, Haryana, India

Received 22 *November* 1986; *revised* 19 *May* 1987

The effect of SO_2 on the bacteriological stability and eating quality of minced goat meat was studied at 7°C and 15°C storage temperature. At 425 and 450 p.p.m., SO_2 conferred maximum bacteriological stability resulting in increased storage life. There was no adverse effect on the eating quality, or on the odour of meat. The results also indicated SO_2 to be a good colour fixative agent for goat meat.

One of the major problems of today's meat industry is to check growth of surface bacteria which spoil the meat. Usually, this is achieved by lowering the temperature of storage. In tropical countries, refrigeration, however, is quite often undependable and an additional use of some other preservative might be beneficial. Use of sulphurdioxide, especially in comminuted meats is permitted in U.K., Australia and India. However, not much information is available regarding its efficacy as a meat stabilizing agent. Besides the stabilizing effect, SO_2 being a reducing agent, might also act as a colour fixative thereby providing an alternative for nitrites. The present studies were, therefore, undertaken to assess the efficacy of SO_2 in retarding the spoilage of meat at 7 and 15°C, and to fix the colour of the meat.

Materials and Methods

The lean meat from leg and shoulder regions of adult male goat carcasses (one to two years old) was purchased and minced at the retail shop with the hand mincer within two hours of slaughter and brought to the laboratory in polythene bags kept in ice box. The samples were kept at refrigeration temperature till processed, which usually was within two hours of its mincing.

**Present address:* Department of Meat Science, College of Veterinary Sciences, Tirupati, Andhra Pradesh, India.

Sodium disulphite ($Na_2S_2O_5$. Sarabhai Chemicals, Baroda) was used as the source of SO_2 and the dose was calculated on the basis of its expected yield. A near uniform level of SO_2 (indicated by SO_2 estimation[1] in samples drawn from three different places) in one to two kg of minced meat was obtained in approximately 10 min. of mixing. The treated samples were divided into smaller lots of about 50g each, packed in polythene bags and kept for storage. Minced meat samples from the same source not treated with SO_2 but handled, packed and stored similarly served as controls.

Effect of various SO_2 doses on meat stabilization: The minced goat meat obtained from the same source at the same time were treated in one experiment with sodium disulphite to get 300, 450 and 600 ppm. of SO_2 and in another to get a SO_2 concentration of 375, 400, 425 and 450 ppm. The samples were incubated at 7 ± 0.5 and 15 ± 0.5°C along with the untreated controls till spoilage was detected. Samples from both treated and untreated meat were drawn daily to perform sensory tests like odour, colour and boiling tests[2]. The sensory evaluation was done by six persons and meat was considered spoiled even if a single evaluator indicated development of abnormal (sour or any other) odour. The colour was judged on a three point hedonic scale. For bacteriological evaluation (SPC 30°C and 7°C)[3] and pH and SO_2 determination, the samples were drawn on 0,3,5,7,9,11 and 13 days from 7°C stored and 0,2,4 and 6 from 15°C stored samples. The pH of the samples was determined thrice by introducing the electrodes (Systronic digital pH meter) in the minced meat sample and the average value taken. The total residual SO_2 in uncooked and cooked minced meat (15 lb pressure, 10 min) was determined by iodimetric titration method[1] by taking 10 g sample homogenised in 200 ml of distilled water, 10 ml of saturated sodium bicarbonate solution and 5 ml of concentrated HCl for direct distillation into iodine solution. Blanks (untreated control) were also run simultaneously.

Effect of initial level of bacterial contamination on SO_2 treatment: Three minced meat samples obtained from three different sources, were treated with SO_2 at 450 ppm and stored at 7 ± 0.5 and 15 ± 0.5°C along with untreated controls. The samples were drawn for sensory (odour, colour changes) evaluation, pH determination and bacteriological (SPC) (30C) examination as described above.

All the experiments were conducted in triplicate and the average values are reported. The data of all the experiments conducted, were subjected whenever necessary to appropriate statistical analysis[4].

Sensory evaluation of treated minced meat: The semi trained panel members who were able to identify the odd sample in the set of three at least twice in a trial of three replicates were enlisted for the triangle test[5]. A set consisting of one odd treated (300, 450, 600 ppm) and two untreated minced meat samples were drawn on zero and 3rd day of storage at 7°C, sodium chloride was added, to get a final concentration of one per cent, cooked in a domestic pressure cooker for 5 min, offered to the panel members to identify the odd sample and the results analysed following the procedure of Ranganna[6]. Uncooked samples of both treated (450 p.p.m.) and untreated minced meat stored for zero and three days at 7°C were also presented to the same panel in screw capped glass bottles for evaluation of colour and odour using a 5 point hedonic scale.

Paired test on consumers: About 50g of each treated and untreated minced meat was provided to each of the 60 individuals who were requested to cook the samples separately but under identical conditions for consumption. A sample score card was provided to indicate the preference if any, amongst the two samples and the results analysed[6].

Results and Discussion

The treatment of minced goat meat with 300, 450 and 600 p.p.m. of SO_2 was found to decrease the rate of multiplication of the spoilage bacteria. In untreated meat samples, the standard plate count (30°C) of 20×10^5/g present on the zero-day of storage increased to 33333×10^5/g during the log phase of five days (zero to 5th day of storage) of bacterial growth with a multiplication rate of approximately 250 colony forming units (CFU)/hr. In 450 and 600 ppm of SO_2 treated meat samples, the rate of multiplication was reduced to 2 CFU/hr and 0.7 CFU/hr. respectively. During the log phase of growth of 5 days (9th to 13th day of storage), the CFU increased to 143×10^5/g and 47×10^5 from 8×10^5 and 4×10^5/g, respectively. It was, however, difficult to assess how much the reduced rate of multiplication of bacteria contributed to the increased keeping quality of the meat since both 450 and 600 p.p.m. of SO_2 treated meat samples spoiled on the same day of storage.

The treatment also increased the bacteriological stability as after an initial reduction, the number of bacteria did not vary significantly for a considerable period of time. In contrast, the bacterial flora in untreated samples revealed significant continuous increase in its number till the day of spoilage. The enhanced bacteriological stability was found to be directly associated with the delay in spoilage changes. Treated meat revealed spoilage changes on the 7th (300 ppm) and 13th (450, 600 ppm) day of storage while the untreated did so on the 5th day of storage. The increased bacteriological stability of meat appeared to be due to bacteriostatic action of SO_2 because, a prolonged lag phase (3 to 9 days) of growth was observed in treated meat samples but not

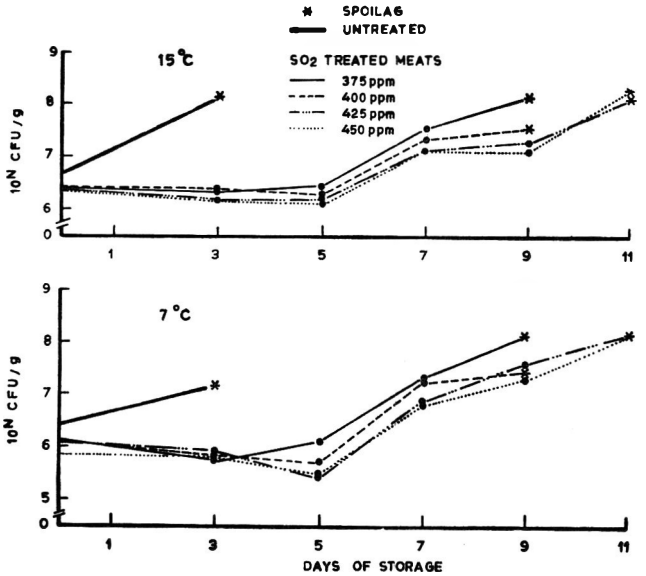

Fig. 1. Effect of different levels of SO_2 on storage life of minced meat (Initial = pH 5.75)

in untreated controls. The increase in SO_2 dose beyond 450 ppm level, however, did not appear to have any beneficial effect as the meat treated with 450 and 600 ppm of SO_2 spoiled on the same day of storage. Therefore, to find out the maximum level of SO_2 beyond which any increase in the dose would have no additional effect, the minced meat sample was treated with 450 and 375, 400, 425 ppm of SO_2 and was stored at 7°C (Fig. 1). The samples treated with 375, and 400 ppm of SO_2 behaved similarly in that both spoiled on the 9th day of the storage and the spoilage bacteria revealed dormancy or prolonged lag phase of growth upto 5th day of storage while the samples treated with 425 and 450 ppm SO_2 spoiled on 11th day. It thus, appeared that the SO_2 dose could be lowered to as low as 425 ppm to obtain maximum preservative effect.

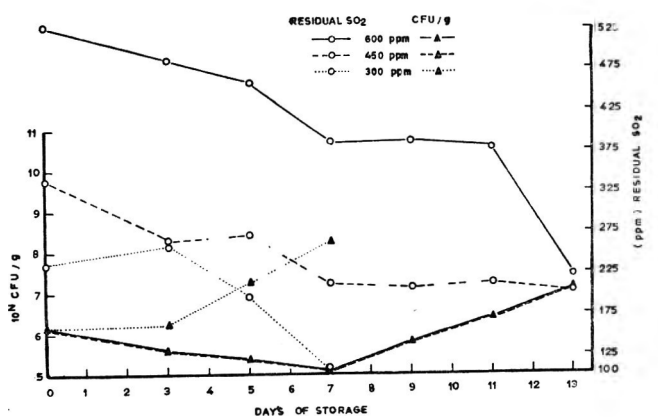

Fig. 2. Relationship of residual SO_2 with microbial growth in treated minced meat stored at 7°C

Bacteriological analysis of treated and untreated meat samples stored at 15°C also revealed the same trend as was observed in the treated and untreated samples stored at 7°C, except the period of bacteriological stability and the rate of growth of bacteria. The former was found to be comparatively reduced while the latter increased indicating the influence of storage temperature on the bacteriostatic action of SO_2. The action was observed to be more pronounced at 7°C than at 15°C.

The initial level of bacterial contamination was also found to affect the preservative efficacy of SO_2. The sample with initial contamination level of 7.6×10^7 CFU/g on treatment spoiled as early as on 6th day of storage while the samples with lower degree of contamination (7.1 and 6.9×10^5 CFU/g) under similar conditions spoiled on 13th day at 7°C. Christian[7] also, reported that the stability of SO_2 treated mince depended upon the initial level of bacterial contamination.

Correlation between the residual SO_2 and the bacterial growth during storage at 7 and 15°C is depicted in Fig. 2 and 3 respectively. The decrease in SO_2 contamination started with immediate effect from the zero-day of storage. This reduction was probably on account of conversion of SO_2 to reversibly bound form during processing. However, in general, a negative correlation,

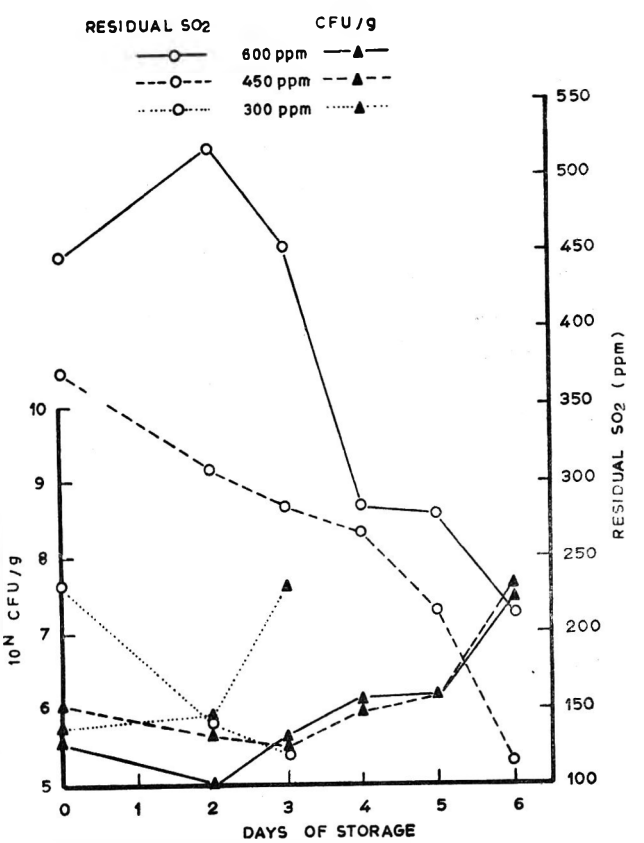

Fig. 3. Relationship of residual SO_2 with microbial growth in treated minced meat stored at 15°C

TABLE 1. ACCEPTABILITY OF TREATED MINCED MEAT

	No. of judges	Difference in treated and non treated meat		Indicated preference		Accepted treated‡ meat+ (No)
		Not detected (No)	Detected (No)	Treated (No)	Not treated (No)	
			Triangle test			
Trial I	14	12(86)***	+2(14)	—	—	14(100)***
Trial II	12	10(83)***	2(17)	—	—	12(100)***
Trial III	9	7(78)***	2(22)	—	—	9(100)***
			Paired test			
	38 (Males)	12(32)	26(68)	26(68)**	0	38(100)***
	13 (Females)	4(31)	9(69)	7(59)	2(15)	11(85) ***
	10 Children	4(40)	6(60)	5(50)	1(10)	9(90) ***
Total	61	20(33)	41(77)	48(62)*	3(5)	58(95) ***

*P<0.05; **P<0.01 ***P<0.001

+Two judges were the same individuals who detected difference in treated and not treated meat in all the three trials.

°Average values of the results of three replicates of each trial

§Figures in parentheses indicate per cent

‡Includes preference; + no preference.

though insignificant, was found to exist between SO_2 concentration and bacterial growth. A critical level of SO_2 at which antimicrobial activity would cease, could not be fixed because the residual SO_2 concentration upto which bacterial multiplication was inhibited varied in all the three doses used. The residual SO_2 was 197.53, 206.52 and 378.60 ppm in meats treated with 300, 450 and 600 ppm doses of SO_2, respectively and stored at 7°C. The reduction in SO_2 concentration was comparatively faster at 15°C and similarly the increase in bacterial numbers was also faster. The SO_2 level in cooked meat samples was found to be less by half to one third as compared to the corresponding uncooked ones during the storage.

The colour in all the treated samples turned to be attractive bright pink immediately after SO_2 treatment and the colour score was very high and continued to be so till the day of spoilage on which it dropped down. Also the colour of all the treated meat samples at different storage periods was preferred over the untreated samples at the corresponding storage periods by the panel members without exception. The preference was found to be highly significant indicating that SO_2 was a good colour fixative for goat meat. The colour fixative property of SO_2 was probably due to its being a reducing agent. The presence of the reducing agent in meat is known to preserve the desired colour of fresh meat by maintaining the iron of heme in the reduced state as in oxymyoglobin[8]. The reducing action of SO_2 appeared to be very quick as the meat samples developed bright pink/red colour immediately after the treatment. The period of maintenance of this colour appeared to depend upon the dose of SO_2 upto a certain level, since it dropped below the critical level earlier in 300 ppm SO_2 treatment than in 450 and 600 ppm SO_2 treatment.

SO_2 treatment was not found to alter the meaty flavour as no abnormal odour or vapour odour in the boiling test was detected. Similarly, the SO_2 treatment was not found to alter the overall acceptability of meat in the triangular test (Table 1). The acceptability of SO_2 treated meat was further proved when a paired trial on consumers was conducted. The treated meat was not only found to be acceptable and as good as untreated fresh meat but was preferred by 62 per cent consumers as they reported it to be more tender than the untreated one (Table 1). It is, however, difficult to conclude on the basis of these results that SO_2 enhanced the eating quality of the meat. A more detailed study is necessary to understand the effect of SO_2 on the enhancement of eating quality of the meat.

In view of the results obtained in the present study, it was very much evident that SO_2 could be used in meats as in other foods, to prolong its storage life without affecting its eating quality.

References

1. Amerine, M. A., Berg, H. W. and Cruess, M. V., *The Technology of Wine Making* The Avi Publ. Com., Inc. West Port, Conn., 1972, 2nd Edn.
2. Thornton, H. and Gracey, J. F., *Text Book of Meat Hygiene* Billiere Tindall, London. 1974, 6th Edn. 148.
3. *Standard Methods for the Examination of Dairy Products*, American Public Health Association, New York, 1966, 11th Edn.,
4. Snedecor, C. W. and Cochren W. C., *Statistical Methods.* Onford and IBH Publishing Co. Calcutta, 1967. 6th Edn.
5. Weir, C. W., *The Science of Meat and Meat Products.* W. H. Freeman and Co., San Francisco, 1960, 213.
6. Ranganna, S., *Analysis of Fruit and Vegetable Products'* McGraw-Hill Publishing Co. Ltd., New Delhi, 1979, 299.
7. Christian, J.H.B., Preservation of minced meat with sulphur dioxide. *C.S.I.R.O. Fd Pres. Q.*, 1963, **23**, 30.
8. Fox, J. B. Jr. Chemico-physical properties of meat pigments as related to color. in *Proceedings of the Meat Industry Research Conference*, held at University of Chicago, March 21-22. 1968, 12.

Changes in Flesh Lipids of Seer Fish During Frozen Storage

A. A. FAZAL AND L. N. SRIKAR

Department of Biochemistry,

University of Agricultural Sciences, College of Fisheries, Mangalore-575 002, India

Received 22 *November* 1986; *revised* 2 *June* 1987

Changes in total lipid (TL), phospholipid (PL), neutral lipid (NL), peroxide value (PV) and free fatty acid (FFA) were studied in seer fish, *Scomberomorus guttatus* during frozen storage (180 days) at -20°C. Initially, seer fish had a TL content of about 4%, PL 16% of TL and NL 84% of TL. On storage, there was a decrease in PL and increase in NL fractions. PV and FFA increased but were within acceptable limits during the period of frozen storage. Gas chromatographic analysis revealed that the total saturated fatty acids (SFA) of NL were higher (54.47%) than the unsaturated fatty acids (45.51%), while the polyunsaturated fatty acids (PUFA) were more (25.85%) than the mono-unsaturated fatty acids, (MUFA) (19.66%). During frozen storage, there was a drastic decrease in PUFA while an increase in SFA and MUFA was noticed. PL showed highest amount of SFA (67.25%) followed by MUFA (20.65%) and PUFA (11.95%). During frozen storage, SFA and MUFA decreased but there was an increase of PUFA.

Seer fish is a highly cherished food fish of India. It also takes a good share in the export market. The quality of this fish is impaired as lipid decomposition and protein denaturation occur during frozen storage. An understanding of the changes in lipids and proteins is, therefore, important both from the point of view of nutrition and of proper utilisation. Comparatively, very limited information is available on these aspects. However, a number of reports are available on the changes in lipids and proteins of other fish *viz.*, Indian oil sardines[1-3] Atlantic mackerel[4], Cod[5], Herring[6] and Skipjack[7]. The present investigation was aimed at analysing the lipid composition of seer fish and study the changes that occur during frozen storage.

Materials and Methods

Seer fish, *Scomberomorus guttatus* purchased from the local market was transported to the processing laboratory in iced condition. Steaks were prepared by cutting seer fish manually to 1 inch thickness. The steaks (500g each) so prepared were wrapped with polythene sheets in trays and immediately frozen in a coli freezer for 48 hr. The frozen steaks were glazed (dipped for 30 sec.) in chilled water, packed in polythene bags and stored at -20°±2°C in a master carton.

The lipids were extracted from the sample according to the method of Bligh and Dyer[8] in an atmosphere of nitrogen. Lipid samples were preserved at -18°C under nitrogen until further analyses. The neutral (NL) and phospholipids (PL) were separated by silicic acid column chromatography[9]. PL were digested using 70 per cent perchloric acid until the digest became colourless and clear[10]. Phosphorous content in the digest was determined according to Fiske and Subba Row[11]. Fatty acid composition of NL and PL were determined by gas chromatography. Methyl esters of fatty acids were prepared according to AOAC[12] and analysed with a varian gas chromatograph model 3700 equipped with a

TABLE 1. TOTAL LIPID (TL), NEUTRAL LIPID (NL), POLAR LIPID (PL), PEROXIDE VALUE (PV) AND FREE FATTY ACID (FFA) CONTENTS OF SEER FISH DURING STORAGE AT -20°C

Storage period (days)	TL (g%)	NL (% of TL)	PL (% of TL)	PV*	FFA (% oleic acid)
0	4.18	84.02	15.98	0.42	2.55
30	3.81	85.09	14.91	0.95	3.91
60	4.10	86.11	13.89	1.53	4.88
90	3.82	86.74	13.26	1.73	5.23
120	4.13	87.03	12.97	2.84	5.42
150	3.77	87.90	12.10	2.59	5.62
180	3.56	88.42	11.58	3.51	5.79

Mean of 3 determinations
*m.moles of O_2 / kg. fat.

hydrogen flame ionisation detector (FID) and computing integrator having sensitivity 1×10^{-10} Amp/mv. A glass column of 1.83 m × 4.0mm (i.d.) packed with 10 per cent Silar 5 CP on gaschrom. Q (100-120 mesh). was used. The column temperature was programmed from 150 to 240°C at 5°C/min.

PV was estimated by the method of Hills and Thiel[13] modified as follows. An aliquot of chloroform extract containing about 10 mg lipid was vacuum dried and dissolved in 4 ml Analar benzene and 2 ml methanol; colour development was made using a drop each of ammonium thiocyanate and ferrous chloride. FFA was determined by improved titrimetric method of Ke *et al*[14].

Results and Discussion

The TL content in the frozen sample before storage was 4.2 per cent; the contents of PL and NL were about 16 and 84 per cent of TL respectively (Table 1). During frozen storage for 180 days, the PL content in the sample decreased to 11.6 per cent while the NL increased to about 88.4 per cent. The decrease in PL is probably due to phospholipase(s) activity as reported for Cod[5], Skipjack[7] and Carp muscle[15]. Sarvadeva and Srikar[16] demonstrated the release of FFA from PL into NL during frozen storage. Our observations are in agreement with the above results.

An increase in PV and FFA was observed during the storage (Table 1). PV increased from an initial value of 0.4 to 3.5 m. moles of oxygen per kg lipid at the end of 6 months storage. An 8.4 fold increase in PV was indicative of autooxidation. Similar observations have been made by Srikar and Hiremath[1] in oil sardine during 16 weeks storage at -20°C. The increase in FFA was from 2.55 to 5.79 per cent during the period of storage (Table 1). The increase was about 127 per cent, steady for the first 90 days and rather slow during the latter period.

The changes in fatty acid composition in NL and PL are given in Table 2. The most prominent fatty acids in PL were 16:0, 22:0, 16:1, 18:1, 18:2 and 20:5. After storage for 6 months, 16:0, 18:0, 16:1, 18:1 and 18:2 were found to be decreasing while, 22:0, 20:2, 20:4,20:5 fatty acids increased. NL contained 16:0 (25 per cent), 18:0 (6.4 per cent), 22:0 (17 per cent), 18:1 (19 per cent) 20:4 (6.3 per cent) and 22:6 (7.1 per cent) as major fatty acids. During frozen storage, the fatty acids 14:0, 20:4, 22:5 and 22:6 decreased while 16:0, 18:0, 22:0 and 18:1 increased. A considerable increase in 16:0 was noticed indicating that it is released by the hydrolysis of lipids.

These results suggest that during frozen storage, PL having certain fatty acid combinations are preferentially hydrolysed by lipolytic enzymes during frozen storage.

TABLE 2. FATTY ACID COMPOSITION OF NEUTRAL LIPID (NL) AND PHOSPHOLIPID (PL) OF SEER FISH STORED AT—20°C FOR SIX MONTHS

Fatty acid	Neutral lipid		Phospholipid	
	Initial (%)	Final (%)	Initial (%)	Final (%)
12:0	0.01	tr	0.25	tr
13:0	0.01	tr	—	—
14:0	2.31	1.61	1.25	0.02
15:0	0.63	0.43	0.77	0.01
16:0	24.97	28.97	41.92	11.72
17:0	2.07	2.11	—	—
18:0	6.44	7.02	17.59	5.46
20:0	0.96	0.79	—	—
22:0	17.07	17.85	5.47	38.12
Total SFA	54.47	58.78	67.25	55.33
16:1	0.69	0.77	4.76	0.50
18:1	18.97	25.42	15.89	5.64
Total MUFA	19.66	26.19	20.65	6.14
18:2	1.50	0.95	6.57	0.71
18:3	2.08	2.02	—	—
20:2	2.05	2.00	0.11	10.30
20:4	6.34	5.37	0.08	8.37
20:5	3.98	3.33	5.19	14.08
22:5	2.78	1.33	—	—
22:6	7.12	tr	tr	tr
Total PUFA	25.85	15.00	11.95	38.42

SFA: Saturated fatty acid,
MUFA: Monounsaturated fatty acid
PUFA: Poly unsaturated fatty acid,
tr: traces
All values are means of 2 determinations

This may be surmised from the fact that during storage, the total SFA and MUFA in the PL fraction decreased while PUFA increased. Ohshima *et al*[7], employing HPLC found that fatty acid combinations of 16, 22, 16, 20, 22, 22, 18, 22, 20, 22, 16, 18, and 18, 20 were prominent in fresh skipjack flesh before storage. During storage, the percentages of (22, 22), (18, 22) and (20, 22) of the PC increased gradually and that of (16, 22) decreased distinctly indicating the preferential hydrolysis of PC. A similar preferential hydrolysis may be present in seer fish also. Further studies on PL classes and substrate specificity of phospholipase need to be carried out to confirm this view.

Acknowledgement

We thank Dr. Gopakumar, Dr. Devadasan and Dr. Mukundan of CIFT, Cochin for their help in GC analysis and Prof. H.P.C. Shetty for his interest in this study. The fellowship awarded to the first author by ICAR is gratefully acknowledged.

References

1. Srikar, L. N. and Hiremath, G. C., Fish preservation. I. Studies on changes during frozen storage of oil sardine. *J. Fd Sci., Technol.*, 1972, 9, 191.
2. Nair, P.G.V., Gopakumar, K. and Nair, M. R., Lipid, hydrolysis in mackerel during frozen storage. *Fish. Technol.*, 1976, **13**, 111.
3. Nair, P.G.V., Antony, P. D., Gopakumar, K. and Nair, M. R. Lipid breakdown in oil sardine during frozen storage. *Fish. Technol.*, 1978, **15**, 81.
4. Ackman, R. G., in *Advances in Fish Science and Technology*, by J. J. Connell (Ed), Fishing News (Books) Ltd., England, 1980 86.
5. Ohshima, T., Wasa, S. and Koizumi, C., Enzymatic hydrolysis of phospholipids in cod flesh during storage. *Bull. Jap. Soc. sci. Fish.*, 1983, **49**, 1397.
6. Ohshima, T., Wada, S. and Koizumi, C., Effect of accumulated free fatty acid on reduction of salt soluble protein of cod flesh during frozen storage. *Bull. Jap. Soc. sci. Fish.*, 1984. **50**, 1567.
7. Ohshima, T., Wada, S. and Koizumi, C., Preferential enzymatic hydrolysis of phosphotidyl choline in skip jack flesh during frozen storage. *Bull. Jap. Soc. sci. Fish.*, 1984, **50**, 2091.
8. Bligh, E. G. and Dyer, W. J., A rapid method of total lipids extraction and purification. *Can. J. Biochem. Physiol.*, 1959, **37**, 911.
9. Rouser, G., Kritchvsky, G. and Yamamoto, A., Lipid chromatographic analysis, Dekker Inc. New York, 1967, Vol. I, 99.
10. Allen, R.J.C., in *Laboratory Techniques in Biochemistry and Molecular Biology* by T. S. Work, and E. Work, (eds), North Holland Publishing Co., Amsterdam, 1972, Vol. III, 355.
11. Fiske, N. and Subba Row, Y., The colorimetric determination of phosphorus. *J. biol. Chem.*, 1925, **66**, 375.
12. *Official Methods of Analysis*, Association of OfficialAnalytical Chemists. Washington, D. C. 1975, 12th Edn.
13. Hills, L. G. and Thiel, G. C., Ferric thiocyanate method of estimation peroxides in the fat of butter milk and dried milk. *J. Dairy Res.*, 1946, **14**, 340.
14. Ke, P. J., Woyewoda, A. D., Regier, L. W. and Ackman, R. G. *Env. Canada Fish. Marine Serv. Technol. Branch.*, Halifax, New Series Circular, 1976, **61**, 1.
15. Wu, C., Nakagawa, H. and Sataka, K., Formation of glycery phosphoryl choline by enzymatic decomposition of phosphatidyl choline in carp ordinary muscle. *Bull. Jap. Soc. sci. Fish.*, 1974, **40**, 835.
16. Sarvadeva, A. and Srikar, L. N., Changes in fatty acids of frog legs during frozen storage, *J. Fd Technol.*, 1982, **17**, 79.

Selective Grinding as a Basis for Separating White Pepper

P. P. Thomas, A. Nirmala Menon, A. V. Bhat and A. G. Mathew
Regional Research Laboratory (CSIR), Trivandrum-695 019, India

Received 13 *October* 1986; *revised* 2 *March* 1987

Difference in the behaviour of black skin and inner white core of dry black pepper to compressive forces was made use of to produce white pepper powder instead of the conventional retting and scrubbing. In the new technique using roller mill, the white pepper fraction, (yield of which can be varied by controlling the parameters) was separated by selective sieving. The dry black skin fraction was found to be suitable for oil and oleeresin extraction. Due to the squeezing action on cells during roller milling and subsequent evaporation loss of essential oil, especially the monoterpene fraction, was noticed.

White pepper is usually prepared by retting of ripe pepper (*Piper nigrum* L) berries for 3 to 4 days followed by scrubbing to remove the skin[1]. It can also be prepared by the retting of black pepper which takes 12 to 15 days[2]. Retting brings about foul smell because of intense microbial action. Blackening of pepper is an enzyme catalysed reaction involving phenolics and this is limited only to the skin[3]. If the skin is removed, the inner core will come out as whole white pepper.

Although world demand of white pepper is estimated to be between 10,000 and 25,000 tonnes per year, India is not one of the significant producers mainly because there is a weight loss of about 25 per cent due to removal of skin.

Lewis *et al*[4]. have standardised a process for making "buff coloured" whole pepper by arresting enzyme action and bleaching. While the weight loss has been prevented, the product does not meet the specifications for starch and fibre contents[2].

The skin of the pepper berry is more fibrous and the white inner core is more starchy. Studies were undertaken to find out whether these changes in composition will bring about physical changes especially when ground, which could then be made the basis for a possible method of separating the inner core from the outer skin. If the skin is separated without much damage, it can be used for extraction of oil and oleoresin.

Materials and Methods

Raw materials: Dry black pepper procured from a trader in Trivandrum was graded to uniform size of 4.25 to 4.75 mm diameter equivalent to Tellicherry Garbled Extra Bold (TGEB) grade and was used for the grinding experiments[5]. The pepper samples of different moisture content were prepared by exposing them to moisture in a humid chamber for different periods.

Grinding: Samples at different moisture levels were ground separately in hammer mill, plate mill, pin mill, roller mill and ball mill. The hammer mill was of the swinging hammer type. In the pin mill, the pins were fixed to both plates, one stationary and the other rotating. The plate mill was of standard design with iron grooved plates with one plate rotating and the other stationary. The roller mill was of standard make with two rollers rotating against each other with different speeds. The ball mill consisted of a stainless steel, cylindrical bowl rotating on rubber rollers. Ceramic balls were used for grinding.

Analysis: Moisture content was determined by the toluene distillation method and volatile oil by the Clevenger distillation apparatus[6]. Starch by acid hydrolysis followed by Lane Eynon's procedure; ash, acid-insoluble ash, non-volatile ether extract and crude fibre were determined as per standard AOAC methods[6]. Piperine content was determined by the spectrophotometric method[7].

The colour of the white pepper powder was determined by reflectance reading taken in a Carl Zeiss spectrophotometer with reflectance attachment against a reading of 100 per cent for magnesium oxide.

Gas chromatographic studies were carried out in a Hewlett Packard gas chromatograph using 6 ft, 1/8 in. OV 17 column with temperature programming from 80 to 200°C at 5°C/min.

Histochemical location of essential oil was done using Sudan IV stain as a 4 per cent ethanolic solution[3]. Sections of 30 μm were prepared using a microtome and dipped in the stain for 30 min. The sections were washed

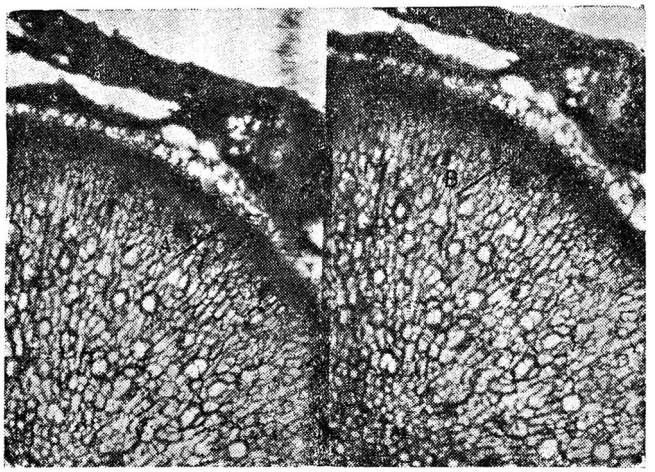

Fig. 1. Whole black pepper section treated with Sudan IV solution

A, Section squeezed and kept overnight; B, Control. Magnification x 60; arrow indicates the oil zone.

free of alcohol and seen under Nikon optiphot optical microscope (Fig. 1).

Determination of the compressive force required to break the pepper berries was carried out in an Instron Universal Testing Machine Model 1195. The cross head speed was 2 mm/min. In each case, a total of 30 readings were taken and average calculated.

Results and Discussion

Separating the skin carefully by hand (using four batches), it was found that the skin or find accounted for 26 to 32 per cent, while the remaining portion was the core which is usually separated as white pepper. Keeping the removal of tissues to the minimum to get a white product, it was found that at least 25 per cent loss was inevitable. As seen from Table 1, white pepper had a higher starch but lower fibre content than black pepper. The skin was markedly higher in ash and acid insoluble ash content.

TABLE 1. ANALYSIS OF WHITE AND BLACK PEPPER AND THEIR FRACTIONS

Chemical constituents (%)	Commercial samples		Hand separated fractions	
	Black pepper	White pepper	Skin fraction	White inner core
Moisture	13.0	14.0	13.0	13.5
Volatile oil (v/w)	4.1	3.8	2.3	1.2
Piperine	2.3	3.2	1.8	2.7
Nonvolatile ether extract	12.0	8.2	11.5	11.5
Oleoresin (EDC)	9.6	7.2	9.6	9.3
Starch	40.5	48.0	30.0	59.0
Crude fibre	14.0	4.0	23.2	4.1
Ash	7.0	2.0	6.0	2.5
Acid insoluble ash	1.5	0.6	0.7	0.4

TABLE 2. COMPRESSIVE FORCE REQUIRED FOR BREAKING PEPPER

Black pepper diameter (mm)	Breaking force in Newtons	
	Black pepper Mean ± SD	White pepper Mean ± SD
4.41 - 4.60	61.4 ± 9.26	31.60 ± 11.60
4.61 - 4.80	68.60 ± 11.59	40.43 ± 12.78
4.81 - 5.00	65.40 ± 11.80	35.20 ± 15.04
5.01 - 5.50	49.5	27.9

Moisture content of the sample — 11%

Among the different types of grinding tested, clear separation and selective grinding was noticed only with the roller mill. By using sieves of 200 to 300 μm range as much as 10 to 59 per cent could be separated from the rest as white pepper powder. Thus, compression and to a limited extent attrition appear to be involved[8].

For the determination of compressive force, pepper berries were separated into four groups depending upon their size. In each group, the whole white pepper was prepared by removing the outer skin manually. The breaking forces for the whole black pepper and inner white core were determined in each group (Table 2). It was seen that while the force required to crush the white core by compression was only 27.9 to 40.43 Newtons, the force for whole pepper was 49.5 to to 68.6 Newtons. The wide difference in the resistance to compression between the white core and the skin explains the differential grinding of the two when it was ground by a roller mill. The size reduction of the skin was also contributed by attrition. The net result was an easier and finer size reduction of the white core which was more starchy, in comparison with the outer skin which was more fibrous. This enabled easy separation employing selective sieving.

Air classification was not successful for separating the white powder and the black skin particles. As determined by water displacement, the density of black pepper was 1.04 g/ml as against 0.78 g/ml observed for white pepper. Bulk density studies with separated white and skin powders were confusing. While white pepper powder granules were smaller and more compact, black skin pepper particles were bigger and fluffy.

The relationship between moisture content and selective grinding was studied using roller mill (Table 3); percentage extraction of white pepper powder increased

TABLE 3. INITIAL MOISTURE CONTENT, YIELD AND REFLECTANCE READING OF WHITE PEPPER POWDER

Moisture (%)	Yield (%)	Reflectance reading
7.0	50.3	37.25
9.0	58.9	38.60
9.5	51.3	39.20
10.0	49.5	42.34
12.0	43.0	50.92
14.0	47.4	49.42
17.5	43.4	52.30

upto 9 per cent moisture and then decreased as the moisture content further increased.

Eventhough the maximum yield of white pepper powder was obtained at 9 per cent moisture level as shown in Table 3, the colour of white pepper powder was inferior compared to other samples. By visual examination and reflectance comparison, it was seen that the samples ground at 17.5 per cent and 12 to 14 per cent moisture levels were better.

In conformity with earlier reports the white core had more piperine (Table 1). However, oil content was more in the skin. Histochemical observation had also showed that most of the oil was concentrated in the skin[9]. While the oil content of the white core in several separations was more or less a steady low value, the skin fraction showed considerable variation in oil content in most cases showing significant loss of oil. Repeated grindings with roller mill showed an increasing loss of oil to the extent of 11 to 28 per cent in 4 stages of grinding.

When microtome sections of the black pepper were treated with Sudan IV solution, and viewed under microscope, the oil containing cells appeared as a continuous ring in the skin. When the sections were squeezed by rolling with a glass ruler two or three times, considerable smearing of the oil was noticed. When the rolled sections were kept overnight, they showed loss of oil compared to control (Fig. 1). In the roller mill, besides the size reduction, there appeared to be a squeezing effect of oil from the cells thereby resulting in substantial loss due to evaporation. Such a situation did not occur when pepper was ground with an impact mill when only size reduction occurred.

Gas chromatographic studies of the oils distilled from white pepper powder and skin fraction showed that both the oils were very low in monoterpene content viz. 25 and 19 per cent, respectively as against 60 to 75 per cent in pepper oil. This is in agreement with the fact that the low boiling monoterpenes escape accounting for the loss of oil in the grinding process. Sesquiterpene content was high in both the oils compared to whole black pepper oil and white pepper oil, respectively.

Since the lining of the inner core is rich in piperine, usually a lot of piperine will be separated along with the skin. The piperine and volatile oil in the skin therefore make it a useful raw material for oleoresin extraction. Thus, white pepper powder could be selectively separated in the grinding of black pepper meant for oleoresin extraction. It may be however, pointed out that white pepper can only be prepared in ground form by this technique and not in the traditional whole form.

It can be seen that because of the use of skin for extraction purposes the process, being continuous and simple, will be economically viable and will more than compensate for the shortcoming of getting only powdered white pepper. Invariably, white pepper is consumed only after grinding. It is also possible to combine the production of white pepper powder with grinding of black pepper, provided the extraction of white inner core fraction by compressive grinding is limited to a low level.

Acknowledgement

Acknowledgements are due to Mrs. K. P. Padma Kumari, for assistance in gas chromatographic studies, to Mr. S.G.K. Pillai for optical micrograph studies and to Mr. K. K. Ravi Kumar for his assistance in breaking force measurements.

References

1. Lewis, Y. S., Nambudiri, E. S., Krishnamurthy, N. and Natarajan, C. P., White Pepper. *Perf. Essent. Oil Rec.*, 1969, **60**, 53.
2. Govindarajan, V. S., Pepper—Chemistry Technology and Quality Evaluation. *CRC Crit. Rev. Fd Sci. Nutr.*, 1977, **9**, 115.
3. Mangalakumari, C. K., Sreedharan, V. P. and Mathew, A. G., Studies on blackening of pepper (*Piper nigrum*, Linn.) during dehydration. *J. Fd Sci.*, 1983, **48**, 604.
4. Lewis, Y. S. Nambudiri, E. S., Krishnamurthy, N., Sankari Kutty, B., Shivashankar, S. and Mathew, A. G., Need for growing pepper cultivars to suit pepper products. *Indian Spices*, 1976, **13**, 4.
5. Agmark Grade Specification of black pepper and ground black pepper. *Indian Spices*, 1975, **12**, 12.
6. *Official Methods of Analysis.* Association of Analytical Chemists, Washington, D. C. 1975, 12th Edn.
7. Tauzig, F., Sozuki, J. I. and Ray, E., Black Pepper I. Analysis of bite principles. *Fd Technol.*, 1956, **10**, 151.
8. Warren, L., McCabe and Julian, C. Smith, *Unit Operations of Chemical Engineering*, McGraw Hill Book Co., London, 1967, 2nd Edn.
9. Mangalakumari, C. K., *Histological and Histochemical Studies on Plantation Crops and Spices.* 1983, Ph.D. thesis Kerala University.

Colorimetric Method for the Determination of Carbendazim (MBC), Benomyl and Their Degradative Product— 2 — Aminobenzimidazole

J. R. RANGASWAMY, Y. N. VIJAYASHANKAR AND S. R. PRAKASH*
Infestation Control and Protectants Discipline
Central Food Technological Research Institute, Mysore-570 013, India

Received 14 *August* 1986; *revised* 31 *March* 1987

A new method has been developed for determination of Carbendazim (MBC-methyl benzimidazol-2 yl carbamate) based on the determinations of methanol and 2-amino-benzimidazol (2-AB), the two products of alkaline hydrolysis. Methanol after oxidation to formaldehyde, was coupled with chromotropic acid reagent and determined. By this method as low as 1.5 μg methanol can be estimated. 2-AB on alkaline iodination forms reddish brown N-iodo-2-AB with an absorption maximum at 400 nm and serves as a measure of MBC with a lower determinable limit of 3 μg.

Carbendazim and benomyl are the two important benzimidazole carbamates used as protective and eradicant systemic fungicides controlling a wide range of pathogens of fruits, vegetables, cereals, ornamental plants and grapes. Suspensions of upto 1000 ppm. are used as a soak in several agricultural practices to prevent diseases caused by *Fusarium*, *Penicillium* and other species of fungi[1]. MBC is absorbed by the roots and green tissues of plants. It has extremely low toxicity to mammals.

Miller *et al*[2]. have estimated benomyl in water. 2 AB, the product of hydrolysis of benomyl or MBC was detected on TLC[3]. Average recoveries of about 87 per cent have been reported on all samples used by the colorimetric method[4]. This method has been improved to measure the fluoroscence of benomyl both in acidic and basic media[5].

A colorimetric method suitable for routine determination of benomyl, MBC and 2-AB has been reported herein.

Materials and Methods

(*a*) *Reference materials*: Benomyl (methyl-1 (butyl carbamoyl) benzimidazol-2 yl carbamate) was obtained from Agri-chemical Sales Division, E.I. du Pont de Nemours & Co. Inc., Wilmington, Delaware, U.S.A. Carbendazim (MBC-Methyl benzimidazol-2yl-carbamate) was obtained from Hindustan Mineral Products Pvt. Ltd., Bombay, India. Benomyl-50 per cent Wettable powder was purchased locally. 2-amino benzimidazol (2-AB) was synthesised by us in this laboratory.

(*b*) *Reagents* (*BDH grade*):

Chromotropic acid (*1,8-dihydroxy napthalene-3, 6-disulphonic acid*) *reagent*: 0.1 per cent solution was prepared by dissolving 100 mg sodium salt of chromotropic acid in 100 ml 50 per cent H_2SO_4.

Stock solution of 2-AB: Prepared by dissolving 40 mg 2-AB in 100 ml 0.2 per cent NaOH solution. Working standards of 2-AB solution were obtained by suitably diluting aliquots with 0.2 per cent NaOH solution so as to have 5-40 μg 2-AB per ml.

Stock solution of methanol: Methanol standard solution was prepared by dissolving 50 mg ice cold methanol in 50 ml cold water. The stock solution was stored in a refrigerator. Working standards were prepared by diluting aliquots of the stock solution suitably so as to have 5-25 μg methanol in 0.5 ml solution.

(*c*) *Hydrolysis of MBC*: To 10 mg MBC was accurately weighed into a long necked B_{24} 50 ml round bottom flask 7.5 ml of 6.5N NaOH solution was added and the mixture was refluxed under double walled water cooled condenser for 15 min either on a hot plate or on a bunsen flame underneath a wire gauze. The inner wall of the condenser was washed down with 3-4 ml ice cold distilled water and the flask with the contents was cooled in ice. The reaction mixture was neutralised with 7.5 ml of 6.5 N H_2SO_4 and the resultant hydrolysate was

**Present address:* Department of Animal Drug Metabolism, Merck Sharp & Dhome Research Lab., P.O. Box 2000, Rahway, New Jersey, 07065, USA.

made upto 25 ml with water. Aliquots (0.5 and 1.0 ml) of this hydrolysate were employed for determination of methanol and 2-AB separately. Quantitative hydrolysis of small amounts of MBC (0.75-2.0 mg) was done by employing its solution in acetone.

(*d*) *Hydrolysis of benomyl*: 1.5 to 8 mg benomyl was hydrolysed with 2.5 ml 0.1 N HCl on a water bath for 15-30 min to cleave t.butyl-amino carbamoyl group. The resultant MBC was alkali hydrolysed as above.

(*e*) *Determination of active ingredient in benomyl 50 per cent wettable powder*: Known weights of wettable powder (1.5-9.0 mg) was either directly used for hydrolysis or stirred well with chloroform (3×10 ml) and filtered. The combined filtrate was evaporated under a stream of N_2 to dryness, and the residue was acid and alkali hydrolysed as in (*c*) and (*b*) and aliquots of the final solution were used for determination of CH_3OH,

(*f*) *Synthesis of 2-AB*: Using cyanogenbromide prepared by the method of Blatt[6], 2-AB was synthesised by the method of Biddle *et al*[7]. Its purity was checked by m.p. (222°C) and by TLC and used as standard.

(*g*) *Calibrations*: To 0.5 ml methanol (5 to 25 μg) solution in test tubes 0.1 ml 5 per cent H_3PO_4 and 0.1 ml 1 per cent $KMnO_4$ solutions were added. Shaken for a min. and excess of $KMnO_4$ was destroyed by drops of 5 per cent Na_2SO_3. Chromotropic acid reagent (1.5 ml) and 0.5 ml con. H_2SO_4 were added, tubes were shaken well and kept in a water bath at 60-70°C for 10 min. The tubes were cooled, volume made upto 5 ml with 50 per cent H_2SO_4 and absorbance was read at 580 nm against blank similarly prepared. Methanol content in MBC (10-150 μg) hydrolysate (as in *c*) was similarly analysed.

Two millilitres of 0.1 N iodine and 0.5 ml 4N NaOH solutions were added to 1.0 ml 2-AB (5-40 μg) solution. Excess of iodine was destroyed with a drop of 0.05N Na_2SO_3 after shaking for 3 min. The absorbance was read at 400 nm against the blank similarly prepared using 1.0 ml of 0.2 per cent NaOH for drawing the calibration curve. This method was applied for assaying 2-AB content in MBC hydrolysate (as in *c*) after neutralising with 7.5 ml of 6.5 N NaOH.

Results and Discussion

The linear relationship between the absorbance at 580 nm and the concentration of methanol and MBC are valid upto 25 and 150 μg/5 ml reaction mixture respectively. But in the case of 2-AB, this relationship at 400 nm is obeyed upto 40 μg. The methanol determination method has a lower estimatable limit of 1.5 μg (9.96 μg MBC) with a detectable limit of 1 μg. Alkaline iodination method for 2-AB has a lower determinable limit of 3 μg (4.31 μg MBC) with a detection limit of 2 μg. So, by this method it is possible to determine 4.31 to 8.96 μg MBC depending on the product of hydrolysis analysed.

Sodium hydroxide (1.5 ml) of 6.5 N was found to be necessary and sufficient for complete hydrolysis of MBC in the range of 2-10 mg and to retain the hydrolysed methanol quantitatively. The hydrolysis was incomplete when the mixture was hydrolysed on a boiling water bath instead of a hot plate or an open flame. Dry formulation of benomyl can either be accurately weighed or taken as $CHCl_3$ solubles for hydrolysis. Oxidation of methanol to formaldehyde under the conditions employed was complete and quantitative. Chromotropic acid in 50 per cent H_2SO_4 (0.1 per cent solution) coupled with formaldehyde smoothly at hot water bath temperature. Chromotropic acid reagent in sulphuric acid of strength lower than 50 per cent afforded either yellow or pale pink chromophore. When the reaction mixture was made upto 5 ml with water instead of 50 per cent H_2SO_4, the resultant solution lost its characteristic violet colour by slowly turning to yellow.

2-AB underwent quantitative iodination within 3 min. under the alkaline conditions employed. When a mixture of 2-AB and MBC was iodinated, MBC did not undergo iodination indicating the specificity of the reaction to 2-AB. So, this method can be employed even

TABLE 1. RECOVERY EXPERIMENTS WITH TECHNICAL MBC

Weighed for hydrolysis (mg)	Determined (mg) Mean±S.D.	Recovery %
10.0	10.11±1.70	101
7.50	7.40±0.49	99
6.00	6.05±0.48	101
5.00	5.03±0.44	100
3.75	3.64±0.15	97
3.33	3.15±0.35	94
3.00	2.98±0.29	99
2.50	2.50±0.39	100
2.20	1.97±0.14	89
2.00	1.95±0.19	97
Used as acetone solution for hydrolysis, (mg).		
1.88	1.76±0.09	93
1.60	1.50±0.12	94
1.50	1.43±0.35	95
1.25	1.22±0.04	97
0.75	0.72±0.01	96
0.55	0.52±0.04	94
0.40	0.38±0.04	95

*Mean of 6 hydrolysis

for the determination of 2-AB in the presence of MBC or benomyl without any pre-clean up. Half ml of 2.5 to 4 N NaOH was found to be sufficient for iodination as increase of either volume or normality of alkali did not result in a corresponding increase in absorbance of N-iodo-2-AB. Aqueous solution of 2-AB was slightly milky but became clear on warming. However, when such stock aqueous solution of 2-AB was stored in a refrigerator turbidity was found to form at times, so stock solution of 2-AB in 0.2 per cent sodium hydroxide solution has been employed throughout this work.

By this method, it has been possible to quantitatively estimate methanol cleaved from carbamoyl group of MBC. As seen from Table 1, 87-100 per cent recoveries were obtained based on methanol determination. Quantitative recoveries were obtained by hydrolysing 100 μg MBC. By this method, 93-111 per cent recoveries were obtained by hydrolysing 1.5-7.5 mg benomyl. The method could also be applied for analysis of benomyl dust or wettable powder formulations. Table 2 shows almost 100 per cent recovery of active ingredient from 50 per cent benomyl wettable powder.

When amounts of MBC smaller than 8 mg were hydrolysed, the amount of methanol formed would be below the determinable limits of chromotropic acid method. In such cases, MBC could be estimated as 2-AB after rendering the hydrolysate alkaline, 87-100 per cent recoveries of MBC were obtained as 2-AB (Table 3). In this study, acidic acetone instead of ethyl acetate has been employed for extraction, as ethyl acetate is immiscible with the aqueous reagent employed in the determinations.

TABLE 2. RECOVERY EXPERIMENTS*

Weighed (mg)	Found (mg) Mean±S.D.
50% Benomyl wettable powder	
9.00	4.80±0.29
5.00	2.34±0.17
3.33	1.89±0.06
2.50	1.81±0.09
2.00	0.91±0.12
1.67	0.87±0.09
As $CHCl_3$ soluble residue for hydrolysis	
5.52	5.16±0.08
4.13	4.30±0.42
3.18	3.16±0.07
2.62	2.68±0.08
Technical Benomyl	
7.5	7.42±0.44(99)**
6.0	6.12±0.39(102)
5.0	4.91±0.23(98)
3.0	2.79±0.08(93)
2.0	1.93±0.16(96)
1.5	1.66±0.21(111)

*Mean 6 hydrolysis

**Values in parenthesis are per cent recoveries.

TABLE 3. 2-AB CONTENT OF THE MBC HYDROLYSATE[a]

Calculated (μg)	Determined (μg) Mean±S.D.	Recovery (%)
5.0	4.42±0.20	88
7.0	6.11±0.27	87
10.0	9.80±0.52	98
20.0	19.39±0.88	97
30.0	30.00±0.0	100
40.0	38.67±1.21	97
50.0	48.62±1.40	97
52.5	52.00±0.92	99
60.0	58.82±1.03	74
80.0	79.02±2.68	99
100.0	99.99±2.12	100
105.0	102.43±0.95	98

[a]Mean of 6 hydrolysis

Acknowledgement

The authors thank Shri S. K. Majumder, Deputy Director for his encouragement and Dr. B. L. Amla, Director, for his interest in the work.

References

1. Gould, C. J. and Miller, V. L., Effective methods for controlling Fusarium basal rot of Narcissus with Benomyl and Thiabendazole. *Pl. Dis. Rep.*, 1971, **55**, 428.
2. Miller, V. L., Gould, C. J. and Csonka, E., Estimation of benomyl in the milligram range. *J. agric. Fd Chem.*, 1974, **221**, 93.
3. Vogel, J., Corvi, C. and Veyrat, G., Thin layer chromatographic method for the determination of Benomyl residues in fruits and vegetables. *Mitt. Geb. Lebensm. Hyg.*, 1972, **63**, 453.
4. Pease, H. L. and Gardiner, J. A. Fluorimetric and colorimetric procedure for determining residues of Benomyl, *J. agric. Fd Chem.*, 1969, **17**, 267.
5. Pease, H. L. and Holt, R. F., Improved method for determining Benomyl residues. *J. ass. off. analyt. Chem.*, 1971, **54**, 1399.
6. Blatt A. H., (Ed.) *Organic Synthesis*. John Wiley & Sons, Inc., London, 1943, Vol. 12, 150.
7. Biddle, P., Slane, E. and Willians. J. L., Organic complex forming agents for metals. IV. Some new heterocyclic complexions. *J. Chem. Soc.*, 1960, 2369.

RESEARCH NOTES

APPLICATION OF GARCINOL AS A COLOURANT FOR BUTTER AND GHEE AND METHOD OF ITS ESTIMATION

N. KRISHNAMURTHY AND S. R. SAMPATHU
Central Food Technological Research Institute, Mysore-570 013, India

Received 30 *September* 1986; *revised* 28 *April* 1987

Garcinol, a light yellow coloured fat-soluble pigment, obtained from the fruit rind of kokum (*Garcinia indica*), has been evaluated for use as a colourant in butter. A spectrophotometric method has been standardised for the estimation of garcinol added to butter. The pigment added at 0.3% level imparted an acceptable yellow colour to butter. Its retention was 98.4% after 90 days storage at 4°C and was 99.0% after 15 days storage at 25°C. On conversion of butter into ghee by heating, 94.0% of the added garcinol was retained.

There is considerable variation in the colour of butter sold commercially. The natural colour varies from white or ivory white to light yellow depending on its carotenoid content. In the dairy industry where butter is produced on a large scale, the colour varies from batch to batch. In order to get a uniform colour, a small amount of a colourant like annatto or turmeric or a combination of both is added to the butter[1].

The present study describes the application of garcinol, a light yellow coloured fat-soluble pigment isolated from the fruit rind of an edible tropical fruit kokum (*Garcinia indica*) for colouring butter. Garcinol is a polyisoprenyl phenolic pigment present to the extent of 2-3 per cent in the dried rind of kokum. The isolation and identification of garcinol are reported elsewhere[2,3]. Acute oral toxicity studies on garcinol conducted at this institute using albino rats and mice at dose levels of 2.5 and 5 g per kg body weight did not show any abnormal changes[4].

Pure white butter was obtained from the local market and garcinol isolated in this laboratory was used for colouring. Garcinol (0.9g) was dissolved in 5 g of melted butter and mixed with 295 g of butter using a pestle and mortar. This resulted in a light yellow coloured butter comparable in colour with commercial samples. One hundred gram portions of coloured butter were packed in transparent bottles and storage stability was studied at 4°C (refrigerator) and at 25±2°C (diffuse day light). Control (uncoloured) samples were also maintained under similar conditions. For colour evaluation, 2-3 g portions of butter were withdrawn at intervals of 15, 45 and 90 days and subjected to spectrophotometric analysis for garcinol content according to the following method.

Butter was melted on a hot water bath and then solubilised in 20 ml of ethanol: chloroform mixture (1:1 v/v) and filtered under suction. The funnel was washed twice with 10 ml portions of the solvent. The filtrate and the washings were collected into the same flask and finally the volume was made upto 50 ml with the solvent; five millilitre of this was diluted to 10 ml with the solvent and its absorbance recorded at 360 nm, using ethanol: chloroform solvent mixture as blank. An initial colour reading of the butter sample was taken and recorded as the zero day value.

A calibration curve was prepared by determining the absorbances at 360 nm (pure garcinol in ethanol: chloroform mixture gave an intense peak at this wave length) of different concentrations of pure garcinol in the solvent mixture (Fig. 1). Using this standard graph, garcinol concentration in butter samples was estimated.

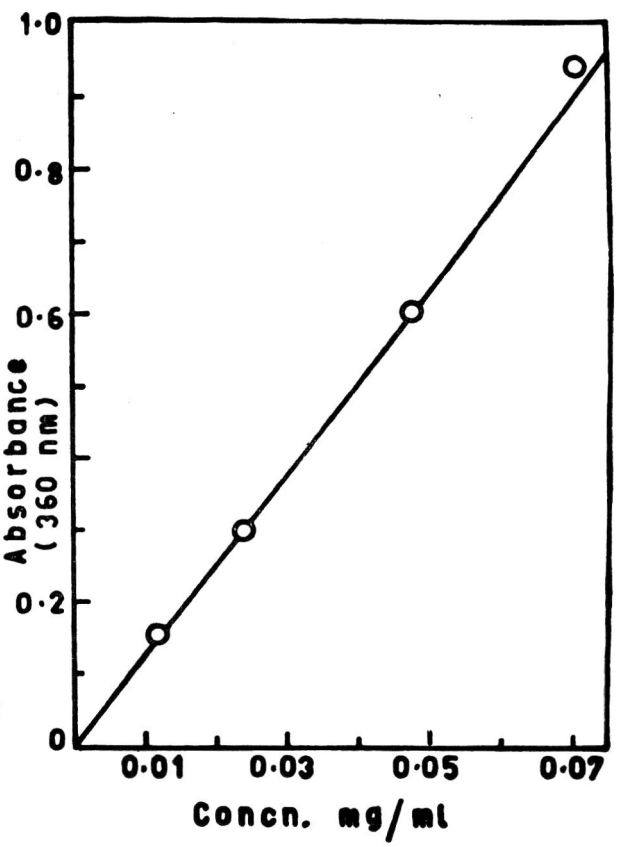

Fig. 1. Calibration curve for garcinol estimation

TABLE 1. STORAGE STABILITY OF GARCINOL IN BUTTER

Storage period (days)	% garcinol retention at 4°C	25°C
0	99.1	99.1
15	99.1	99.0
45	99.0	98.5
90	98.4	—

Values are averages of duplicate determinations.

The butter sample kept at 4°C was withdrawn after 90 days storage and melted into ghee in which garcinol was estimated as above.

Direct mixing of garcinol with butter resulted in uneven distribution of colour. This problem was overcome by dissolving the requisite amount of garcinol in a small quantity (5-10 g) of melted butter and then mixing with the bulk sample.

The retention of yellow colour was satisfactory in the samples stored both at 4 and 25°C. The sample at 25°C had developed putrified smell along with mould growth at 45 days storage period. However, the colour retention was 98.5 per cent (Table 1) and acceptable with respect to colour only. Mould growth was noticed in the control sample also. In the cold stored sample there was practically no loss of colour over the 90 days storage period and the sample was acceptable.

In the estimation of garcinol, the butter sample had to be melted first before adding ethanol: chloroform mixture. Chloroform alone could not be used for this purpose because of the moisture present in the butter and the consequent immiscibility. This problem was overcome by using chloroform in combination with ethanol, which resulted in a clear solution. The control sample of butter did not show any absorption at 360 nm.

Garcinol content in 50 g of butter stored at 4°C for 90 days was found to be 148 mg. The ghee obtained from this butter had 139 mg of garcinol, thereby accounting for 9 mg loss during processing. The ghee thus obtained was light yellow in colour and appealing.

It is concluded that garcinol is a promising yellow colourant for use in butter; the limiting factor is the low shelf life of the butter itself at 25°C. Garcinol would also be useful in other fat-based foods.

The authors are thankful to Dr. Y. S. Lewis, Project coordinator (Retd) of the Institute, and to Dr. B. Ravindranath, presently Head, Chemicals Division, United Breweries, Bangalore for useful discussions while carrying out this work.

References

1. Coulson, J., in *Development in Food Colours*. by J. Walford, (Ed), Applied Science Publishers Ltd., London, 1980, **1**, 189.
2. Krishnamurthy, N., Lewis, Y. S., and Ravindranath, B., On the structures of garcinol, isogarcinol and camboginol, *Tetrahedron Lett.* 1981, **22**, 793.
3. Krishnamurthy, N., Ravindranath, B., Guru Rao, T. N. and Venkatesham, K., Crystal and molecular structure of isogarcinol, *Tetrahedron Lett.*, 1982, **23**, 2232.
4. *Annual Report*, Central Food Technological Research Institute, Mysore (India), 1982.

GENETIC VARIABILITY IN THE MILLING AND VERMICELLI QUALITY OF SOME INDIAN DURUM WHEAT CULTIVARS

D. INDRANI, S. SUKANYA AND J. S. SIDHU*
International School of Milling Technology & Baking Technology, Central Food Technological Research Institute, Mysore-570 013. India

Received 8 *October* 1986; *revised* 12 *February* 1987

Forty four samples of durum wheat cultivars collected from different parts of the country were analysed for chemieal, milling and vermicelli quality characteristics. The total and fine semolina yields ranged from 44.5 to 74.0% and 25.6 to 41.2%, respectivity. Similarly, a wide variation in cooking qualty of vermicelli was observed. The gruel solids loss ranged from 3.8 to 9.6% in these samples.

Durum wheat cultivation in India is considered to be quite old, and durum semolina is used for the preparation of a number of sweet and savoury dishes. Recently evolved varieties of durum have been shown to possess better disease resistance mainly towards rusts and Karnal bunt and give higher yields[1]. Due to wheat surpluses, the country is in a position to export wheat and its products. The premium price offered for durum wheat by the world trade could serve as a good proposition to earn valuable foreign exchange for our country. Moreover, a large number of Indian farmers are getting interested in cultivating durum than bread wheats, mainly because of their better resistance to rusts and diseases.

There are some reports[2-4] on the physico-chemical, rheological and spaghetti making quality of durum wheats of India. The present paper discusses the genetic

**Present address:* Department of Food Science & Technology, PAU, Ludhiana, India.

variability in the chemical, milling and vermicelli quality characteristics of some promising cultivars of durum wheats being tested for uniform regional trials under the All India Coordinated Wheat Improvement Project of the ICAR. Forty four samples of durum wheat cultivars comprising of 32 varieties (1985 crop season), grown under the above project were obtained from different parts of the country. A few of these varieties were received from more than one station.

The cleaned wheat was conditioned to 16 per cent moisture content for 12 hr. Just 30 min prior to milling, 0.5 per cent moisture was added to the wheat and mixed thoroughly. The wheat was milled in a Buhler laboratory mill (Model MLU-202) as per procedure reported earlier[2]. The total semolina was sifted through 32, 45 mesh and 6×× sieves. The yield of coarse semolina (–32,+45), fine semolina (–45+6××) and flour (–6××) were computed on 14 per cent moisture content on the clean wheat basis. Total yellow pigment content of wheat grain was determined by standard AACC method[5]. Only the fine semolina was used for the preparation of vermicelli. Dough was prepared with 35 per cent water absorption using farinograph and mixed for 5 min after the peak time. Vermicelli were then extruded through a hand operated vermicelli press. These were dried at 60°C for 3 hr and then at 100°C for 1 hr in a hot air oven. Cooking quality, water uptake (g of water taken up per 100g of raw vermicelli) and gruel solids loss of these samples were determined by the ISI methods[6]. Semolina quality grade (based on total semolina yield, per cent) and the appearance and texture of the cooked vermicelli were evaluated as follows.

Total lemolina yield (%)	Score
>65	5
60.01–65.0	4
55.01–60.0	3
50.01–55.0	2
<50.0	1

Cooked vermicelli lcore		Description
(*a*) Appearance	1	Dull whitish colour
	2	Appealing light creamy/ whitish colour
	3	Appealing creamy yellow colour
(*b*) Texture	1	Mushy, sticky
	2	Somewhat firm and slightly sticky
	3	Firm, without stickiness

The data for milling and cooking quality characteristics of durum cultivars are presented in Table 1. A wide variation was observed in semolina and flour yields, total yellow pigments and semolina quality grade. The fine semolina yield, flour yield and total yellow pigments for these samples ranged from 25.6 to 41.2 per cent, 7.7 to 22.5 per cent and 2.3 to 5.5. p.p.m. respectively. Similarly, a wide variation in the cooking quality of vermicelli from these cultivars has been observed. The dough consistency, appearance and

TABLE 1. SEMOLINA MILLING AND VERMICELLI COOKING QUALITY OF DURUM CULTIVARS

Quality characteristics	Range	Mean±S.D.	Coeff. of variation
A. Milling quality			
1. Semolina yield (%)			
(*a*) Fine	25.58–41.17	34.30± 2.84	8.28
(*b*) Coarse	18.90–33.70	28.88± 3.97	13.75
(*c*) Total	44.48–74.03	63.20± 5.54	8.77
2. Flour yield (%)	7.65–22.48	12.19± 3.83	31.42
3. Total pigments (ppm of β-carotene)	2.34–5.46	3.55± 0.77	21.76
4. Semolina quality grade	1–5	4.16± 1.03	24.76
B. Vermicelli cooking quality			
1. Farinograph dough consistency (BU)	200–610	354.77±92.77	26.15
2. Sensory analysis			
(*a*) Appearance	1–3	1.64± 0.49	29.88
(*b*) Texture	1–3	2.30± 1.62	70.43
3. Water uptake (%)	207–619	468.68±71.16	15.18
4. Gruel solids loss (%)	3.8–9.6	2.33± 1.53	65.67

TABLE 2. DISTRIBUTION OF DURUM WHEAT CULTIVARS ACCORDING TO THE RANGE OF DIFFERENT PARAMETERS OF MILLING QUALITY

Fine semolina		Total semolina		Flour yield		Total pigments	
(%)	No. of samples	(%)	No. of samples	(%)	No. of samples	(ppm)	No. of samples
25.01–30.0	3	40.01–50.0	2	5.01–10.0	18	2.01–3.0	10
30.01–35.0	21	50.01–60.0	8	10.01–15.0	15	3.01–4.0	23
35.01–40.0	19	60.01–70.0	33	15.01–20.0	9	4.01–5.0	8
40.01–45.0	1	70.01–80.0	1	20.01–25.0	2	5.01–6.0	2

[+]One sample had a value of 1.61 ppm.

texture of cooked vermicelli, water uptake and gruel solids loss ranged from 200 to 610 BU, 1 to 3, 207 to 619 per cent and 3.8 to 9.6 per cent respectively. The higher values for standard deviation and coefficient of variation also indicate the wider dispersion of the data on the milling and cooking quality of these cultivars.

The distribution of durum cultivars according to the range of different parameters of milling quality is shown in Table 2. Indian durum wheats are known to contain low levels of yellow pigments[1]. The data in Table 2 confirm this fact, because a great majority of the samples had yellow pigment contents lower than 4 p.p.m. A high frequency of samples for the fine semolina content falling between 30-40 per cent was observed. About 75 per cent of the samples gave a total semolina yield of 60 to 70 per cent. Looking at the water uptake data in Table 3, one can see that about 84 per cent of the samples had quite a high value of 400-600 per cent. Similarly, a little over 50 per cent of the samples gave considerably low value of gruel solids loss (4.5-7.5 per cent). The appearance of about 64 per cent of the cooked vermicelli samples had a score of 1 to 2, whereas for texture 43 per cent of the samples fell in the higher range of 2-3.

It is concluded that this data can be utilized for the development of wheat varieties better suited to the industry as also for export.

The authors wish to thank Dr. B. L. Amla, Director of the Institute for encouragement during the course of this investigation.

References

1. Upadhyaya, Y. M., Durum wheat. *Indian Fmg*, 1977–1978, **27**(11) 28.
2. Kathuria, D. K. and Sidhu, J. S., Indian durum wheat. I. Effect of conditioning treatments on them illing quality and composition of semolina. *Cereal Chem.*, 1984, **61**, 460.
3. Rahim, A., Prabhavathi, C., Haridas Rao, P. and Shurpalekar, S. R., Suitability of Indian durum wheats for Semolina milling and vermicelli preparation. *J. Fd Sci. Technol.*, 1976, **13**, 249.
4. Kathuria, D. K. and Sidhu, J. S., Indian durum wheats. II. Effect of conditioning treatments on the quality of spaghetties. *Cereal Chem.*, 1984, **61**, 463.
5. *The Approved Methods.*, American Association of Cereal Chemists. St. Paul, Minnesota, 1972.
6. *Vermicelli Making*, IS: 1485–1976, Indian Standards Institution, New Delhi.

TABLE 3. DISTRIBUTION OF DURUM WHEAT CULTIVARS ACCORDING TO THE RANGE OF DIFFERENT PARAMETERS OF COOKING AND SENSORY QUALITY OF VERMICELLI

Appearance		Texture		Water uptake		Gruel solids loss	
Score	No. of samples	Score	No. of samples	(%)	No. of samples	(%)	No. of samples
0.0 –1.0	12	0.0 –1.0	9	200–300	1	3.01– 4.5	7
1.01–2.0	28	1.01–2.0	19	300–400	3	4.51– 6.0	13
2.01–3.0	4	2.01–3.0	16	400–500	28	6.01– 7.5	12
				500–600	10	7.51– 9.0	11
				600–700	2	9.01–10.5	1

FLOUR MILL STREAMS. IV. STUDY OF PHYTIC ACID DISTRIBUTION

D. INDRANI, K. LEELAVATHI AND J. S. SIDHU*
International School of Milling Technology and Baking Technology, Central Food Technological Research Institute, Mysore-570 013
India

Received 8 *October* 1986; *revised* 16 *April* 1987

The wheat grain, straight run flour and different flour streams as obtained in ISMT mill were analysed for total phosphorus, inorganic phosphorus and phytic phosphorus. The total, inorganic and phytic phosphorus contents differed significantly among these flour streams and increased progressively towards the tail end flour streams. The flours from B_4, C_5 and C_3 were found to contain significantly higher (142.6, 108.7 and 95.3 mg P/100g, respectively) amounts of phytic phosphorus.

In wheat grain, like in other seeds, about 60 to 90 per cent of the phosphorus is present as phytic acid[1] The phytic acid content of wheat is not constant but varies with the variety and other factors and is located mainly in the bran and germ with relatively little in the inner endosperm or white flour[2–4]. The phytic acid content of wheat flour has attracted a lot of attention and the concern of nutritionists because of the uncertain availability of phytic phosphorus to humans as well as the degree of its interference with divalent ion absorption[5]. Depending upon the processing conditions, phytic acid contents of these flours get further reduced during bread making[6,7].

Physico-chemical, rheological and baking characteristics of these flour streams as obtained in ISMT mill (Buchler Roller flour Mill, Capacity 20 tons per day), have been reported earlier[8,9]. Nine different flour streams viz. first break (B_1), second break (B_2), third break (B_3), fourth break (B_4), first reduction (C_1), second reduction (C_2), third reduction, (C_3), fourth reduction (C_4), fifth reduction (C_5) and straight run flour (SRF) consisting of all the above flour streams were analysed in quadruplicates for total phosphorus (TP), inorganic phosphorus (IP) and phytic phosphorus (PP), according to the procedure reported earlier[6] and the results (mean value$\pm$standard deviation) are expressed, on 14 per cent moisture basis. The data were subjected to analysis of variance and the F values as well as standard error of mean are reported.

The distribution of different forms of phosphorus in wheat grains and flour mill streams is presented in Table 1. A highly significant ($P<0.05$) difference was observed in total, inorganic and phytic phosphorus contents of different flour streams. C_1 and C_2 flour

TABLE 1. DISTRIBUTION OF DIFFERENT FORMS OF PHOSPHORUS (MG P/100G FLOUR) IN WHEAT AND FLOUR MILL STREAMS

Flour mill stream	Total P (Tp)	Inorganic P (Ip)	IP as % of Tp	Phytic P (Pp)	Pp as % of Tp
Wheat grain	321.3 ± 9.3^{d}	64.0 ± 3.6^{d}	19.9	193.8 ± 3.6^{c}	60.3
SRF	89.1 ± 5.8^{e}	15.6 ± 1.0^{g}	17.5	39.3 ± 0.8^{i}	42.1
B_1	132.6 ± 3.5^{b}	$18.5\pm1.4^{b,c}$	14.0	61.7 ± 3.5^{b}	46.5
B_2	125.7 ± 6.0^{b}	$20.5\pm0.5^{a,c}$	16.3	61.6 ± 1.9^{b}	48.9
B_3	141.2 ± 1.6^{f}	20.9 ± 1.1^{a}	14.8	62.6 ± 7.9^{b}	44.3
B_4	217.7 ± 3.1^{g}	24.8 ± 0.5^{e}	11.4	142.6 ± 1.2^{d}	65.5
C_1	68.2 ± 5.0^{a}	10.3 ± 0.0^{f}	15.1	22.8 ± 0.8^{e}	33.5
C_2	68.1 ± 2.0^{a}	12.6 ± 1.6^{g}	18.5	27.9 ± 0.9^{a}	41.0
C_3	149.4 ± 12.2^{h}	$18.9\pm0.5^{b,c}$	12.6	95.3 ± 3.8^{f}	63.8
C_4	98.6 ± 5.0^{i}	17.5 ± 1.2^{b}	17.7	34.2 ± 2.0^{g}	34.7
C_5	177.3 ± 3.1^{j}	22.4 ± 1.3^{a}	12.6	108.7 ± 3.0^{h}	61.3
F value (df: 10,33)	1700.85***	34.41***	—	1463.87***	—
S.Em (df: 33)	2.49	0.7	—	1.18	—

Any two means in the same column having different superscripts differ significantly. Calculated as per Duncan's new multiple range test ($P<0.05$)

**Present Address:* Department of Food Science & Technology, PAU, Ludhiana, India.

streams were obviously the lowest in all these forms of phosphorus, because these streams come from the purified middlings having the least amount of bran contamination. The break flours were found to be richer in total, inorganic and phytic phosphorus contents when compared with reduction flours. Moreover, as we approached towards the last break or reduction flour, the level of different forms of phosphorus increased very significantly. The bran, germ and outer endosperm of wheat are known to be very rich in ash content including phosphorus[10]. The break flours contain little or no germ. Their relatively higher content of phytic phosphorus, progressively greater in approaching the later breaks is due to the increasing content of the outer endosperm and more so of bran powder. The reduction flours, particularly last reductions (C_4 and C_5) contain much higher level of total and phytic phosphorus than the other flour streams. Depending upon the extent of incorporation of any or each of the three-germ, outer endosperm and bran—the total and phytic phosphorus contents of individual flour stream would vary.

It can be concluded from the study that during milling, the phytic acid is largely distributed among those streams which constitute the by-products. A few of these streams (B_4, C_4, and C_5) are generally combined to yield a low grade flour, which is called as resultant *atta* or red dog and is normally used by the consumer to prepare, *chapatis* and *poories*. If 0.57 to 2.2g of phytic phosphorus is consumed daily in the diet, it combines with divalent ions (Ca, Fe, Cu, Zn and Mg) to form an almost insoluble complex, which is not readily absorbed by the human system[5]. Keeping in view the higher level of consumption of phytic acid by these consumers, who mostly use resultant *atta*, a suitable enrichment of their diet with additional amount of these minerals would be desirable. The patent flour which the bakers normally use for the production of bread and other baked goods, is reasonably low in phytic acid and does not pose any serious health problem.

Although the phytic phosphorus content in different flour mill streams from a given wheat would also depend upon roll setting, flow sheet and sieve clothing employed by a particular mill, the pattern of distribution of phosphorous would more or less remain same.

The authors wish to thank Dr. B. L. Amla, Director, CFTRI, Mysore for encouragement during the course of this investigation.

References

1. Barre, R., Distribution of phytic aid in wheat kernel. *Ann. Pharm. Franc.*, 1956, **14**, 1982.
2. Hay, J. G., The distribution of phytic acid in wheat and a preliminary study of some of the calcium salts of this acid. *Cereal Chem.*, 1942, **19**, 326.
3. Pringle, W.J.S., and Moran, T., Phytic acid and its destruction in baking. *J. Soc. Chem. Ind.*, 1942, **61**, 108.
4. McCance, R. A., Widdowson, E. M., Moran, T., Pringle, W.J.S. and Macrae, T. F., The chemical composition of wheat and rye and of flours derived therefrom. *Biochem. J.*, 1945, **39**, 213.
5. Ranhotra, G. S., Loewe, R. J. and Puyat, L. V., Phytic acid in soy and its hydrolysis during bread making. *J. Fd Sci.*, 1974, **39**, 1023.
6. Chhahra, P. and Sidhu, J. S., Fate of phytic acid during bread making. *Die., Nahrung* (In press).
7. Harland, B. F. and Harland, J., Fermentative reduction of phytic acid in rye, white and whole wheat bread. *Cereal Chem.*, 1980, **57**, 226.
8. Dube, R., Indrani, D., and Sidhu, J. S., Flour mill streams. I. Physico-chemical and rheological characteristics. *Indian Miller.*, 1987, **1**, 17.
9. Parthasarathy, A., Leelavathi, K., Selvaraj, A. and Sidhu, J. S. Flour mill streams. II. Suitability for different baked products. *Indian Miller.*, 1987 **1**, 35.
10. Jones, C. R. and Moran, T., A study of the mill streams comprising 80% extraction flour with particular reference to their nutrient content. *Cereal Chem.*, 1946, **23**, 248.

USE OF NON-CONVENTIONAL COAGULANTS IN THE MANUFACTURE OF *PANEER*

S. SACHDEVA AND S. SINGH
National Dairy Research Institute, Karnal-132 001, India

Received 8 *July* 1986; *revised* 14 *April* 1987

Certain non-conventional, low cost coagulants (0.6% solution of hydrochloric or phosphoric acid and acidophilus whey) were effectively used in the manufacture of *paneer* as substitutes for citric acid. Hydrochloric acid is the most economical among the chemical coagulants. The use of citric acid solution in naturally soured whey (0.6%) or acidophilus whey reduced the requirement of citric acid and increased the solids recovery without any loss of *Paneer* quality.

Paneer is an indigenous milk product prepared by the acid and heat coagulation of milk. It is defined as a product obtained from cow's or buffalo milk or a combination thereof, by precipitation with sour milk, lactic or citric acid[1]. Citric acid is the most common coagulant. Due to the high cost of citric acid, the feasibility of using low cost coagulants in the manufacture of *paneer* was explored.

Standardised buffalo milk (5.8 per cent fat) was used for *paneer* manufacture by the modified method of Bhattacharya *et al*[2]. The modification involved heating

TABLE 1. EFFECT OF DIFFERENT COAGULANTS ON THE QUALITY, YIELD AND SOLIDS RECOVERY OF PANEER

Coagulant					Moisture	Yield	Total solids recovery	Total solids in whey	Penetrometer reading
Name	Level (%)	pH	% Titratable acidity	Amount (l/100 l. milk)	(%)	(%)	(%)	(%)	
Citric acid	1.0	2.51	1.28	20.50	55.74	21.80	62.31	5.98	140
Tartaric acid	1.0	2.34	1.08	20.00	55.47	21.67	62.22	6.22	137
Lactic acid	1.0	2.61	0.76	21.00	54.99	22.58	65.67	5.84	134
Phosphoric acid	0.6	1.77	1.47	19.00	56.14	22.77	64.45	5.87	145
HCl	0.6	1.31	0.58	25.50	56.83	23.42	65.29	5.83	149
Citric acid in sour whey	0.6	3.27	1.79	20.50	55.08	23.20	67.31	7.08	135
Acidophilus whey	—	3.41	1.22	19.00	54.83	22.75	66.40	7.22	133

Each value is the mean of four replicates.

of milk to a temperature of 90°C instead of 82°C. The titratable acidity (TA) of whey and coagulant[3] and total solids (TS) content of whey[4] were determined by the ISI procedures. The pH was determined by using Global digital pH meter. Moisture was determined by the ISI method for cheese[5] with slight modification which involved addition of 5 ml of hot distilled water to break the curd and disperse it uniformly. The hardness was determined by the precision penetrometer (Central Ignition Co., London). Sensory evaluation of *paneer* was done on a 9-point Hedonic scale in raw, fried and cooked form. The frying of *paneer* was done in refined groundnut oil heated to a temperature of 175-185°C. The fried *paneer* was cooked in double the quantity of water with 1.5 per cent salt by boiling for 5 min.

The effecst of different coagulants, including citric, tartaric, lactic, phosphoric, hydrochloric, citric acid in sour whey and acidophilus whey on the quality, moisture retention, yield, total solids recovery, total solids loss in whey and penetrometer reading were studied. The flavour score of all the raw *paneer* samples ranged from 7.1 to 7.5 regardless of coagulants used except in *paneer* prepared from phosphoric acid which scored 6.6 and was criticised to be acidic. The frying and cooking had improving effect on acceptability of *paneer* the scores ranging from 7.2 to 7.6 and 7. 4 to 7.8, respectively. The body and texture scores of raw *paneer* ranged from 7.0 to 7.4 without any significant effect of specific coagulants. Similarly, the scores for appearance of *paneer* varied from 7.2 to 7.4 irrespective of the coagulants used.

The results for moisture, yield, total solids recovery, total solids loss in whey and penetrometer reading are presented in Table 1. The pH and TA of various coagulants ranged from 1.31 to 3.41 and 0.58 to 1.79 per cent, respectively The greatest drop in pH was in hydrochloric acid solution followed by phosphoric acid. The amount of coagulant used varied over a narrow range of 19-21 per cent except for hydrochloric acid which was required to an extent of 25.5 per cent. The moisture varied from 54.83 to 56.83 per cent. The yield ranged from 21.67 to 23.42 per cent depending upon the moisture retention and the total solids recovery in *paneer*. The total solids recovery was highest (67.31 per cent) in case of citric acid dissolved in sour whey, followed by acidophilus whey, lactic acid, hydrochloric acid, phosphoric acid, citric acid and least (62.22 per cent) in tartaric acid. Bhattacharya *et al*[2] reported maximum yield and total solids recovery of 21 and 61 per cent, respectively when 1 per cent citric acid was used as coagulant. The solids loss in whey was highest (7.22 per cent) in case of acidophilus whey and minimum (5.83 per cent) in case of hydrochloric acid. The penetration values ranged from 133 to 149, being directly related to the moisture content.

The data presented in Table 1 represent the optimum concentration/level of coagulants. To arrive at these levels, different concentrations of coagulants were tried initially. Phosphoric acid solution of different concentrations viz. 0.4, 0.6 and 0.8 per cent were used. This coagulant imparted a slightly acidic flavour which increased in intensity with increasing concentration of acid. There was no appreciable difference in the body and texture and appearance characteristics between the experimental *paneer* samples and the control. The yield and total solids recovery were inversely related to the concentration of phosphoric acid. The total solids loss in whey increased with increasing concentration. The different concentrations of hydrochloric acid viz. 0.4, 0.6 and 0.8 per cent showed similar results as in case

of phosphoric acid except that the samples were free from acidic flavour defect. The hydrochloric acid concentration of 0.4 per cent resulted in a soft and weak body. The amount of coagulant required was also more when lower concentration of acids was used.

Sour whey, a by-product of *paneer* manufacture was used as a coagulant to effect economy. When whey soured overnight at 30°C was used as such, the amount required to coagulate was too much (90 per cent) posing problems in handling. To cut down the bulk and the requirement of citric acid, it was dissolved in sour whey instead of water at the rate of 0.2, 0.4 and 0.6 per cent. The yield and total solids recovery increased with increase in citric acid concentration, reaching maximum at 0.6 per cent (23 and 67 per cent respectively). The quantity of 0.6 per cent citric acid solution in sour whey, required for coagulation, was 20.50 per cent of the total milk quantity which is the same as when 1 per cent citric acid solution in water was used. In order to completely eliminate the need of citric acid, the whey was cultured with *L. acidophilus* at 2 per cent and incubated overnight at 30 and 37°C. Naturally soured whey reached an acidity of 0.44 per cent and pH of 4.42 after 24 hr incubation at 30°C. In contrast, the cultured whey reached an acidity of 0.92 per cent and pH 3.65 at 30°C and 1.22 per cent acidity and pH 3.41 at 37°C. The acidophilus whey with the highest acidity resulted in the best *paneer* with maximum yield and TS recovery.

It may be concluded that inorganic acids like hydrochloric and phosphoric can be used in the manufacture of *paneer* without loss of its yield and quality. Hydrochloric acid is the most economical and suitable, while organic acids are costly. The *paneer* production can also be economised by replacing citric acid completely with acidophilus whey with which the yield and quality are both superior.

References

1. *Commentary on the Prevention of Food Adulteration Act.* Jain Law Agency, Chandigarh, 1976, 605.
2. Bhattacharya, D. C., Mathur, O. N., Srinivasan, M. R. and Samlik, O., Studies on the method of production and shelf life of *paneer*, *J. Fd Sci. Technol.*, 1971, 8, 117.
3. *Methods of Test for Dairy Industry, Part I. Rapid Examinations of Milk*, IS: 1479-1960. Indian Standards Institution, New Delhi.
4. *Methods of Test for Dairy Industry, Part II. Chemical Analysis of Milk*, IS: 1479-1961. Indian Standards Institution, New Delhi.
5. *Specification for Natural Cheese (Hard Variety), Processed Cheese, Processed Cheese Spread and Soft Cheese*, IS: 2785-1979. Indian Standards Institution, New Delhi.

UTILIZATION OF WHEY FOR THE PRODUCTION OF BETA-GALACTOSIDASE BY *HELMINTHOSPORIUM ORYZAE*

RAKESH MATHUR, S. K. GARG

Department of Dairy Bacteriology, College of Dairy Science, Sukhadia University, Udaipur-313 001, India

and

SANJEEV AGRAWAL

Department of Plant Physiology and Biochemistry, S.K.N. College of Agriculture, Jobner-303 329, India

Received 20 *March* 1986; *revised* 16 *March* 1987

Twelve mold cultures were screened for beta-galactosidase production; *Helminthosporium oryzae* was found to be the most efficient. Beta-galactosidase production was maximum in the temperature range of 25-30°C, pH 4.5 and with 5% (v/v) inoculum of a week-old culture of *H. oryzae*. Among the organic and inorganic nitrogen sources, ammonium chloride was found to be the best followed by urea. Incubation for 8 days was optimum.

Whey is a by-product recovered after cheese and casein manufacture. It contains about 4.9 per cent lactose, 0.9 per cent protein and 93 per cent water[1]. Enormous quantities of whey are drained off as wastage causing serious pollution problems. Though disposal and/or utilization of whey in India has not yet assumed serious proportions, with the rapid expansion of dairying the problem will surely crop up. It can be solved by culturing microorganisms in whey to produce enzymes like beta-galactosidase which catalyses the hydrolysis of lactose into glucose and galactose[2]. Although beta-galactosidase can be obtained from plants and animals, its commercial production from microorganisms has attracted much attention due to ease of cultivation and manipulation of cultural conditions.

The enzyme from yeast, *Kluyveromyces fragilis* has been most widely studied for food applications[3,4] but it is active at low temperature and around neutral pH which is not suitable for acid whey and yoghurt. However, the enzyme from fungal origin is not only active at low pH but can sustain high temperature exposure[5,6] and can therefore be directly used.

In view of these facts, fungal cultures were screened and the most suitable culture was standardized to study the production of beta-galactosidase.

Organisms: Mold cultures of *Alternaria parascicola*, *Aspergillus awamori*, *A. luchiensis*, *A. nidulans*, *A. oryzae*, *A. terreus*, *Helminthosporium oryzae*, *H. sativum*, *Macrophomina phaseoli*, *Mucor pusillus*, *Myrothecium verrucaria* and *Syncephalosporium* sp. were obtained from the Culture Collection Centre of National Dairy

Research Institute, Karnal and maintained on Czapek Dox Agar[7] with lactose substituted for free sugar in the medium.

Propagation: Whey powder, obtained from the Experimental Dairy of National Dairy Research Institute, Karnal, was used as a medium for the propagation of fungal cultures. It was deproteinised by adjusting the pH to 4.5 with 1N HCl and boiling for 10 min. Coagulated proteins were removed by centrifugation at 3000 ×g for 15 min and the clear supernatant was sterilized at 121°C for 20 min. All the cultures were inoculated at 5 per cent (v/v) level (approximately 10^8 spores/ml) and incubated at 28±1°C for 7 days. At the end of the incubation period, mycelia were separated by filtering through Whatman No. 1 filter paper and washed twice with distilled water. One set of mycelium was dried at 60°C to constant weight to study the growth; another set was used for preparation of cell free extract by grinding it in a pestle and mortar with 0.1M citrate buffer of pH 4.6 and centrifuging at 20,000 ×g for 20 min. This was used for assaying beta-galactosidase activity.

Assay of enzyme activity: Beta-galactosidase activity was assayed using a modified procedure of Wendorff and Amundson[8]. Cell free extract prepared as above was incubated with 1mM orthonitrophenyl-beta-D-galactopyranoside (ONPG) in a total volume of 3 ml at 45°C for 10 min. At the end of the incubation period, reaction was stopped by addition of 2 ml of 1M sodium carbonate and the colour developed was measured at 420 nm. A unit of enzyme activity was expressed as the amount of enzyme required to liberate 1 micro mole of O-nitrophenol in 10 min under the conditions specified above. Protein in the cell free extract was estimated colorimetrically by the method of Lowry *et al*[9].

Effect of cultural factors viz., temperature (20-40°C), pH (2-9), inoculum size (2-10 per cent) and different organic and inorganic nitrogen supplementations viz., cornsteep liquor, yeast extract, molasses, urea, beef extract, proteose peptone, ammonium chloride, amomnium nitrate, ammonium sulphate and sodium nitrate, at 0 to 700 mg N/L whey, was studied on beta-galactosidase production.

Among the twelve fungal cultures screened, *Helminthosporium oryzae* was the most efficient producer of beta-galactosidase (Table 1). Hence, *H. oryzae* was selected for studying optimum cultural and nutritional conditions which were found to be: temperature, 25-30°C period of incubation, 8 days; initial pH of whey, 4.5; inoculum, 5 per cent (v/v); and 500 mg N/L equivalent of ammonium chloride. The highest enzyme units of 690 were produced under optimum cultural and nutritional conditions.

TABLE 1. PERFORMANCE OF MOLDS IN THE PRODUCTION OF BETA-GALACTOSIDASE FROM WHEY

Organisms	Dry cell wt. (g/L whey)	Enzyme (units/L whey)	Enzyme (units/mg protein)
Alternaria parascicola	9.8	264	0.880
Aspergillus awamori	7.8	92	0.328
A. luchiensis	11.8	333	0.833
A. nidulans	7.3	150	0.267
A. oryzae	13.8	70	0.194
A. terreus	11.6	190	0.558
Helminthosporium oryzae	13.6	380	1.428
H. sativum	13.1	375	1.041
Macrophomina phaseoli	13.2	138	0.363
Mucor pusillus	7.9	148	0.493
Myrothecium verrucaria	7.0	160	0.533
Syncephalosporium sp.	9.2	88	0.231

*All the experiments were conducted in duplicate.

Authors are thankful to the National Dairy Research Institute, Karnal, for the supply of cultures and whey powder. Facilities provided by the College of Dairy Science, Sukhadia University, Udaipur are thankfully acknowledged.

References

1. Agrawal, S. Garg, S. K. and Dutta, S. M., Many uses of whey, *Sc. Rep.*, 1984, **21**, 348.
2. Garg, S. K., Agrawal P. and Agrawal, S. Dairy effluents-treatment, disposal and utilization, *Sci. Rep.*, 1985, **22**, 184.
3. Tikhomirova, A. S., Kulikova, A. K. and Feniksova, R. V., Conditions for biosynthesis of beta-galactosidase in *Saccharomyces fragilis*, *Microbiology*, 1972, **41**, 204.
4. Wendorff, W. L., Amundson, C. H., Olson, N. F. and Garvar, J. C. Use of yeast beta-galactosidase in milk and milk products, *J. Milk Fd Technol.*, 1971, **34**, 294.
5. Wierzbecki, L. E. and Kosikowski, F. V., Lactase potential of various microorganisms grown in whey, *J. Dairy Sci.*, 1973, **56**, 26.
6. Woychick, J. H. and Windolowski, M. V., Lactose hydrolysis in milk and milk products by bound fungal beta-galactosidase, *J. Milk Fd Technol.*, 1973, **36**, 31.
7. Haynes, W. D., Wickerham, L. J. and Hesseltine, C. W., Maintenance of cultures of industrially important microorganisms, *Appl. Microbiol.*, 1955, **3**, 361.
8. Wendorff, W. L. and Amundson, C. H., Characterization of beta-galactoisidase from *Saccharomyces fragilis*, *J. Milk Fd Technol.*, 1971, **34**, 300.
9. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with folin phenol reagent, *J. biol Chem.*, 1951, **193**, 265.

DISINFESTATION OF WHOLE AND GROUND SPICES BY GAMMA IRRADIATION

S. R. PADWAL-DESAI, ARUN SHARMA AND S. V. AMONKAR*

Food Technology & Enzyme Engineering Division
Bhabha Atomic Research Centre, Bombay-400 085, India

Received 23 *July* 1986; *revised* 28 *February* 1987

Number of insect species were identified in chilli (*Capsicum annum* Linn), turmeric (*Curcuma longa* Linn), ginger (*Zingiber officinale* Roscoe), pepper (*Piper nigrum* Linn) and coriander (*Coriandrum sativum*) and also in two commercial brands of prepacked ground spices. *Lasioderma serricorne* (Cigarette beetle), *Oryzaephilus surinamensis* (Saw toothed grain beetle), *Rhizopertha dominica* (Lesser grain borer), *Sitotroga cerealella* (Angoumois grain moth) and *Tribolium castaneum* (Red flour beetle) were the predominant pest species found in these spices. Exposure of spices to Co^{60} gamma irradiation at 1 kGy dose level did not show adult emergence of insects in these spices during storage at ambient temperature (28-30°C) indicating that the radiation dose (10 kGy) that has been shown to be effective for microbial decontamination of spices destroys insect pests as well.

Earlier reports from this laboratory have shown that exposure to gamma-irradiation in the dose range of 7.5-10 kGy was adequate to sterilize some of the whole spices[1]. This method was also effective in eliminating microbes in commercial brands of prepacked ground spices, without affecting their quality attributes[2]. A major problem with Indian spices is insect infestation during storage[3], particularly due to tropical conditions. This not only affects the shelf-life but also marketability often resulting in huge economic losses[4]. Experiments were therefore, undertaken to identify the common pests in chilli, turmeric, ginger, pepper and coriander. Commercial brands of prepacked ground spices were also examined during storage to ascertain the effective dose of gamma-irradiation for the elimination of the insects.

Whole spices and prepacked ground spice products were purchased from local retailers. Each of these spices was kept in a closed glass jar for 90 days at ambient temperature (28-30°C) and was examined for the, emergence of insects at monthly intervals. In another set of experiments, the same spices were exposed to gamma-irradiation (doses 1 to 10 kGy). For identification, the insects were isolated by sieving the spice and removed on to a watch glass. Pest species were identified according to the procedure of Borror and Delong[5].

The irradiation procedure using a Gamma-cell 220 (AECL) has been described earlier[1]. The dose rate was 27 kGy/min during these experiments.

Table 1 shows the insect pests identified in the unirradiated whole and ground spices. The irradiated spices did not show the presence of any insect at all the dose levels studied. The predominant species differ in different spice samples. Thus, chilli showed emergence of four insect species, while turmeric had two and ginger three. Cardamom and coriander showed only presence of red flour beetle. Pepper, either whole or ground, did not show presence of any insects.

Radiation sensitivity of these insects has been reported earlier[6,7]. The values for lethal dose of gamma irradiation for some of the common insect pests have been included in Table 2. In those studies, the effects of gamma-irradiation on various pests including *L. serricorne* *O. surinamensis*, *R. dominica*, *S. cerealella* and *T. castaneum* were examined at different developmental stages. All these insects are known to be highly susceptible to gamma-irradiation. The eggs and middle larval instars were eliminated at a dose of 0.2 kGy. Any adult that emerged was either killed or rendered sterile by a dose upto 0.1 kGy[8]. A minimum dose of 1 kGy was

TABLE 1. PREDOMINANT INSECT SPECIES FOUND IN SPICES UNDER STORAGE

Pest	Whole spices					Ground spices		
	Chilli	Turmeric	Ginger	Cardamom	Coriander	Chilli	Turmeric	Coriander
L. serricorne	+	+	+	—	—	+	+	—
O. surinamensis	+	—	—	—	—	+	—	—
R. dominica	—	—	+	—	—	—	—	—
S. cerealella	+	—	—	—	—	+	—	—
T. castaneum	+	+	+	+	+	+	+	+

*Bio-Organic Division, BARC.

TABLE 2. LETHAL DOSE OF GAMMA IRRADIATION FOR INSECT PESTS

Pest	Dose (kGy)
Sitophilus oryzae	1.6
Sitophilus granarius	2.0
Rhizopertha dominica	2.0
Calandra granaria	2.0
Calandra oryzae	2.0
Tribolium castaneum	2.0
Lasioderma serricorne	2.0
Sitranus surinamensis	2.5
Sitotroga cerealella	2.5
Tribolium confusum	3.0
Stegobium paniceum	3.0
Oryzaephilus surinamensis	3.0

therefore, sufficient to prevent insect emergence in the spices and spice products. The present report thus not only identifies the insect species infesting various spices but also confirms that doses for disinfestation are far below those required for the elimination of microbes. Gamma irradiation of spices should, therefore, hold considerable promise as an effective treatment both for disinfestation and disinfection.

The authors thank Dr. G. B. Nadkarni, Head, Food Technology & Enzyme Engineering Division, BARC, for his keen interest in this work.

References

1. Sharma Arun, Ghanekar, A. S., Padwal-Desai, S. R. and Nadkarni, G. B., Microbiological status and antifungal properties of irradiated spices. *J. agric. Fd Chem.*, 1984, **32**, 1061.
2. Munasiri, M. A., Parte, M. N., Ghanekar, A. S., Sharma Arun, Padwal-Desai, S. R. and Nadkarni, G. B., Sterilization of prepacked ground spices by gamma irradiation. *J. Fd Sci.* 1987, 52 823.
3. Seenappa, M. L., Stobbs, L. W. and Kempton, A. G., The role of insects in the biodeterioration of Indian red peppers by fungi. *Int. Biodet. Bull.*, 1979, **15**, 96.
4. Seenappa, M. L., Factors of microbial origin influencing national and international marketing of major Indian spices. *Proceedings of the Seminar on Spices, Oleoresins and Flavours*, Association of Food Scientists & Technologists India, Bombay, 1981, 22.
5. Borror, D. J. and Delong, D. M., *An Introduction to the Study of Insects*, Holt, Rinehart & Winston, New York, 1964, 819.
6. Pendlebury, J. B., Jefferies, D. J., Banham, E. J. and Bull, J. O., Some effects of gamma radiation on the Lesser Grain Borer, Tropical Warehouse Moth, Indian Meal Moth and Cigarette Beetle. AERE-4003, Harwell, 1962.
7. Titton, E. W., *Ionizing Radiation for Insect Control in Grain and Grain Products*, IFFIT-Training Course, Wageningen, The Netherlands. 1986, 29.
8. Rahalkar, G. W. and Lewis Anne, Status of project on grain disinfestation with gamma rays. *Proceedings of BARC/IAEA/FAO Symposium on Food Irradiation*, Bhabha Atomic Research Centre, Bombay, 1969, 21.

THYMOL—A SECONDARY PRODUCT FROM CALLUS CULTURES OF AJOWAN (*CARUM COPTICUM*)

RENU AGRAWAL AND M. V. PATWARDHAN
Central Food Technological Research Institute, Mysore-13, India

Received 23 *July* 1986; *revised* 25 *February* 1987

Callus cultures have been established from mature seeds of Ajowan (*Carum copticum*). A semidifferentiated callus was raised from the undifferentiated callus which produced thymol (20% as compared to the seeds)—a major constituent of the essential oil. A colorimetric method has been developed to estimate thymol.

In recent years, there has been an increased interest in plant tissue culture for the production of secondary metabolites[1,2] Nabeta *et al*[3] studied the volatile oils from callus and suspension cells of *Perilla frutescens* with the help of GCMS Similar studies have been carried out by Ishikura *et al*[4], with cultures of *Cryptomeria japonica*. Ajowan (*Carum copticum*) is one of the major Indian spices that is relished the world over. The main constituents contributing to its essential oil content are thymol and cymene. Ajowan seeds (market sample) were selected as one of the spices for the production of secondary metabolites by tissue culture.

A qualitative spot test for thymol has been described by Feigl[5]. Thymol has also been estimated quantitatively in the essential oil of *Thymus capitatus* by high performance liquid chromatography[6]. This is not convenient for routine analysis, particularly in laboratories where HPLC would not be available. Hence, the spot test described by Feigl was modified and developed for the quantitative determination of thymol, one of the flavour ingredients obtained from callus cultures of Ajowan.

Explant source: Seeds of Ajowan collected from the local market were sterilized by treating with $MgCl_2$ (0.5 per cent) for 2 min and subsequently washed 3 times with distilled water to remove excess $HgCl_2$. The surface sterilized seeds were inoculated aseptically on to solid agar medium.

TABLE 1. EFFECT OF 2,4-D AND IAA ON THE CALLUS GROWTH OF *CARUM COPTICUM*

Auxins (μg/ml)		Callus characteristics
2,4-D	IAA	
1	—	Fragile callus
2	—	Fragile callus
3	—	Compact undifferentiated white callus
2	1	Undifferentiated white callus with few green patches
1	2	Undifferentiated white callus with few green patches
—	3	Chlorophyllous callus
—	3*	Semi-differentiated callus

Medium used: Basic Murashige and Skoog's medium with 10% coconut milk

*The medium was supplemented with 1 μg/m NAA and kinetin along with 5% sucrose.

TABLE 2. EFFECT OF TIME ON THE THYMOL CONTENT IN UNDIFFERENTIATED AND SEMI-DIFFERENTIATED CALLUS CULTURES OF *CARUM COPTICUM*

No. of weeks	Thymol content (% dry wt.)	
	Undifferentiated white callus[a]	Semidifferentiated green callus[b]
2	0.02* (0.018 – 0.021)	0.06 (0.059 – 0.062)
4	0.025 (0.024 – 0.026)	0.08 (0.078 – 0.082)
6	0.06 (0.058 – 0.060)	0.17 (0.16 – 0.18)
8[c]	0.08 (0.078 – 0.081)	0.20 (0.19 – 0.22)

[a] Medium used: Basic Murashige and Skoog's supplemented with 3 μg/ml of 2,4-D.

[b] Basic Murashige and Skoog's medium supplemented with 3 μg/ml of IAA, 1 μg/ml NAA and kinetin 10% coconut milk and 5% sucrose.

[c] Prolonged growth of the callus for more than 8 weeks led to fast deterioration and culture turning brown.

*Sample size for each estimation was 5 gms of fresh callus. Each reported value is an average of 4 replicates.

Figures in the parenthesis indicate the range of values.

Culture medium: Murashige and Skoog's[7] (MS) medium was used as the basal medium for callus growth. The growth hormones 2,4 dichlorophenoxy acetic acid (2, 4-D), indole acetic acid (IAA), naphthalene acetic acid (NAA) and kinetin were added as mentioned in Table 1. The inoculated flasks were incubated at 26±1°C and were illuminated at 1000 lux for a period of 8 hr a day.

Reagents: Standard thymol solution in methanol 5 mg/50 ml, 2:6 dichloroquinone-4-chlorimine in methanol 2:6 DC:10 mg in 100 ml, methanolic ammonia (v/v:10:1).

Chemicals: A standard medium was purchased from Himedia Laboratory Rt. Ltd., Bombay. The growth hormones and thymol were from Sigma Chem. Co., USA. The reagent 2:6-DIC was obtained from Aldrich Chemical Co., USA.

Thymol estimation from callus cultures: Five grams of fresh culture were extracted with 10 ml of methanol. Four replicates each of undifferentiated and semi-differentiated cultures, subcultured at the same time (over eight passages) were employed for the estimation of thymol after an incubation period of 2,4,6 and 8 weeks in both the cases. The filtrate was recovered and an aliquot was taken in a glass stoppered test tube. A filter paper strip impregnated with the 2:6 DIC reagent was suspended from the stopper. The test tube was placed in a glycerol bath which was then heated to 165°C. After 20 min heating, the test tube was removed from the bath and the filter paper was immediately dipped in 5 ml of methanolic ammonia solution. The filter paper strip was discarded and the stable blue colour developed was read at 600 nm in a spectronic 20 colorimeter. Recovery experiments indicated more than 85 per cent recovery of the thymol added to the methanolic extract. Variation in thymol per cent from sample to sample was negligible.

The values for thymol, a secondary metabolite synthesized by the undifferentiated and the semi-differentiated callus (where organogenesis was initiated) of *Carum copticum* were estimated by this method. The results are given in Table 2.

Callus induction: Undifferentiated callus was obtained from the seeds using MS as basal medium supplemented with 3 μg/ml of 2, 4-D. The absence of 2,4-D and increased concentration of IAA in the medium resulted in the formation of chlorophyllous (undifferentiated green culture) callus. When the medium was supplemented with NAA and Kinetin along with IAA, the chlorophyllous callus led to semi-differentiation (initiation of organogenesis) (Table 1). The thymol estimations have been carried out only with undifferentiated callus and semi-differentiated callus.

The undifferentiated and semi-differentiated callus were found to contain 0.104 and 0.173 per cent DWB of total phenols estimated by the method of Swain and Hills[8]. The moisture content of the samples was 91.7 per cent.

With progress in incubation, there was increase in the thymol content both in the undifferentiated callus and in the semi-differentiated callus (Table 2). At every stage, the thymol content was more in the semi-differentiated callus than in the undifferentiated one.

This study leads to the conclusion that initiation of organogenesis (semi-differentiation) is one of the prerequisites for the production of secondary metabolites. This has also been suggested by other workers[9,10].

The senior author expresses her gratitude to Council of Scientific and Industrial Research, New Delhi, for the award of research associateship.

References

1. Nabeta, K. and Sugisawa, H., Terpene biosynthesis by the callus tissues from *Perilla* sp., in *Plant Tissue Culture* by A. Fujiwara, (Ed) Japanese Association for Plant Tissue Culture, Tokyo, 1982, 289.

2. Rodov, V. I. S. and Reznikova, S. A., Production and Characteristics of peppermint (*Mentha piperita* L.) cell suspension culture in connection with biosynthesis of terpenoids. *Feziol. Rest.*, 1982, **29**, 644.

3. Nabeta, K., Ohnishi, Y., Hirose, T. and Sugisawa, H., Monoterpene biosynthesis by callus tissues and suspension cells from *Perilla* species., *Phytochem.*, 1983, **22**, 423.

4. Ishikura, N., Nabeta, K. and Sugisawa, H., Volatile components in cell suspension cultures of *Cryptomeria japonica*, *Phytochem.*, 1984, **23**, 2062.

5. Feigl, F., *Spot Tests in Organic Analysis*, Elsevier Publ. North Holland, Amesterdam, 1960, 6th Edn, 202.

6. Solinas, V. and Gessa, C., High performance liquid chromatographic analysis of carvacrol and thymol in the essential oil of *Thymus capitatus*. *Chromatography*, 1981, **14**, 186.

7. Murashige, T. and Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Pl.*, 1962, **5**, 473.

8. Swain, T. and Hills, W. E. Qualitative analysis of phenolic constituents. *J. Sci. Fd Agric.*, 1959, **10**, 63.

9. Collin, H. A. and Watts, M., Flavour production in culture in *Hand book of Plant Cell Culture* by D. A. Evans, W. R. Sharp, P. V. Ammirato and Y. Yamada, (Eds) Mcmillan Publ. Co. New York, 1983, **1**, 729.

10. Freeman, G. G., Whenham, R. J., Mackenzie, I. A. and Davey, M. R., Flavor components in tissue culture of onion (*Allium cepa* L.) *Pl. Sci. Lett.*, 1974, **3**, 121.

MYCOTOXINS IN SPICES. III. INVESTIGATION ON THE NATURAL OCCURRENCE OF AFLATOXINS IN *CORIANDRUM SATIVUM* L.

N. MISRA

Botany Department, University of Gorakhpur, Gorakhpur-273 001 India

Received 31 *October* 1986; *revised* 20 *April* 1987

Aflatoxins were isolated from ten seed samples of *Coriandrum sativum* L. Of these, only four were found to be BGY positive; aflatoxins B_1, B_2 and G_1 were isolated from BGY positive samples. The concentrations of various aflatoxins were determined after development with chloroform-acetone from observation on their flourescence emission on silica gel plates.

Secretion of some toxic metabolites by certain fungi during the storage of foodstuffs has created an alarming situation as they are consumed by human beings[1]. Coriander (*Coriandrum sativum* L.) vernacular name, *dhania* is an annual herbaceous plant belonging to the family *umbelliferae*. Its seeds/fruits are used for flavouring various dishes. Commercially, these are employed in the manufacture of processed meats, cheese, pickles and sauces. In India, these seeds are used for the preparation of certain ayurvedic medicines. The seeds are stored mostly in gunny bags after sun-drying, The conditions most conducive for their deterioration prevail throughout the year. A large number of microorganisms have been reported on the seeds of coriander[2,3]. Some of them viz. *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. have been reported to secrete toxins. Other workers[1–5, 7–9] have also isolated aflatoxins from cotton, cayenine, pepper, Indian chilli powder, dried pepper, black pepper, capsicum and nut meg. This paper reports for the first time results of qualitative and quantitative analysis of aflatoxins isolated from stored seeds of coriander.

Fresh seeds were collected immediately after harvest from ten different fields and were observed under UV light. Samples which showed fluorescence were identified as BGY positive, others BGY negative. All the samples were separately stored in the laboratory at room temperature (18-40°C) and 60-100 per cent R.H. At regular intervals of three months, the samples were observed under UV light.

The BGY positive samples were analysed for aflatoxins quantitatively and qualitatively[6]. The extracts (2.5, 5 and 10 μl) were quickly chromatographed on silica gel TLC plates (0.50 mm thick). For each sample, six TLC plates were developed. Standards containing about 0.5 μg/ml of B_1 and G_1 and 0.1 μg/ml of B_2 and G_2 were also

TABLE 1. AFLATOXINS IN CORIANDER SEEDS DURING STORAGE

Sample No.	Aflatoxin type	Aflatoxin (μg/kg) during indicated storage periods (months)				
		0	3	6	9	12
I	B_1	37	59	72	72	72
	B_2	5	11	19	19	19
	G_1	3	8	12	12	12
4	B_1	21	37	37	37	37
	B_2	—	—	—	—	—
	G_1	4	7	10	10	10
6	B_1	19	35	35	35	35
	B_2	—	—	—	—	—
	G_1	4	7	10	10	10
7	B_1	—	17	33	33	33
	B_2	—	8	11	11	11
	G_1	—	5	16	16	16
8	B_1	—	62	75	75	75
	B_2	—	12	21	21	21
	G_1	—	7	10	10	10

chromatographed along with unknowns. The plates were developed in chloroform:acetone solvent (9:1) with a solvent run of about 14 cm. The plates were dried in darkness and Rf values were subsequently compared in UV light.

For quantitative estimations, fresh TLC plates were prepared for each sample. Extracts of the BGY positive samples and standards were chromatographed on each plate. The concentrations of aflatoxins in the samples were calculated by visual comparison of fluorescence intensities of the spots in the sample with those of standards.

Table 1 reveals the fluorescence emission from ten seed samples of coriander collected from different fields in Gorakhpur and stored upto 12 months. Of the ten samples examined, five showed fluorescence, they were BGY positive, three being positive immediately after harvest i.e. after nearly fifteen days. This may be due to the humidity (R.H. 90 per cent) and temperature (35°C) at the time of collection from fields which experienced rains just a week earlier. The other two samples showed fluorescence after storage for three months. This may be because of low seed moisture content i.e. 6 and 8 per cent. All the samples except one exhibited strong blue, blue and green fluorescence; one sample exhibited only strong blue and green fluorescence. No spot corresponding to aflatoxin G_2 was observed on any chromatoplate.

Table 1 also represents the quantity of aflatoxins in BGY positive samples. From the data, it is evident that highest amounts of aflatoxins were isolated from sample 8 and least from sample 7. Concentration of B_1 was highest in all the samples and that of G_1 was least. The concentration of B_1 and G_1 in sample I and 8 are about the same but quantity of B_2 extracted from sample I was more than from sample 8. Only, aflatoxins B_1 and G_1 were extracted from sample 4 and sample 6. Concentractions were almost the same.

During 6 months storage, concentrations of the aflatoxins increased in all the samples. Beyond six months, there was no further increase. These results are perhaps because of high R.H. (80-100 per cent) and moderate temperature (30-40°C) during the first six months after which there was a decline both in R.H. (60-75 per cent) and in temperature (18-28°C). Other reason for the above observations may be the moisture content of the seeds. It increased during the first six months after which it was static (i.e. 14, 18, 21 per cent in sample I; 9, 9.7, 13 per cent in sample 4; 9, 9.5, 12.8 in sample 6, 6, 9.7, 9.7 per cent in sample 7 and 8, 13.2, 18 per cent in sample 8 at 0, 3, 6 months, respectively).

References

1. Shank, R. C., Wogan, G. N., Gibson, J. W. and Nondasuta, A., Dietary aflatoxins and human liver cancer. II. Aflatoxin in Marker food and feedstuffs of Thailand and Hong Kong. *Fd Cosmet. Toxicol.*, 1972, **10**, 61.
2. Misra, N. and Bhargava, K. S., A study of mycoflora of spices and condiments in storage. *Int. Mycological Congr.*, Tampa, Florida, U.S.A. 1977, 444.
3. Misra, N., New records of seed borne fungi from four, umbelliferous spices. *Nat. Acad. Sci. Lett.*, 1981, **4**, 195.
4. Ashworth, L. J., Mc Means, J. L., Fyle, J. L., Brown, C. M., Osgood, J.W. and Ponton, R. E., Aflatoxins in cotton seeds: Influence of weathering on toxin content of seeds and on a method for mechanically sorting seed lots. *Phytopath.*, 1968, **58**, 102.
5. Scott, P. M. and Kennedy, B.P.C., Analysis and survey of ground black, white and capsicum peppers for aflatoxins. *J. Asso. off. analyt. Chem.*, 1973, **56**, 1452.
6. Suzuki, J. I., Dainins, B. and Kilbuck, J. H., A modified method for aflatoxin determination in spices. *J. Fd Sci.*, 1973, **38**, 949.
7. Mirocha, C. J. and Christensen, C. M., Fungus metabolites toxic to animals. *Ann. Rev. Phytopath.*, 1974, 303.
8. Beljaars, P. R., Shumans, J.C.H.M. and Koken, P. J., Quantitative fluorodensitometric determination and survey of aflatoxins in nutmeg. *J. Ass. off. analyt. Chem.*, 1975, **58**, 263
9. Bilgrami, K. S., Prasad, T. and Sinha, R. H., *Changes in Nutritional Components of Stored Seeds Due to Fungal Association*. Report. International Biosciences Monograph. Today and Tommorow's Printers and Publications, New Delhi, 1979.

BOOK REVIEWS

Traditional Foods—Some Products and Technologies: C.F.T.R.I., Mysore-570 013, India, 1986; pp: 292; Price: Rs. 150.

The book is a collection of papers presented during a UN University Workshop on "Traditional Food Technologies—Their Development and Integrated Utilisation with Emerging Technologies" held at Central Food Technological Research Institute, Mysore in June 1983.

Twenty one papers have been contributed by experts from sixteen developing countries on the preparation of their traditional foods. These including Ethiopia, Nigeria, Sudan, Senegal, Pakistan, India, Burma, Nepal, Thailand, Malaysia, Indonesia, Philippines, Korea, Republic of China, Japan and Nigeria have described methods of mechanising the manufacture of traditional foods so that they could be mass produced. Two papers, one each from the U.S.A. and Canada have been presented to show the work carried out in the laboratories of their countries to modernise and mechanise traditional preparations from many developing countries. The application of biotechnology in the food industry forms the subject of a paper from Hungry. The contribution of FAO in the upgrading of traditional techniques for food consumption and processing has been described. Cultural aspects of traditional foods of India, the part played by tradition in the development, preparation and consumption of foods have been presented in two short papers. The last paper describes the Research and Development in Indian traditional foods being carried out at the CFTRI, Mysore.

The papers reveal that there is a wealth of information available from developing countries on the utilisation of indigenous raw materials for the preparation of palatable, nutritious and familiar foods. All the contributors agree that more work should be concentrated on the mechanisation of the preparation of such traditional foods, their hygienic manufacture and packaging. Attempts to import blindly technologies into developing countries which are mainly suitable for the manufacture of Westernised foods should be discouraged.

The cover page design of the book is very pleasing and the get up of the book is quite attractive.

DR. (MRS) SUMATI R. MUDAMBI
CONSULTANT NUTRITIONIST,
BOMBAY

Engineering Properties of Foods:: Ed. by M. A. Rao and S.S.H. Rizvi: Marcel Dekker Inc., New York-10016; 1986; pp: 416, Price: $ 69.75 (US and Canada) $ 83-50 (All other Countries).

This book on engineering properties of foods will be useful in design and operation of food processing equipments. Most equipments perform the unit operations like heat transfer, mass transfer, flow of fluids, including concentration and dehydration. Engineering properties of foods such as rheological properties, thermal mass transfer properties, thermodynamic properties during drying of foods, rheology of solid foods, physico-chemical and engineering properties of foods in reverse osmosis and ultra filtration and electrical properties of foods are discussed in detail.

In the first chapter, an attempt is made to describe foods under specific rheological behaviour, like pseudo-plastic, time dependent types like thixotropic and rheopectic. Effect of temperature on apparent viscosity has been discussed. Experimental methods for determination of flow properties are outlined. Implications of rheological properties in fluid flow and heat transfer of foods are discussed.

Chapter two discusses thermal properties of foods viz. specific heat, thermal conductivity, heat content, convective heat transfer co-efficient. Measurement methods are discussed. Data on thermal conductivity for different foods are listed and data sources indicated. Model concepts for these properties are also outlined.

Chapter three covers the importance of mass transfer in extraction, drying, distillation and absorption. Basic equations covering phase equilibrium in gas-liquid, liquid-liquid/solid, gas-solid systems are discussed. Diffusion in gases, liquids and solids is covered. Mass transfer aspects of water vapour and volatile flavours in food packaging materials are outlined and data for oxygen and water vapour permeabilites through plastic films listed.

Chapter four covers thermodynamics of food water systems, during dehydration of foods. Implications of water activity have been discussed and measurement methods are outlined. Moisture sorption isotherms and their implications are discussed. Basic principles of dehydration processes have been discussed in detail in addition to covering hot air drying of foods.

Chapter five discusses rheology of solid foods. Fundamental tests for the evaluation of elasticity-modulus, Poisson ratio, relaxation time and shear modulus are

outlined. Empirical and imitative tests for texture, appearance and flavour are discussed. Instruments like shear press, penetrometer and tenderometer and their uses in evaluation of tenderness, toughness, maturity and firmness are explained.

Chapter six on membrane food processing discusses in detail the fundamental principles governing food concentration by membrane processing. Basic transport equations are discussed which will help in design of membrane systems. Practical problems in membrane separation processes are outlined.

The last chapter on electrical properties of foods, covers the heating characteristics of food in high frequency radiation energy transfer in an electromagnetic field. The dielectric properties which cover energy coupling from the field source and its absorption in the product are discussed. Basic equations relating to measurement of dielectric properties are covered.

The book will serve as a good source of reference and provides theoretical background for students who want to take up advanced research. Plenty of references are listed at the end of each chapter.

B. S. RAMACHANDRA
C.F.T.R.I., MYSORE

With Our Own Hands: Research for Third World Development: Canada's contribution through the International Development Research Centre, 1970–1985, IDRC, Box 8500, Ottawa, Canada KIG 3H9, 1986; pp: 206: Price: Not stated.

Ten completed projects of the IDRC, among the 'hundreds under way' every year, are here reviewed as case studies. Three prefactory essays that occupy 55 pages are entitled: Introduction and critical review; The role of research in Third World development; and IDRC and Third-World researchers: The products of partnership. The ten projects themselves derive from as many countries and include a wide range of activities: ceramic stoves, animal production systems, primary school teachers, rural sanitation, oral rehydration therapy, a national information system, cropping systems research, firewood and polewood and the storage of seed potatoes. The project that is set in India is concerned with West Bengal and Orissa. It sought to verify whether the composite fish culture package of practices for raising three Indian and three exotic types of carp in the same pond, developed by the Central Inland Fisheries Research Institute (ICAR), was profitable and acceptable to farmers. The input of IDRC, though relatively modest, was claimed to be made at a critical stage of moving from station conditions to village life. In the event, the effects of the project were found to have been sustained among those originally involved in it, and also to have spread to neighbours in other villages and far beyond. Similar successes are noted in studies which involved the adoption of diffused light in rural storage of seed potatoes in Peru and of cropping systems developed in Indonesia for rainfed and partially-irrigated rice.

An external agency does bring to bear on a problem not just finance but critical evaluation, vitality and-sometimes related experience. One is, however, led to question how far, in a large country, small catalytic inputs can really go in overall development. However successful, do they not smack of do-gooding, with a touch of neo-colonisation thrown in? There is the danger always of neglecting one's own support systems which, properly fostered, are the only answers to harmonious development and self-sufficiency. Accounts like those in the present book help us realise the need for constant review and analysis, modification of action plans, and monitoring to raise the functional efficiency of our own systems.

DR. K. T. ACHAYA
BANGALORE

Ice Cream by W. S. Arbuckle, The AVI Publishing Company, Inc., Westport, Connecticut, 1986; pp: 483; Price: $ 49.50.

This fourth edition of the book has 22 chapters, one less (Soda Fountain Recipes) than the second edition. In the preface to the book, the author states that this edition is a full revision of previous editions and includes an updating of the areas that have been affected by changes and new technology. He also states "Chapters that include basic information on ice cream technology remain for the most part unchanged in order to accommodate beginners in the industry and the smaller processors. In other chapters, major revisions and the incorporation of new material have been made. Atleast to this reviewer, these major revisions were not visible in the contents of the book. Updating of information, if any, relates to the standards and statistics of production. In quoting the production in different countries, cross check of information appears to be wanting e.g., Pakistan's production 19,000 gallons and per capita 1.71 pts works out to a population of 90,000. Chapters 13 and 14 incorporate photographs of modern

equipment, unfortunately there is hardly any related discussion.

Most disappointing from the point of view of food research scientists and technologists is the bibliography section. There are references dating back to thirties which perhaps have been retained as key classical references, but, references directly related to manufacturing process, packaging and storage and other aspects, of late seventies and early eighties are hard to find. If the earlier editions are not already on the shelf, this book could be a useful addition to the library.

J. V. PRABHAKAR
C.F.T.R.I., MYSORE

AFST(I) News

Jammu Chapter

The Annual General Body Meeting was held on 24th April 1987. The following office bearers were elected for the year 1987:

President	:	Shri S. S. Langar
Vice-President	:	Dr. J. S. Chawla
Hony. Secretary	:	Dr. B. L. Raina
Hony. Treasurer	:	Dr. Siba Mukhopadhyaya
Councillors	:	Dr. P. Pushpangadan and Shri Shiv Dass Dubey

Jabalpur Chapter

The Annual General Body Meeting was held on 11th June 1987. The names of the new executive members are:

President	:	Prof. Y. K. Sharma
Vice-President	:	Dr. O. P. Gupta
Hon. Secretary	:	Dr. M. B. Bera
Joint Secretary	:	Dr. R. S. Y. Yadav
Treasurer	:	Dr. S. K. Sharma

Ludhiana Chapter

The Annual General Body Meeting was held on 30th March 1987. The following new Executive has been elected for the year 1987–88.

President	:	Dr. S. K. Berry
Vice-President	:	Dr. K. L. Sehgal
Hony. Secretary	:	Dr. K. L. Bajaj
Joint Secretary	:	Dr. M. S. Teotia
Hony. Treasurer	:	Dr. (Mrs) Shyama Wadehra
Auditor	:	Dr. R. K. Raheja
Councillors	:	Dr. R. Pal Singh Dr. (Mrs) S. Verma.

Palayamkottai Chapter

A Workshop on "Bakery Demonstration" will be conducted in collabaration with the U.S. Wheat, Associates, New Delhi, Society of Indian Bakers (Southern Regional Chapter), and the District Science Centre, Tirunelveli during 18-20 November 1987. A Souvenir also will be brought out and released during the Workshop. For further information about the advertisement and other matters, please contact Sri. V. Theetharappan, Hon. Secretary, AFST(I), Palayamkottai Chapter, A-115, N. G. O. Colony, Perumalpuram P. O., Tirunelveli-627 007, Tamilnadu.

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INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (in triplicate) should be typewritten in double space on one side of bond paper. They should be complete and in final form, since only minor corrections are allowed at the proof stage. The submitted paper should not have been published or data communicated for publication anywhere else. Only invited review papers will be published.

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. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Superscripts and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.

4. **Abstract:** The abstract should indicate the principal findings of the paper. It should be about 200 words and in such a form that abstracting periodicals can readily use it.

5. **Tables:** Tables as well as graphs, 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. They should be typed on separate sheets. Nil results should be indicated and distinguished clearly from absence of data, which is indicated by '—' sign. Tables should not have more than nine columns.

6. **Illustrations:** Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper not bigger than 20 cm (oy axis) $\times$ 16 cm (ox axis). The lettering should be such that they are legible after reduction to column width. Photographs must be on glossy paper and must have good contrast; *three 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 along with title of the paper should be cited completely in each reference. Abbreviations such as *et al.*, *ibid*, *idem* should be avoided.

   The list of references should be included at the end of the article in serial order and the respective serial number should be indicated in the text as superscript.

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

   (*a*) *Research Paper:* Jadhav, S. S. and Kulkarni, P. R., Presser amines in foods. *J. Fd Sci Technol.*, 1981, **18,** 156.

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

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

   (*d*) *Proceedings, Conferences and Symposia Papers:* Nambudiri, E. S. and Lewis, Y. S., Cocoa in confectionery, *Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India*, Mysore, May 1979, 27.

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

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

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

REG. NO. 24918/64

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Vol. 25. No. 1 **Contents of forthcoming issue** **Jan./Feb. 1988**

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Research Notes

Printed and Published by T. S. Satyanarayana Rao, Secretary, AFST(India), CFTRI, Mysore-570 013, at Sharada Press, Mangalore-575 001