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RESEARCH PAPERS

Pressure Extrusion of Indian Maize and Maize-Legume Composite Flours[a]

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Indian maize and maize-legume composite flours were pressure extruded in a Brabender Measuring Extruder Type 20 DN. The products were Organoleptically acceptable. Blending with legume flours decreased the expansion ratio, bulk volume, water absorption and water solubility, and increased the breaking strength of the product, and the torque requirement for extrusion as compared to maize semolina alone. This was attributable to increase in protein and slight reduction in starch in the composite flours.

Extrusion cooking process as applicable to cereal foods is gaining importance during the last two decades. A limited literature is available on extrusion cooking of cereals with high-protein flours of plant origin, such as, Instant Corn-Soy-Milk (CSM)[1], soybeans[2,3], cotton seed and peanut flours[4], as well as products like *Bal Ahar* and Indian Multipurpose Food[5]. However, extrusion processing of Indian maize and maize-legume composite flour blends does not seem to have been studied. Therefore, a study in this respect was initiated.

Materials and Methods

Four Indian maize varieties, 'Deccan 101', 'Ganga 5', 'Amber Composite' and 'Vijay', all grown at Hyderabad, Andhra Pradesh, India during *Rabi* 1977, and kindly supplied by the All India Coordinated Maize Improvement Project, New Delhi, were used. The dry-milling of whole maize was carried out using a standardized procedure to obtain semolina[6]. The particle size of semolina used in these extrusion studies was 200-500 μ.

Split pulses of redgram (*Cajanus cajan*), bengalgram (*Cicer arietinum*), greengram (*Phaseolus aureus*) and blackgram (*Phaseolus mungo*) were used. They were ground in a Kamas Laboratory Hammer Mill to pass through 500 μ sieve, and the 200-500 μ fraction of it was used for the study.

Based on preliminary experiments, it was concluded that the minimum moisture in sample required for extrusion was 12 per cent and the maximum legume-component in composite flours is 10 per cent at the above moisture level for a Brabender Measuring Extruder (Laboratory Model) Type 20 DN. Hence, maize semolina and legume flours were mixed in the ratio of 9:1, the moisture content was brought to 12 per cent, tempered overnight, and pressure extruded. Maize semolina was also individually pressure extruded. A Brabender Measuring Extruder, Type 20 DN, which was fitted on to a Brabender Do-Corder (a torque rheometer instrument) for measuring the torque during the extrusion processing, was used along with a 3 mm discharge die[7].

Chemical analysis for starch, protein and fat were done following standard procedures[8]. For estimating soluble nitrogen, a known amount of sample was mixed with a known amount of distilled water, agitated in a rotary shaker, filtered, and nitrogen was estimated in the filtrate by standard procedure[8].

The expansion index of the product was calculated from the diameter of the extrudate strand divided by the diameter of the standard die used in the extrusion (3 mm). Breaking strength of the extrudate strands was measured in a Zwick Zug Machine in 10 replicates for each sample. Bulk volume was obtained through the rape seed displacement method in a standard apparatus. Water Absorption Index (WAI) and Water Solubility Index (WSI) were also determined[9]. Water Absorption Index (WAI) is the weight (as per cent) of gel obtained by suspending a known amount of starch substance in water for a specific time and temperature and subsequent centrifugation. The supernatant water centrifuged out from the gel, when dried gives the Water Solubility Index (WSI as per cent). Water absorption of extrudate was also measured in a Brabender Farinogram using a 30 g extrudate in a 50 ml Farinogram bowl, and

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TABLE 1. CHEMICAL COMPOSITION AND PHYSICAL PROPERTIES OF RAW MATERIALS (DRY BASIS)

Material	Fat (%)	Starch (%)	Protein (%)	Soluble protein (%)	WAI (%)	WSI (%)
Deccan 101	0.77	80.1	12.1	0.25	58.3	3.6
Deccan 101 + Redgram	0.75	76.5	13.7	0.91	59.0	5.5
Ganga 5	0.83	81.2	11.7	0.22	75.4	2.9
Ganga 5 + Bengalgram	1.27	77.1	13.2	0.93	58.1	4.1
Amber Composite	0.47	80.1	11.9	0.19	74.8	2.7
" + Greengram	0.63	79.3	13.9	0.83	50.3	4.6
Vijay	0.86	81.0	11.1	0.54	74.2	2.6
Vijay + Blackgram	0.93	78.2	13.1	0.31	81.9	5.1

WAI: Water absorption index; WSI: Water solubility index.

reported as water absorbed per 100 g dry substance for achieving a value of 500 FU.

Sensory evaluation of the extrudate was done by a panel of four experienced judges as per hedonic scale ratings and terminology from literature[10].

Results and Discussion

Data on chemical analysis and physical properties of raw materials are shown in Table 1, and those of the extrudates in Table 2 and 3.

Characterization of raw materials: Fat content of semolina in the maize varieties was below 1.0 per cent; mixing with pulses however, increased the fat content. But the values were still below 5 per cent, which is said to yield a chewy product[11]. Starch was constant while the protein varied slightly among the varieties. The starch content decreased and the total and soluble protein increased upon blending with pulse flour.

Water absorption and water solubility were different in 'Deccan 101' semolina compared to others. But all composite flours behaved differently from each other in this respect.

Characterization of extrudates: Extrusion cooking resulted in an appreciable reduction in fat, a very slight reduction in starch, and one to two fold increase in soluble-protein contents, while the protein content remained practically unchanged (Table 2). The reduction in fat needs further study, because of the estimation of fat by the standard petroleum ether extraction, an estimation of bound fat, hitherto not carried out, should be explored. There were small differences in the starch content before and after extrusion.

The physical data revealed some interesting aspects with respect to torque requirements in the extrusion process (Table 3). The minimum torque required to start the extrusion process was generally higher in case of maize than their composite flours, indicating the relative ease of extrusion of composite flours to start with. However, the difference between the minimum and maximum torques required in the process (amplitude) was generally higher for composite flours compared to maize extrusions. This indicates the higher amount of energy needed for extrusion processing of composite flours.

The expansion indices of extrudates from composite flours were about 2/3rd of those from maize semolina, evidently due to their higher protein contents. This can also be judged from Fig. 1. There was a progressive reduction in expansion index with increasing legume-content of composite flours (Fig. 2). The composite flour extrudates also showed considerably greater breaking strengths and slightly lower bulk volumes than the pure maize extrudes. Protein was negatively corre-

TABLE 2. CHEMICAL ANALYSIS OF EXTRUDATE (DRY BASIS)

Material	Fat (%)	Starch (%)	Protein (%)	Soluble protein (%)
Deccan 101	0.25	78.3	12.2	1.39
Deccan 101 + Redgram	0.32	74.9	13.6	2.26
Ganga 5	0.18	77.9	11.7	1.19
Ganga 5 + Bengalgram	0.24	76.1	13.3	1.35
Amber Composite	0.14	77.6	11.9	1.11
" + Greengram	0.15	75.9	13.9	1.35
Vijay	0.36	79.2	11.2	1.05
Vijay + Blackgram	0.29	76.0	13.0	—

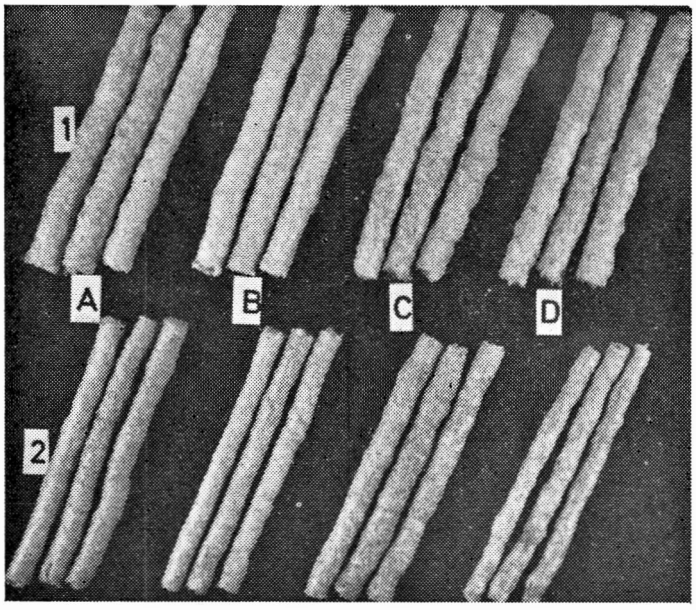

Fig. 1. General view of extrudates from maize semolina from different varieties (1) and maize-legume composite flours (2)

A: Deccan 101; B: Ganga 5; C: Amber Composite; and D: Vijay

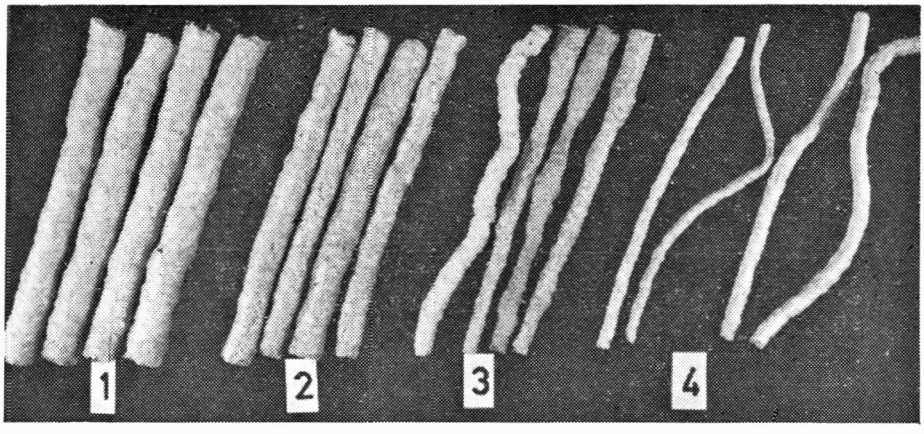

Fig. 2. Progressive reduction in expansion index due to increased legume component in composite flours.

1: Maize semolina + No legume;
2: Maize semolina + 10% legume;
3: Maize semolina + 15% legume; and
4: Maize semolina + 20% legume

Maize variety: Amber Composite, Legume: Greengram

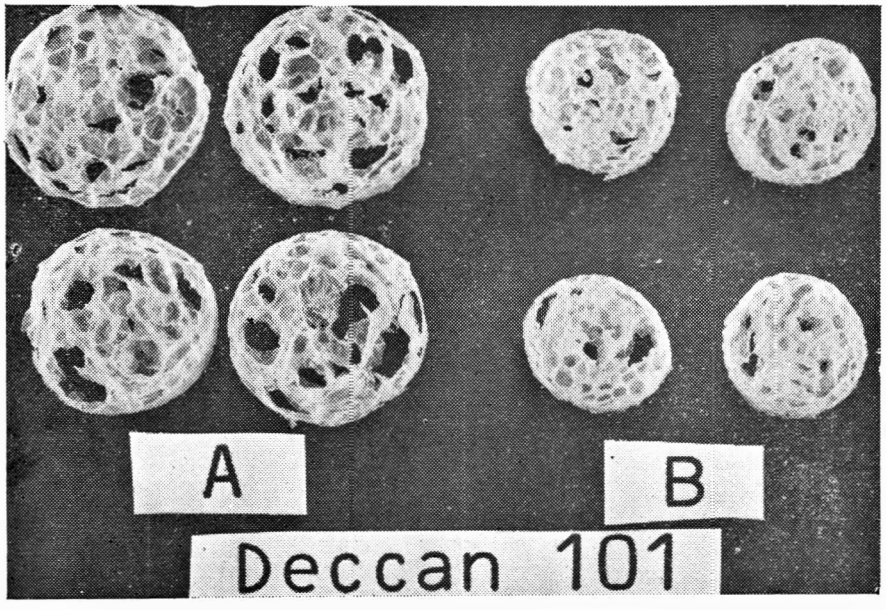

Fig. 3. Appearance of Transverse Sections of Extrudates.

A: Maize; and B: Maize + 10% legume. Maize variety: Deccan 101.

TABLE 3. PHYSICAL PROPERTIES OF EXTRUDATES

Material	Torque required in extrusion (mp)		Expansion index	Breaking strength	Bulk vol.	WAI	WSI	Farinogram water absorption
	Min.	Max.		(Kp/cm)	(ml/g)	(%)	(%)	(ml/100 g)
Deccan 101	1260	3000	3.3	1.64	8.1	526.8	28.4	131.6
Deccan 101 + Redgram	990	1980	2.6	2.01	7.2	464.6	26.0	124.7
Ganga 5	3380	5010	3.2	1.69	9.3	490.0	31.0	139.0
Ganga 5 + Bengalgram	300	2820	2.3	2.25	7.6	460.9	28.5	138.3
Amber Composite	969	1450	3.0	1.78	7.5	508.9	25.3	135.5
,, + Greengram	580	3000	2.6	2.07	6.6	526.5	24.5	130.9
Vijay	750	1740	3.1	1.74	7.6	560.5	24.1	144.8
Vijay + Blackgram	690	2640	2.1	2.49	5.6	486.3	26.7	135.7

WAI: Water absorption index; WSI: Water solbility index.

lated to expansion index ($r = -0.73$) and bulk volume ($r = -0.56$) and positively to breaking strength ($r = 0.68$), while starch was negatively correlated to the latter ($r = -0.73$). Previous observers have noted that addition of soy products to yellow snack meal in extrusion tended to lower the expansion index[2].

The extrudates from maize semolina in general absorbed more water as well as gave more solubles than those from composite flours, but the differences were small. A similar trend was observed in Farinogram water absorption values. The above properties are important in reconstitution and use of extruded products.

Sensory evaluation: The internal structure of a typical extrudate for maize semolina and composite flour are shown in Fig. 3. The results of sensory evaluation can be summarized as follows:

Extrudates from maize had attractive colour and uneven surface and, internally, a uniform distribution (with respect to size of pores) with moderate porosity (number). They were brittle and easily breakable by thumb, acceptable in taste with a good mouth feel.

Though composite flour extrudates revealed a bland taste with brittleness in mouth-feel, they were also easily breakable by thumb with the characteristic sound comparable to extrudates from maize semolina. These were densly pored and pore distribution was from uniform to moderately uneven. Visually they were uneven in surface and recorded dull to appealing in appearance. All the extrudates were organoleptically acceptable.

Acknowledgement

The authors express gratitude to Prof. Dr. H. Bolling, Director, Bundesforschungsanstalt für Getreide und Kartoffelverarbeitung, and Prof. Dr. W. Seibel, Director, Institut für Bäkereitechnologie, Detmold, for their constant encouragement and interest in this project. Thanks are also due to CFTRI, Mysore, and CSIR, New Delhi for the deputation and to German Academic Exchange Service, Bonn Bad-Godesberg, for the award of a Fellowship. Useful discussions with Dr. K. R. Bhattacharya of CFTRI, Mysore, is gratefully acknowledged.

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Process Feasibility of Ghee Making by Direct Contact Heat Exchange

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The process feasibility of ghee making by direct contact heat exchange was explored. The equipment was designed in such a way that it could be operated in batch as well as in continuous operation. Six trials were conducted in batch operation and one in continuous operation. The moisture content, free fatty acids and peroxide value of ghee prepared were within the specified limits. It had acceptable texture, colour and flavour. There was neither foaming nor scale formation on the surface of heat exchanger. The energy requirement was 133 Kcal/kg of butter as compared to 204 Kcal/kg of butter in conventional batch type process using steam in jacketed space. The fat recovery in batch operation trials ranged from 91.7 to 95.2% but it was only 81.6% in continuous operation. This was due to spillage losses caused by splashing of the product.

Ghee is obtained by clarification of milk fat at high temperature. About 43 per cent of the total milk produced in India is utilized for making ghee. The total annual production of ghee in India is estimated to be 280 million kg[1]. Ghee making in India is mostly a home industry. The ghee manufactured in the organised industry has more uniform quality, but this constitutes only a small fraction[2].

Ghee is prepared by different methods, like *desi* (country), creamery butter, direct cream and prestratification methods. In all these methods the product is heated using steam as a heating medium. The raw material, butter or cream is pool boiled in the stainless steel jacketed pan. During the process, proteins caramalise and get deposited on the surface of pan. The temperature of surface is quite high as compared to that of the bulk. Even constant stirring does not prevent burning on the surface. Moreover, due to pool boiling there is lot of foam formation and hence the supernatant liquid has to be drained continuously through a central downcomer. The constant descaling and draining require more labour and thus this operation is tiresome. The present study is aimed at overcoming these difficulties. The nitrogen gas is heated to high temperature (150°–240°C) and then bubbled in to a column containing molten butter. In this case the surface temperature will be lower than that of bulk temperature. Thus local burning of the product is eliminated. Due to direct heat exchange, the requirement of heat energy is minimized. Since the operation will be carried out in an inert atmosphere, the chances of fat oxidation are minimized.

Materials and Methods

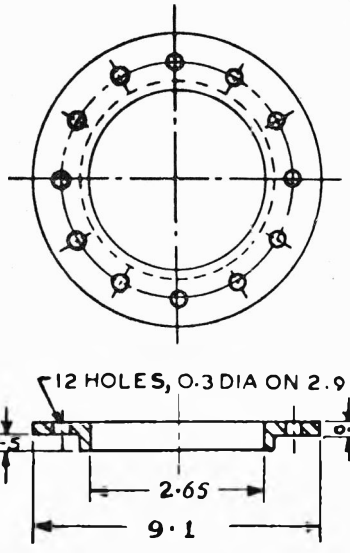
Description of the experimental set up: The equipment was designed and fabricated in the Engineering Workshop of National Dairy Research Institute, Karnal. It consists of the following components:

(*i*) Electric heater. (*ii*) Direct contact heat exchangers. (*iii*) Over head supply tank. (*iv*) Inter-connecting piping and valves.

(*i*) *Electric heater:* A 5 kw, single phase 220 V, immersion type heater was modified to suit the requirement of heating nitrogen gas. The details are shown in Fig. 1. It consists of nichrome wire heating element enclosed in a M.S. pipe of 5.3 cm O.D. and 156.5 cm length. One end of this pipe is closed while from the other end, wire leads are taken out through porcelain connectors. Two hundred fifty aluminium fins were slipped over this pipe. This assembly of heater and fins was placed in 8.6 cm O.D. and 157 cm length M.S. outer pipe and then it is lagged with 2.8 cm thick glass wool insulation to reduce heat losses. Lower end of the outer pipe is provided with a socket in which 0.65 cm nipple is welded for supply of nitrogen gas to the heater. The purpose of providing the aluminimum fins is two fold. Firstly, aluminium has high thermal conductivity and hence has efficient heat transfer and secondly aluminium has got greater affinity for oxygen which is even more pronounced at higher temperatures. Since commercial nitrogen invariably contains some traces of oxygen, this is purged off by the formation of aluminium oxide. Thus pure nitrogen is expected to be bubbled through the product. These fins are perforated and placed in such a way that the path of nitrogen is staggered and hence longer residence time is attained. However, this results in higher pressure drop in the heater.

A 2.5 cm diameter hole is drilled just near the outlet and a thermowell is welded for housing a thermocouple. A 0.65 cm nipple is also welded for the supply of heated nitrogen to the heat exchangers.

ALL DIMENSIONS IN cms.



DETAIL OF FIN

LEGEND:–

1. HEATING ELEMENT (5 KW).
2. COVER PIPE FOR ABOVE.
3. ALUMINIUM FINS (250 NOS.).
4. OUTER PIPE.
5. THREADED COLLER.
6. SOCKET.
7. NIPPLE (0.65). (INLET).
8. GLASS WOOL INSULATION.
9. SOCKET FOR THERMOWELL.
10. NIPPLE (0.65). (OUT LET).
11. COVER.

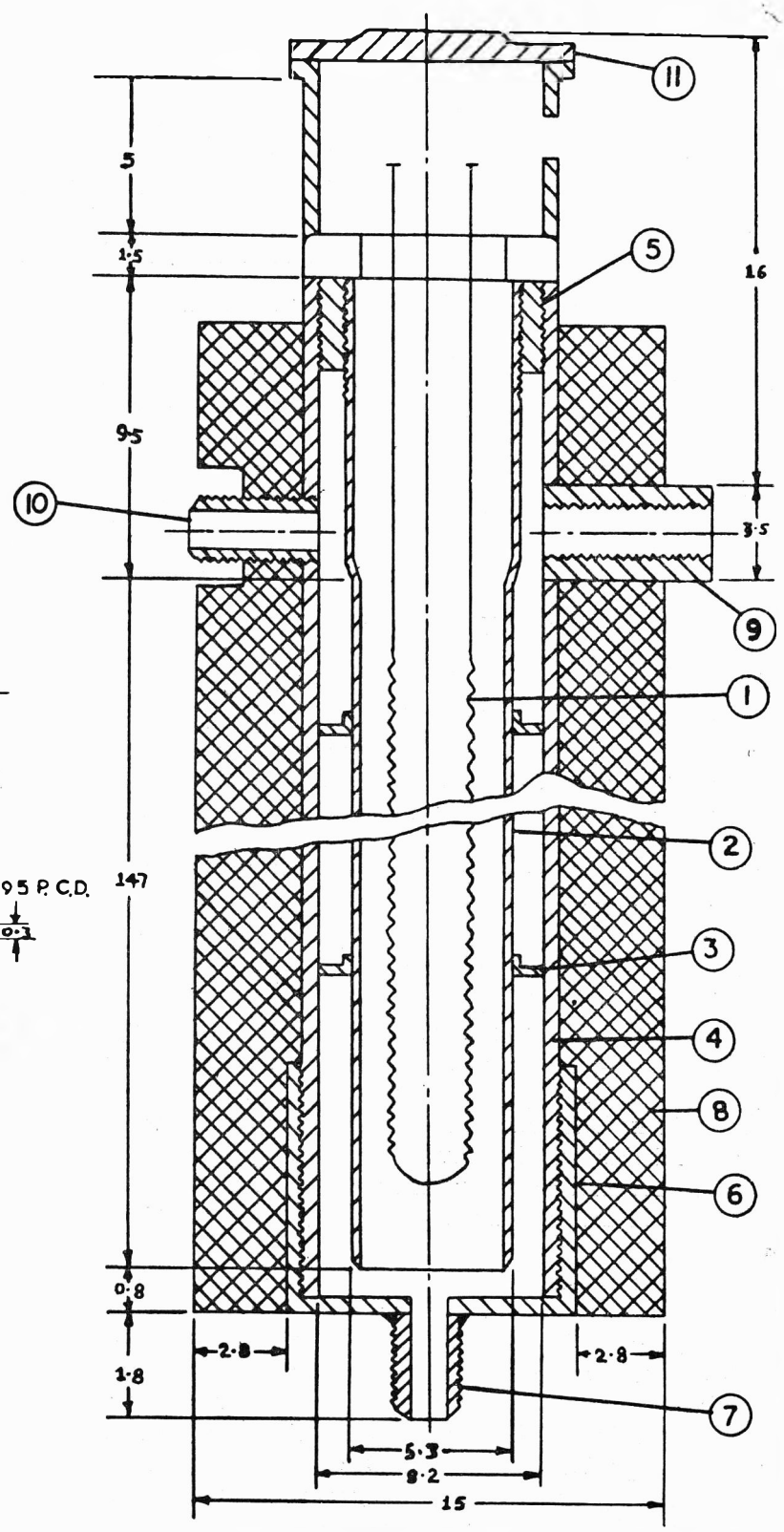


FIG. 1. ELECTRIC HEATER FOR NITROGEN GAS

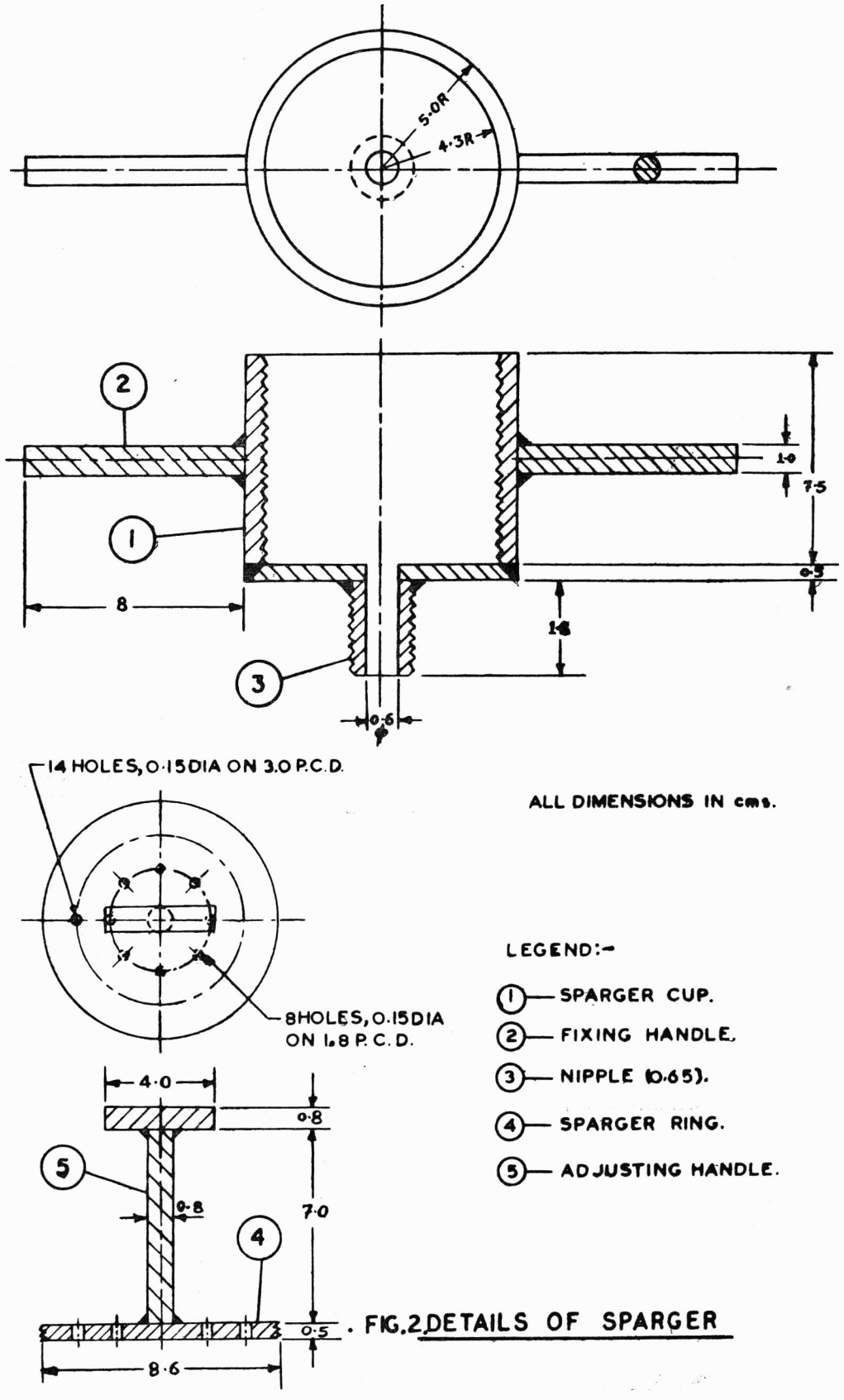
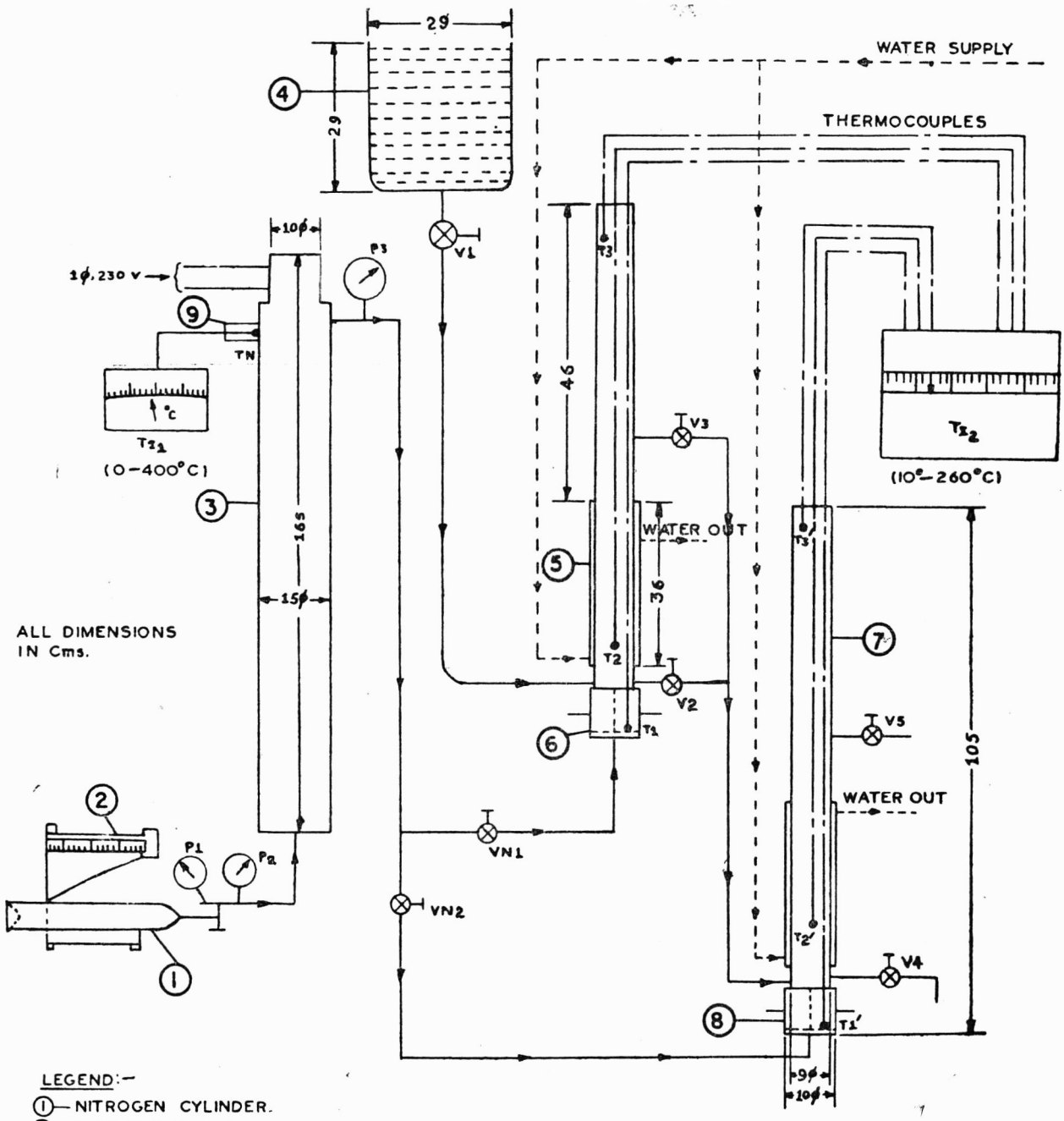


FIG. 2 DETAILS OF SPARGER



LEGEND:–

① — NITROGEN CYLINDER.
② — WEIGHING SCALE (AVERY-500 kg)
③ — ELECTRIC HEATER
④ — OVER HEAD BUTTER TANK (S.S).
⑤ — JACKETED M.S PIPE COLOUMN(I) (9 X 100 cm).
⑥ — SPARGER FOR ABOVE
⑦ — JACKETED M.S PIPE COLOUMN (II)(9x 100 cm).
⑧ — SPARGER FOR ABOVE.
⑨ — THERMO WELL.

P_1, P_2, P_3, – PRESSURE GAUGES.

$T_1, T_2, T_3, T_1', T_2', T_3'$, – THERMOCOUPLES. (COPPER–CONSTANTAN)

TN – THERMOCOUPLE (CROMEL–COPEL) FOR NITROGEN.

TI_1, TI_2, – TEMPERATURE INDICATORS.

VN_1, VN_2 – NITROGEN SUPPLY VALVES.

V_1, V_2, V_3, V_4, V_5 – VALVES.

FIG.3. SCHEMATIC HOOK–UP OF EXPERMIENTAL SET–UP.

(DIRECT CONTACT HEAT EXCHANGER)

(*ii*) *Direct contact heat exchangers:* These comprise of two jacketed M.S. pipe columns (9×100 cm) each provided with a sparger. The columns are arranged in such a way that gravity flow of product is maintained. The water supply connections are provided to the jacket by 1.0 cm plastic pipe. The water circulation is contemplated to keep the vertical contact surface at lower temperature than that of the bulk, if needed.

The details of sparger are shown in Fig. 2. It consists of a M.S. socket, whose one end is closed by welding a M.S. plate, and a movable perforated M.S. disc. This disc has 22 holes of 0.15 cm diameter for uniform distribution of nitrogen gas in the column. A 0.65 cm nipple is weided for supply of nitrogen gas.

Instead of one single column, two columns are provided to have better flexibility in operation and ease in cleaning.

(*iii*) *Over head supply tank:* It is a 29×29 cm stainless steel tank mounted just above the outlet of the first column of direct contact heat exchanger. It was provided with a 1.25 cm stainless steel needle valve for regulating the flow of molten butter.

(*iv*) *Inter-connecting Piping and valves:* Two heat exchangers are interconnected by means of 1.25 cm. G.I. pipe for product flow while nitrogen gas is transported through 0.65 cm copper tubing. V_2, V_3, V_4, and V_5 are 1.25 cm. globe valves provided in product line and VN_1 and VN_2 are 0.65 cm needle valves connected in hot nitrogen gas line.

Instrumentation: The Bourdon type pressure gauges are provided to note the pressure of nitrogen gas in cylinder and at the inlet and outlet of the heater.

The temperature of hot nitrogen gas is measured by a chromel-copel thermocouple connected to a millivoltmeter. The scale of millivoltmeter is directly calibrated in terms of temperature. The specifications are as follows:

Make	:	Instrumentation Ltd.
Range	:	0° – 400°C.
Rint	:	201 Ω

Chromel-copel thermocouple type TCC 03, 320 mm sensing length. Three copper-constantan thermocouples are provided in each column of the heat exchanger to measure the temperature at a point on the sparger surface, bulk temperature and exhaust vapour-gas mixture temperature. All the six thermocouples are connected to a millivoltmeter whose scale is directly calibrated in terms of temperature. The other specifications are as follows:

Make	:	Belliss & Morccom (India)
Range	:	10° – 250°C
Rext	:	88.2 Ω
No of tappings		— Six.

The quantity of nitrogen gas circulated into the system is measured by differential weight principle. The gas cylinder is placed on weighing scale (500 kg). The mass flow rate is computed by dividing difference in weight with length of run i.e. time for which the nitrogen gas was passed through the heat exchanger.

Electrical energy input to the heater is measured by means of energy meter.

Operation of the equipment: The schematic hook-up of the experimental set up is shown in Fig. 3. The equipment can work in batch or in continuous operation.

Six trials were conducted in batch process and one trial in continuous process. The sequence of operation was as follows:

(*i*) *Batch Operation:*

(*a*) Power supply to the heater (3) was switched on and initial reading of energy meter was noted.

(*b*) The heat exchanger column (7), sparger (8) were cleaned with hot water and then by detergent (Teepole) followed by rinsing with hot water.

(*c*) When the temperature indicator TI indicated the desired temperature, the power supply to heater was switched off. The final reading of the energy meter was noted.

(*d*) The nitrogen gas cylinder (1) was weighed on weighing scale (2).

(*e*) A known quantity of butter, prepared in the experimental dairy of National Dairy Research Institute, Karnal, was melted and poured in to the heat exchanger and the valve VN_2 was opened. All other valves were kept closed. The flow rate of nitrogen was adjusted by means of nitrogen gas pressure regulator.

(*f*) The hot nitrogen gas was bubbled for the desired length of run and then valve VN_2 and pressure regulating valve were closed. The temperature at points 1', 2' and 3' were measured by means of copper-constantan thermocouples T'_1, T'_2, and T'_3 respectively, at the interval of 5 min.

(*g*) Valve V_4 was opened and ghee was taken out. The remaining product was drained by means of removing the flare nut at the bottom of sparger (8).

(*h*) After draining, the sparger was unscrewed and the ghee residue was removed. The heat exchanger surface was examined for any scale deposition.

(*i*) The period, during which the nitrogen gas was bubbled, was noted by means of stop watch. The cylinder was weighed again and the difference in weight was recorded.

(*j*) The ghee was filtered through two layer muslin cloth and was filled in sampling bottles.

k) The equipment was thoroughly washed with hot water and detergent.

(*ii*) *Continuous operation.*

(*a*) Same as that in batch operation.

(*b*) Both the heat exchanger columns (5) and (7) and spargers (6) and (8) were cleaned with hot water and then by detergent (Teepole) followed by rinsing with hot water.

(*c*) & (*d*) Same as that in batch operation.

(*e*) A known quantity of molten butter prepared in the experimental dairy of National Dairy Research Instt., Karnal, was put into the over head tank (4).

(*f*) Valves V_1, VN_1, V_3 and nitrogen gas pressure regulating valve were opened and thus molten butter and hot nitrogen were introduced into to the heat exchanger column (5). The needle valve V_1 was adjusted to a flow rate of 0.15 kg/min. The valves V_2 and V_4 were kept closed. The level of butter kept on rising till it reached the outlet valve V_3 and then it flowed to heat exchanger column (7). Valve VN_2 was opened. The final product was taken out from the valve V_5. The samples were drawn at regular intervals for moisture analysis. The product was kept under recirculation until it was converted into ghee. The temperatures at points 1, 2, 3, 1', 2', and 3', were measured by thermocouples T_1, T_2, T_3, T'_1, T'_2, and T'_3 respectively and were recorded at 10 min interval.

(*g*) In the final pass, when no butter was left in the overhead tank, valve V_2 was opened and the product was transferred to the heat exchanger column (7). The valve VN_1 was closed. In the end for complete removal of product, the valve V_4 was opened and flare nuts of both the spargers were removed. This was done after closing the valve VN_2.

(*h*) After draining, the spargers were unscrewed and ghee residue was removed. The heat exchanger surfaces were examined for any scale deposition.

(*i*), (*j*) and (*k*) same as that in batch operation.

Chemical analysis: All the samples drawn were chemically analysed for moisture content, free fatty acids and peroxide value as per the ISI procedure[3].

Results and Discussion

Acceptability of Ghee: Seven trials were conducted, six as batch operation and one as continuous operation. It was observed that the moisture content, free fatty acids and peroxide value of ghee were well within the specified limits prescribed in IS 3508. The colour ranged from pale yellow to bright yellow. The ghee was free from sedimentation though it was filtered only through two layer muslin cloth. Few samples were subjected to sensory evaluation and the average score was 95. As per IS 3508, ghee scoring 90 and above is graded as excellent.

(2) *Foaming and Scale Formation:* In direct contact heat exchange neither foaming nor scale formation on the surface of heat exchanger were observed. This appears to be an advantage over conventional system of ghee making in which during pool boiling there is lot of foam formation and also the surface of ghee pan gets scaled with burnt proteins. The possible reason of non-scale formation was due to the proteins even after caramalisation, kept fluidized with the butter from turbulance created by the nitrogen gas. The ghee residue obtained was in small clumps.

(3) *Comparision of Energy Requirement with Conventional System:* It has been reported that in conventional batch type process, the steam consumption is 0.47 kg/kg of butter with average steam pressure as 2.0 kg/cm^2 and initial temperature of butter 5.5°C. The final temperature of ghee was 115°C[4]. (Shamdas, Private communication) Therefore, heat required per kilogram of butter is 243 Kcal assuming only latent heat transfer and the steam being dry and saturated. However, if the initial temperature of butter is assumed to be 45°C then the heat requirement works out to be 207 Kcal/kg. In the case of direct heat exchange, the heat energy transferred by nitrogen gas to butter in the fifth trial, where similar initial and final conditions of the product were maintained, was only 133 Kcal/kg of butter as shown in Table 1. Thus from energy

TABLE 1. HEAT ENERGY TRANSFERRED BY HOT NITROGEN GAS

Trial No.	Trial period (min)	Mass flow rate of N (kg/m.)	Temp. of hot N gas gas (°C)	Butter taken (kg.)	Moisture. (%)	Temp. of butter (°C)	Temp. of final product (°C)	Av. temp. of exhaust Vap +N gas	Heat transferred by N gas/kg of butter (Kcal)	Qty. of ghee (kg.)
I	15	0.120	155	0.5	15	42	80.8	51.86	89.0	0.392
II	15	0.126	155	0.5	15	40	81.5	50.66	95.0	0.390
III	10	0.130	165	0.5	15	40	65.0	46.10	74.0	0.398
IV	20	0.115	165	0.5	15	65	100.2	54.20	117.3	0.392
V	40	0.100	190	1.0	16	45	115.0	48.00	133.0	0.800
VI	60	0.091	180	1.0	16	40	95.0	40.00	185.0	0.800
VII	180	0.085	235	7.0	16	60	80.0	38.00	105.0	4.800

TABLE 2. PERCENTAGE FAT RECOVERY

Sample No.	Butter used (kg.)	Fat in butter (%)	Ghee obtained (kg.)	Moisture in ghee (%)	Fat recovery (%)
I	0.5	85	0.390	0.13	91.7
II	0.5	85	0.398	0.34	93.6
III	1.0	84	0.800	0.08	95.2
IV	7.0	84	4.800	0.24	81.6

consumption, the direct heat exchange process appears to be promising. But since the steam energy is cheaper than electrical energy, final conclusion regarding economics of the operation can only be drawn after conducting detailed study.

The nitrogen gas can be recycled by providing a water cooled condenser to condense water vapours and then compressing the gas back into the system through heater.

Fat recovery: Table 2 shows the fat recovered in various trials. It varied from 91.7 to 95.2 per cent (on the basis of fat in butter) in batch operation. In continuous operation, the fat recovery was only 81.6 per cent. This was due to spillage losses caused by splashing of the product. However, this can be overcome by providing more free height to the column.

Thus, from the observations made in the present study, it can be concluded that the process of ghee making by direct contact heat exchange is feasible. If explored fully, then this process may take a lead over the conventional methods of ghee making.

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Leg-Twisting: A Method for Improving Beef Muscle Tenderness

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The efficacy of leg-twisting as a method for improving beef muscle tenderness was determined. Five choice grade White Fulani steers were conventionally dressed and split except that the hind leg was retained intact on a randomly selected carcass side. For the leg-twisting treatment, a slit was made at the cranio-ventral tip of the *Rectus abdominis* into which one of the third phalanxes of the retained leg was inserted. The two carcass sides were then suspended conventionally by the achilles tendon and chilled for 48 hours. Of the nine muscles evaluated, seven muscles from the treated carcass sides had lower shear force calues, longer sarcomeres, thicker fibres and smaller number of sarcomers per myofibrillar fragment than the control side The two exceptions were the psoas major and the semitendinosus. The number of muscles showing significant differences as a result of the leg-twisting treatment varied depending on the individual parameters. The beneficial effect of leg-twisting appears to be due to the strain imposed on most of these muscles by a reduction of the orientation angle of the femur on the pelvic axis from 126.7 degrees to 71.2 degrees during rigor development.

Locker[1] first postulated the association between the prevention of muscle shortening during rigor and tenderness improvement. He went further to state that since the final state of contraction appeared to depend on the strain imposed on the muscle in the suspended carcass during rigor onset, it might be possible to improve tenderness of the longissimus dorsi muscle by suspending the carcass in such a manner that the muscles remained stretched through this period. Herring *et al*[1]. demonstrated that the rearrangement in carcass position during rigor onset produced significant changes in sarcomere length, muscle fibre diameter and tenderness. Hostetler *et al*[3]. and Smith *et al*[4]. on the other hand concluded that the extent of shortening in a carcass depended on the physical restrictions imposed by attachment of the muscle to the skeleton of the animal.

Since these observations were made, several methods of improving meat tenderness on the intact carcass have been suggested among which are suspension of the carcass by the aitch bone, the so called tenderstretch method[3,5] suspension of the cacrass in the foetal position[6] the neck-tied, hip-free and hip-tied methods of

carcass suspension[5] the pelvic hanging method[7] and the application of some mechanical tensioning device on the intact carcass[8,9].

Leg-twisting, a traditional Nigerian method of carcass positioning was recently evaluated for the first time using mutton carcass[10]. Muscles from leg-twisted carcass sides were found to be more tender, but with longer sarcomeres, thicker fibres and shorter myofibrillar fragments than the corresponding muscles from the control sides. In the present report, this study has been extended to beef carcasses.

Materials and Methods

Five choice grade White Fulani steers were randomly slaughtered at the Meat Works, University of Ibadan over a 5-week period. Bleeding, legging, skinning, evisceration and splitting were completed with the carcass recumbent on the slaughter floor except that one hind leg was randomly left intact during the legging process for leg twisting purposes. The hoofs on this leg were subsequently removed by peeling after immersion in boiling water for 5 min. After washing, the half carcass with the intact leg was leg-twisted by inserting one of its third phalanxes into a slit made in the flank at the cranio-ventral tip of the Rectus abdominis such that the thin part of the flank formed a sling tucked between the two phalanxes (Fig. 1).

The treatment of the carcasses was accomplished within 45 min after slaughter. The carcasses were moved into a chill room maintained at 1°C. On-rail suspension was by the conventional hanging by the achilles tendon. After chilling for 48 hr, the angle between the femur and the pelvic bone was determined for the five pairs of conventional and leg-twisted carcass halves following the procedure of Abban *et al*[9]. The lateral ischiatic tuber and the tuber coxa served as the reference points on the pelvic bone.

The corresponding points on the femur were the stiffle joint distal to the patella and the point of the greater trochanter.

Steaks of 3.75 cm in thickness were obtained from nine selected muscles as follows: the *longissimus* (LDL) and *psoas major* (PM) were removed together from the shortloin intact with and spanning equal lengths of the 4th and 5th lumbar vertebrae; the *gluteus medius* (GM) was obtained as the roundbone sirloin steak the semimembranosus—adductor (SM-ADD) muscle mass, the *biceps femoris* (BF), the *semitendinosus* (ST) and the sirloin tip containing the *vastus lateralis* (VL) and *rectus femoris* (RF) were in that order dissected off the femur bone and the respective steaks sliced off the thickest portions of these muscles. The steaks were weighed and cooked in pairs (one from each treatment) to medium doneness by broiling on each side for 20 min. The steaks were subsequently cooled to room temperature and reweighed to determine cooking loss.

Three 1.30 cm diameter cores were taken from each broiled muscle in the fibre direction as much as practicable. Three shear values were obtained from each core using a Warner-Bratzler shear press, first across the centre of each core and again across the centres of each of the two half cores making a total of nine shear values per muscle.

Sarcomere length, fibre diameter and fragmentation values as number of sarcomeres per myofibrillar fragment were determined for each muscle using 10 per cent formal infixed samples as described earlier (J. Fd Sci. 1978 (In press).

Results and Discussion

The data presented in Table 1 show significant differences ($P<0.05$) due to the treatments in the shear values of all the muscles except the *psoas major*. The method of leg-twisting significantly reduced the shear force of the *longissimus*, *gluteus medius*, *biceps femoris*, *adductor*, *vastus lateralis and rectus femoris* ($P<0.05$) while that of the *semitendinosus* was significantly increased ($P<0.05$). The results confirm the previous Study (J. Fd Sci. 1978 (In press) from mutton carcasses that the *gluteus medius* and *adductor* were highly tenderized by leg-twisting, improvements of the order of these two muscles. In 22.44 and 21.97 per cent being observed respectively in the mutton carcasses however, the *semimembranosus* and the *rectus femoris* had improvements above 20 per cent, whereas they had lower improvements of the order of 10.10 and 13.3 per cent respectively in the beef carcasses.

TABLE 1. THE EFFECT OF LEG TWISTING ON SHEAR VALUES (KG) OF SELECTED BEEF MUSCLES

Muscle	Conventional Mean±SD	Leg-twisted Mean±SD	% Improvement*
LDL	$3.36^{a}\pm0.87$	$2.85^{b}\pm0.73$	15.17
GM	$4.99^{a}\pm0.28$	$.387^{b}\pm0.52$	22.44
PM	$3.09^{a}\pm0.62$	$3.32^{b}\pm0.64$	-6.92
SM	$4.65^{a}\pm0.64$	$4.18^{b}\pm0.55$	10.10
BF	$6.09^{a}\pm0.85$	$5.23^{b}\pm1.08$	14.12
ST	$6.05^{a}\pm0.95$	$6.62^{b}\pm0.97$	-8.74
ADD	$4.55^{a}\pm1.71$	$3.55^{b}\pm1.16$	21.97
VL	$4.87^{a}\pm0.74$	$4.41^{b}\pm0.77$	9.44
RF	$4.33^{a}\pm0.74$	$3.75^{b}\pm0.44$	13.39

Means of the same row with different superscripts differ significantly ($P<0.05$)

*%Improvement = 100 × (Reduction in shear value due to leg-twisting / shear value due to conventional treatment)

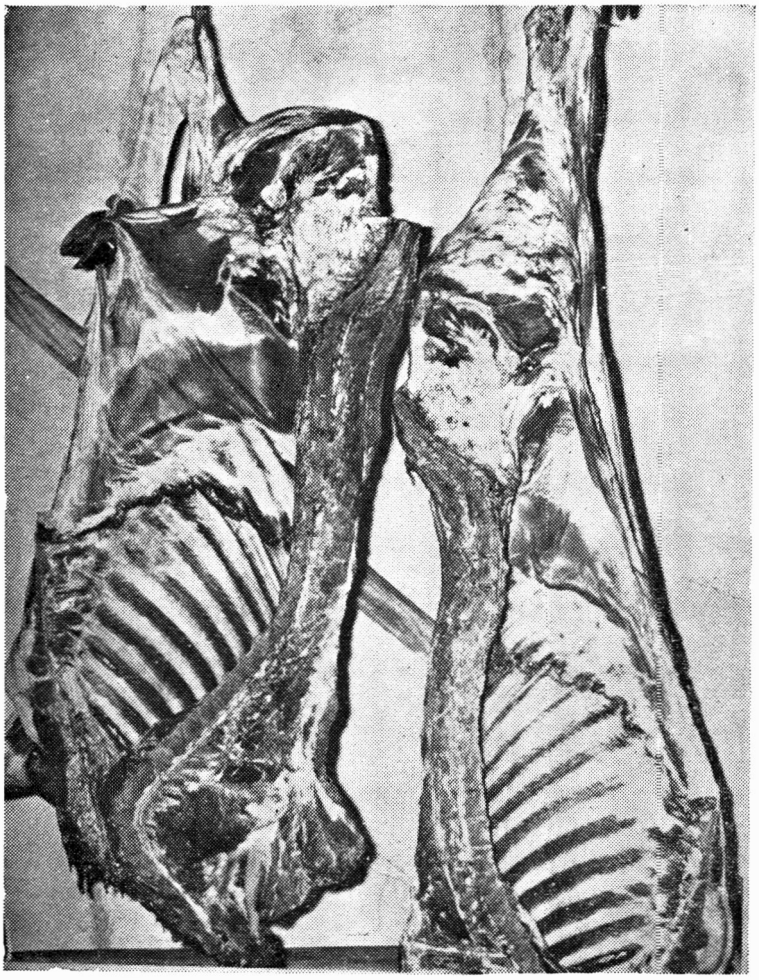

Fig. 1. Leg-twisted and conventional sides of beef carcass.

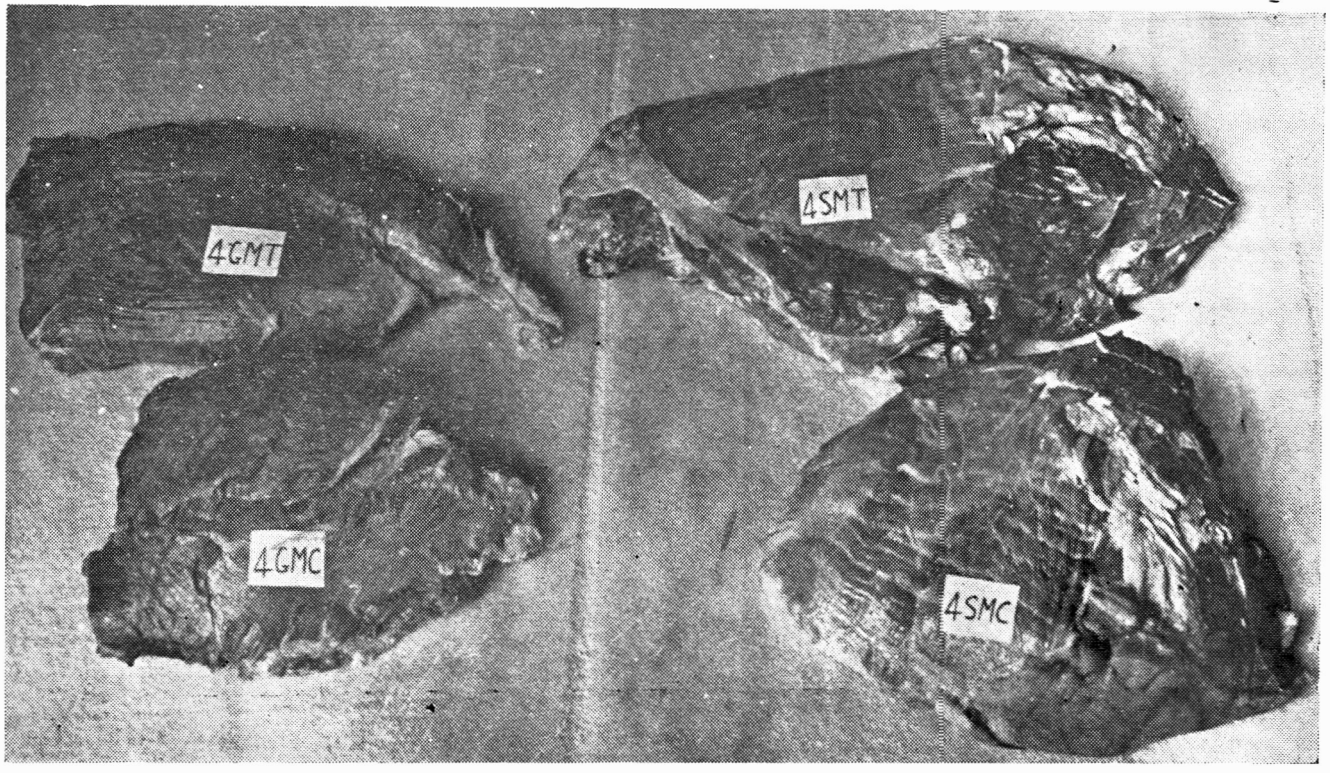

Fig. 2. Effect of leg-twisting (T) and conventional method (C) of carcass suspension on the shape of SM and GM from the same animal.

TABLE 2. VALUES FOR PERCENTAGE COOKING LOSSES OF SELECTED MUSCLES FROM CONVENTIONAL AND LEG TWISTED CARCASSES

Muscles	Conventional Mean±S.D.	Leg twisted Mean±S.D.
LDL+PM	21.92±3.43	22.78±4.15
BF	25.08±2.03	27.04±3.86
GM	28.57±5.44	28.34±6.65
RF+VL	30.30±4.34	33.23±4.84
SM+ADD	27.97±4.52	29.84±6.53
ST	32.95±4.46	31.15±3.54

The *semitendinosus* in the present study recorded a negative improvement of 8.74 per cent as against 24.69 per cent observed in the previous study. The toughening of the *semitendinosus* as a result of leg-twisting was also observed in a previous study using mechanical tensioning device[9] but not in other methods previously designed to improve carcass tenderness[10,5].

No significant difference in cooking loss was observed as a result of leg-twisting (Table 2). However, muscles which exhibited reduction in shear values as a result of the treatment had slight increases in their percentage cooking losses. The above trend would be expected in the light of previous observations. Bouton *et al*[12]. demonstrated with ovine muscle that cooking loss increases as the ultimate pH of muscle decreases. McLoughlin[12] and Abban[13] also reported lower ultimate pH in muscle maintained under tension than in muscle free to contract during rigor development. The latter worker using mechanical tensioning devices on the leg and back regions of the lamb carcass reported an ultimate pH of 5.75 in the semimembranosus of the tensioned carcass side as against 5.95 for the control side.

These results and the present one suggest that leg-twisting and such other methods which put tension on some selected muscles of the intact carcass may cause slight decreases in the ultimate pH and thereby increase the cooking loss but that such losses are only marginal and insignificant.

The effects of leg-twisting on the angle of orientation of the femur bone relative to the pelvic axis and the resulting shear values of the *gluteus medius*, *adductor* and *semitendinosus* are shown in Table 3. The significant reduction in the angle or orientation appears to result from the forward and sideways movement of the pelvic limb. This movement also straightened out the lumbar vertebrae and thereby brought them in straight alignment with the direction of gravity pull on the carcass. The strain thus imposed on the *longissimus* muscle was reflected in a reduction of its shear value from 3.36 to 2.85 kg. Similar strain induced by the skeletal rearrangement on most of the leg muscles and the slack introduced in the *semitendinosus* was reflected in the changes observed in the shear values of these muscles. In physical terms, the lengthening effect of this treatment on the shape of the *semimembranosus* and *adductor* is shown in Fig. 2 and appears to be similar to the effect imposed on these muscles by the tender-stretch method of carcass suspension[6].

Leg-twisting significantly increased the sarcomere lengths of all the muscles evaluated ($P<0.05$) except those of the *semitendinosus* and *psoas major* which were significantly reduced ($P<0.05$) (Table 4). The sarcomere lengths of all the muscles except that of the *semitendinosus* in the leg-twisted carcass side fell in the familiar relaxed pattern (3.7–2.4 μm) or in the upper range of the well defined derivative pattern (2.4–1.8 μ m) of muscle striation as defined by Locker[1]. Even the *semitendinosus* in which the treatment introduced appreciable slack still retained a sarcomere length in the lower range of the derivative pattern suggesting that the toughness induced in this muscle remained within an acceptable range. In contrast, all the muscles of the conventionally treated carcass side except the *posas major* and the semiten-*dinosus* had their sarcomere lengths in the lower range of the derivative pattern. Previous work by Quarrier

TABLE 3. EFFECT OF ANGLE OF ORIENTATION OF FEMUR BONE ON THE SHEARVALUES (KG) OF *GLUTEUS MEDIUS*, ADDUCTOR AND *SEMITENDINOSUS* MUSCLES

Carcass No	Angle (degrees)	Conventional Shear force (kg)			Angle (degrees)	Leg twisted Shear force (kg)		
		GM	ADD	ST	GM	GM	ADD	ST
1	137.5	4.82	4.04	5.29	68.0	3.52	3.38	5.81
2	139.0	4.92	2.26	6.46	79.5	3.73	2.05	7.38
3	137.5	5.28	6.19	4.79	62.0	3.50	3.65	5.37
4	104.5	5.28	6.35	6.83	63.5	4.77	5.31	7.50
5	115.0	4.65	3.94	6.90	83.0	3.85	3.40	7.10
Average	126.7[b]	4.99	4.55	6.05	71.2[a]	3.87	3.55	6.63

Means on same row with different superscript differ significantly ($P<0.01$).

TABLE 4. SARCOMERE LENGTH AND FIBRE DIAMETER IN RELATION TO METHOD OF CARCASS SUSPENSION

Muscle	*Sarcomere length* (*um*)		*Fibre diameter* (*um*)	
	Conventional Mean±SD	Leg-twisted Mean±SD	Conventional Mean±SD	Leg-twisted Mean±SD
LDL	$1.96^{a}\pm0.11$	$2.19^{b}\pm0.06$	$73.79^{a}\pm 8.74$	$54.39^{b}\pm10.47$
GM	$1.83^{a}\pm0.09$	$3.16^{b}\pm0.10$	$70.04^{a}\pm10.74$	$48.40^{b}\pm 2.72$
PM	$3.15^{a}\pm0.24$	$2.17^{b}\pm0.18$	$46.56^{a}\pm 3.62$	$59.28^{b}\pm 4.36$
SM	$1.80^{a}\pm0.07$	$2.94^{b}\pm0.18$	$72.43^{a}\pm 3.45$	$50.02^{b}\pm 1.29$
BF	$1.92^{a}\pm0.19$	$2.88^{b}\pm0.30$	$68.04^{a}\pm 5.59$	$48.14^{b}\pm 2.88$
ST	$2.31^{a}\pm0.30$	$1.89^{b}\pm0.08$	$55.17^{a}\pm 4.29$	$72.64^{b}\pm 6.16$
ADD	$1.81^{a}\pm0.75$	$3.23^{b}\pm0.05$	$73.28^{a}\pm 6.23$	$46.93^{b}\pm 9.46$
VL	$1.95^{a}\pm0.13$	$3.44^{b}\pm0.05$	$68.39^{a}\pm 8.16$	$40.82^{b}\pm 5.07$
RF	$1.92^{a}\pm0.10$	$2.92^{b}\pm0.26$	$72.17^{a}\pm 9.94$	$51.81^{b}\pm 2.36$

a, *b* = Means on same row with different superscripts differ significantly ($P<0.05$).

et al[10]. showed that the conventional method of carcass Suspension tended to stretch the P.M. significantly. Marsh and Leet[14] as well as Herring *et al*[15]. have also shown that little difference in tenderness value was to be expected between a highly stretched muscle and one which was stretched to a lesser extent. Thus the reduced stretching of the *psoas major* in leg-twisted beef was not reflected in a significant increase in toughness, as shown by the fact that the toughening induced was only 6.9 per cent.

The fibre diameters of all the muscles were significantly reduced ($P<0.05$) as a result of leg-twisting while those of the *psoas major* and *semitendinosus* were significantly increased ($P<0.05$). These results are in line with previous reports on sarcomere length-fibre diameter interrelationship[2]. Since muscle in its attempt to maintain a constant volume varies, its sarcomere length and fibre diameter in an inverse manner.

Table 5 shows significant decreases in the number of sarcomeres per myofibrillar fragment in the *longissimusle gluteus medius*, *adductor* and *vastus lateralis* ($P<0.05$), while that of the *psoas major* significantly increased ($P<0.05$). In the previous study on mutton carcasses, (J. Fd. sci., 1978 in press) it was noted that a slight change in the shear force of the *psoas major* was more liable to be accompanied by a significant reduction in the ease of fragmentation while in the *semitendinosus*, a drastic change in the shear force was not accompanied by a similar drastic effect on the fragmentation value. The present findings support this observation. It is also note-worthy that while in the mutton carcass, the myofibrillar fragmentation values were significantly reduced in the *semi-membranosus* and *rectus femoris*, no significance was observed in these two muscles in the present study.

TABLE 5. MYOFIBRILLAR FRAGMENTATION VALUES FOR MUSCLES FROM CONVENTIONAL AND LEG-TWISTED CARCASSES

Muscle	Conventional Mean±SD	Leg-twisted Mean±SD
LDL	$47.42^{a}\pm4.79$	$39.62^{b}\pm3.34$
GM	$44.38^{a}\pm1.3$	$38.14^{b}\pm2.70$
PM	$40.11^{a}\pm3.23$	$44.51^{b}\pm1.66$
SM	$44.35^{a}\pm1.46$	$40.40^{b}\pm5.08$
BF	$46.52^{a}\pm1.79$	$41.89^{b}\pm2.99$
ST	$42.00^{a}\pm3.83$	$43.80^{b}\pm2.60$
ADD	$45.74^{a}\pm3.51$	$37.34^{b}\pm4.65$
VL	$45.93^{a}\pm0.98$	$40.86^{b}\pm4.30$
RF	$43.95^{a}\pm4.80$	$40.06^{b}\pm4.41$

a,*b* = Means on same row with different superscripts differ significantly ($P<0.05$).

Conclusion: The leg-twisting method of beef carcass suspension appears to have put appreciable strain on some of the major muscles of the leg and loin during rigor development resulting in increases in the sarcomere lengths, reduction in the fibre diameter and fragmentation values and ultimately, reduction in shear values of these muscles. The two exceptions among the nine muscles studied were the *psoas major* which is naturally tender and the *semitendinosus* which forms only a small part of the leg muscle mass. The problems of fabrication into wholesale cuts are similar to those of tenderstretch carcasses and the same methods of fabrication is suggested. Although the microbiological aspect was not studied in this work, it seems reasonable to suggest that peeling of the hoofs with boiling water should reduce the microbiological contamination from this source to a minimum.

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A Study on the Rate of Heat Transfer Through Market Milk in the Flexible Single-Service Containers*

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In-pack processing of market milk in flexible single-service containers was developed[1]. Various factors such as quantity of milk in pouch, thickness of plastic containers, temperature of milk, etc, which affect the rate of rise or fall in the temperature of milk during in-pack processing, have been studied.

The published information on the rate of heat transfer or heat penetration through flexible pouches containing market milk is rather scanty[1]. The overall heat transfer coefficient of one litre polyethylene lined pure pak cartons with milk was found to range from 153 W/m 20 c for the stationery full carton to 194W/m20C for a shaken carton[2]. The rate of heat transfer of packaged flexible bags is three times higher than that of parallel canned foods[3]. Some of the studies on the rate of heat penetration through cans containing various food products have been reported[4]. In India work on various factors governing heat penetration has been conducted with cans containing fruit pulp and fruit juice[5].

Materials and Methods

To determine the rate of heat transfer in the plastic pouches during heating and cooling, the milk was heated in plastic pouches with 25 to 30 per cent head space in a thermostatically controlled water bath and cooled by tap water and chilled water separately. To record the temperature a thermometer was inserted in the pouch at one corner and rate of rise and fall in the temperature of milk was recorded every minute. The pouch was shaken by hand continuously to cause proper heat transfer during this period. Triplicate trials were conducted and following factors were studied: (*i*) temperature of heating and cooling mediums; (*ii*) initial

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temperature of milk; (*iii*) quantity of milk in the pouch; and (*iv*) type of pouch.

Effect of varying the temperature of water bath: Milk was filled in thin transluscent polyethylene pouches of capacity 250 ml and 500 ml. These were kept in water bath and the temperature was raised to 70°, 75°, 80°, 85° and 90°C and the corresponding rise in the temperature of milk was noted. In another set, milk was filled in mylar laminated polyethylene pouches of 250 and 500 ml capacities and placed inside the water bath and the temperature was raised to 70°, 75°, 80°, 85°, 90° and 92°C.

These packages subjected to different temperature treatments were then cooled first by tap water of 30°C followed by chilled water (5 to 8°C) and the change in the temperature of the milk by this cooling was noted in both the types of pouches.

Effect of different initial temperatures of milk: The pouches with milk having different initial temperatures (10, 20, 30 and 40°C) were kept in the water bath having 80°C temperature. The time needed to reach maximum temperature was noted. The experiment was conducted with both types of pouches.

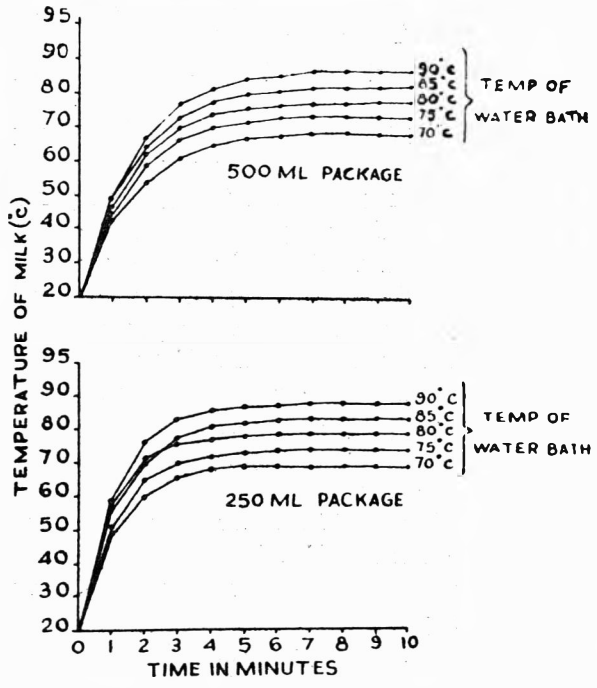
Effect of quantity of milk: Pouches having sizes of 250, 500, 750 and 1000 ml were filled with milk. The initial and final temperatures were kept at 20° and 80°C respectively. Both the types of pouches were used for the study.

The temperature of the milk contained in the pouches of different capacities were cooled from 80°C by using tap water of 30°C and further chilled by circulating chilled water of 5 to 8°C and the transfer of heat from milk in different sizes of pouches was studied.

Effect of different kinds of pouches: Six kinds of pouches were selected. Of these, one pouch was of mylar laminate polyethylene, one was medium (500 gauge) and another thick (800 gauge) non-laminated polyethylene, and the rest three were of thin non-laminated ploye thylene.

Results and Discussion

Effect of varying the temperature of the water bath: A difference of 1.0°C between the temperature of milk in the package on heating at 70°, 75° and 80°C, using both the pouches was found. It will be seen in Fig. 1 and 2 that the temperature of milk rose at faster rate in 250 ml than in 500 ml package and reached to the maximum level in 7 and 8 min in thin polyethylene and 8 and 9 min in mylar laminated polyethylene of 250 ml and 500 ml packages respectively. From Fig 1 and 2 it is clear that while the other conditions are similar it took almost the same time to reach the maximum temperature in the pouches with all the various temperatures of the water bath studied. It means the pouch



Fig, 1. Heating curves—influence of temperature of water bath on the rate of heat transfer in milk through thin translucent polyethylene pouches of 250 and 500 ml capacities.

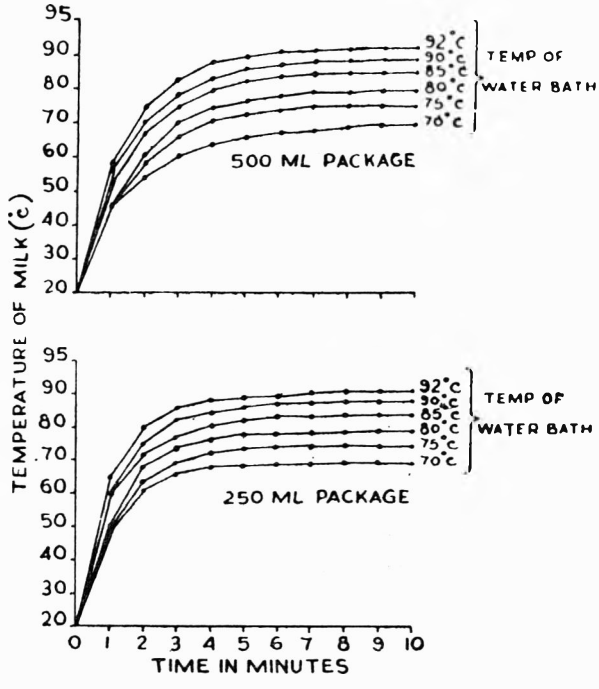


Fig. 2. Heating curves—influence of temperature of water bath on the rate of heat transfer in milk through mylar laminated polyethylene pouches of 250 and 500 ml capacities.

processed at higher temperature will heat more rapidly than the other. A similar observation has also been made with cans in separate reports of different temperatures[4].

As shown in Fig 3 and 4 there was no effect of temperature of hot milk on the final temperature of cooled milk in 250 ml package, but a slight higher temperature of cooled milk in 500 ml package heated to higher temperature is evident. The difference may be due to the change in ratio of cooling medium to milk. It took 5 min to reach the final temperature of the cooled milk. It may also be seen in Fig 3 and 4 that it took almost the same time to reach the minimum temperature during cooling process, that would mean, the pouch processed at higher temperature will cool more rapidly than the other.

Effect of different initial temperatures of milk: It will be observed from the Fig. 5 that there was a definite effect on the rate of heat transfer due to initial temperature of milk upto 1 to 3 min heating, but after 4 to 5 min the difference disappeared. The rate of heat transfer was faster with lower initial temperature than with higher. A similar report has been made with cans[4].

Effect of quantity of milk in the pouch: It will be observed from the Fig. 6 that the rate of heat transfer was slower in larger size packages. A similar observation has been made with cans, larger the can size, longer is the time required to acquire a can-centre temperature[5]. To bring the temperature of milk to the maximum level, it took 7 and 8 m in 250 and 500 ml packages respectively and 9 min in 750 and 100 ml packages. In case the coming-up time of 10 min is kept for in-pack processing of market milk it will provide about 1 min margin of safety to the largest size package and about 2 to 3 min margin to the smaller size packages.

As shown in Fig. 7 the rate of heat transfer was slower in large size packages. On cooling milk directly with chilled water it took almost half the period to reach the same temperature of chilled milk as was reached after cooling with tap water followed by chilled water.

Effect of different kinds of pouches: It will be seen from Fig. 8 that there was not much difference in the rate of heat transfer in different types of plastic pouches, except a little slower heat transfer was observed in thick 800 gauge polyethylene

No variation due to the type of pouches on the rate of heat transfer during cooling with tap water and chilled

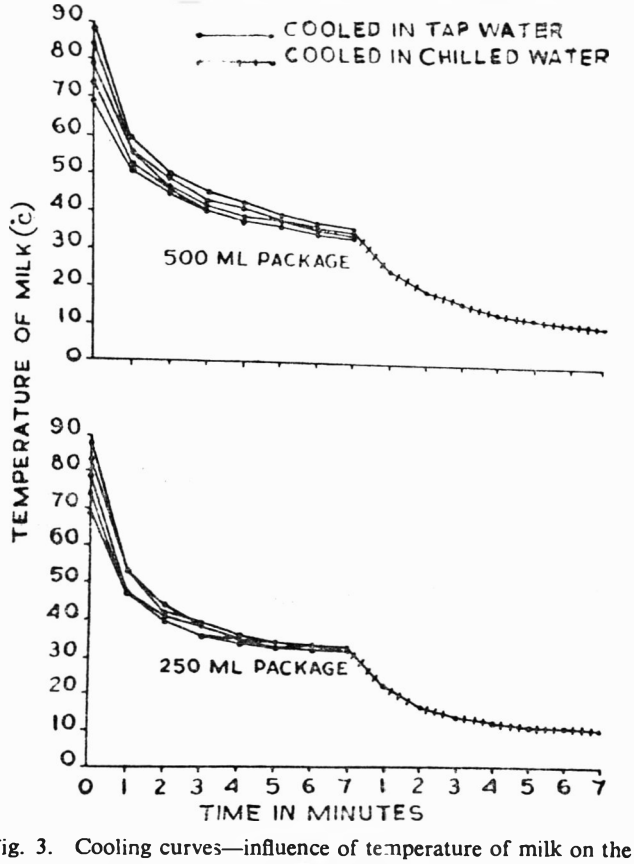


Fig. 3. Cooling curves—influence of temperature of milk on the rate of heat transfer from milk through thin translucent polyethylene pouches of 250 and 500 ml capacities.

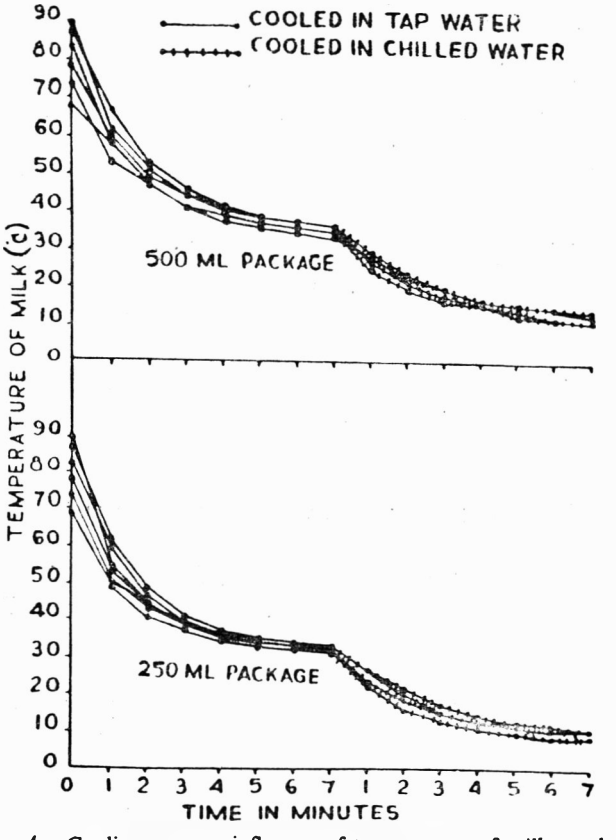


Fig. 4. Cooling curves—influence of temperature of milk on the rate of heat transfer from milk through mylar laminated polyethyiene pouches of 250 and 500 ml capacities.

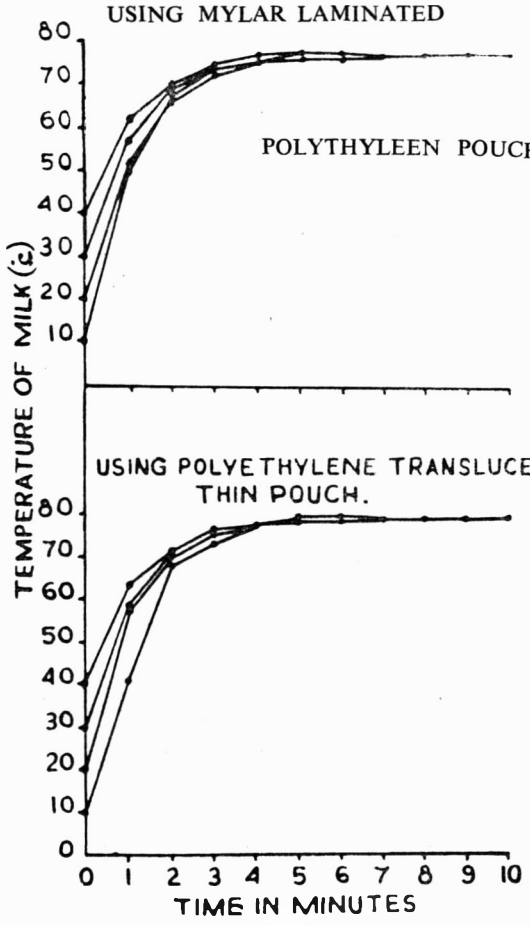


Fig. 5. Heating curves—influence of initial temperature of milk on the rate of heat transfer in milk through pouches of 250 ml capacities.

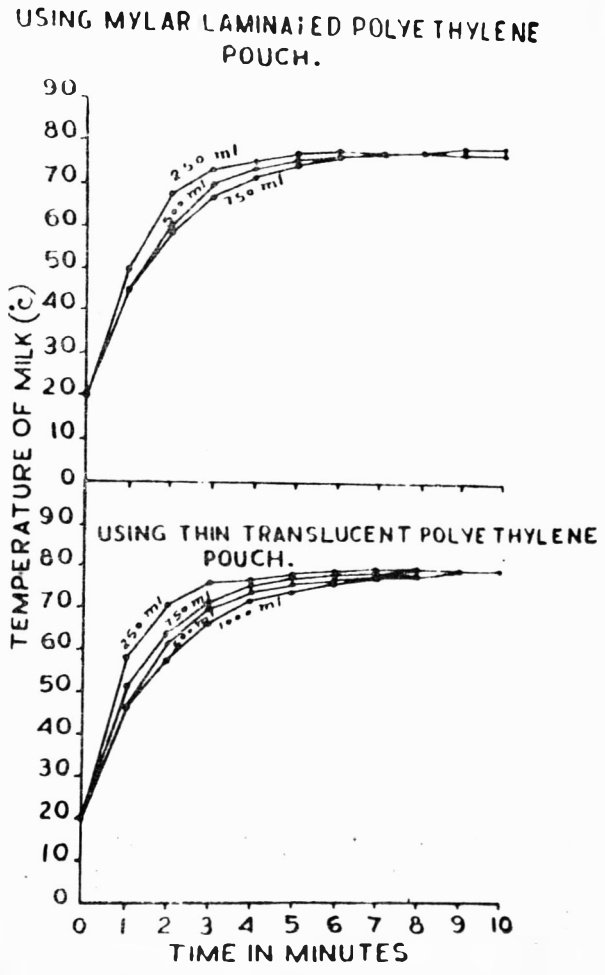


Fig. 6. Heating curves—influence of quantity of milk in the container on the rate of heat transfer in milk through pouches of different capacities.

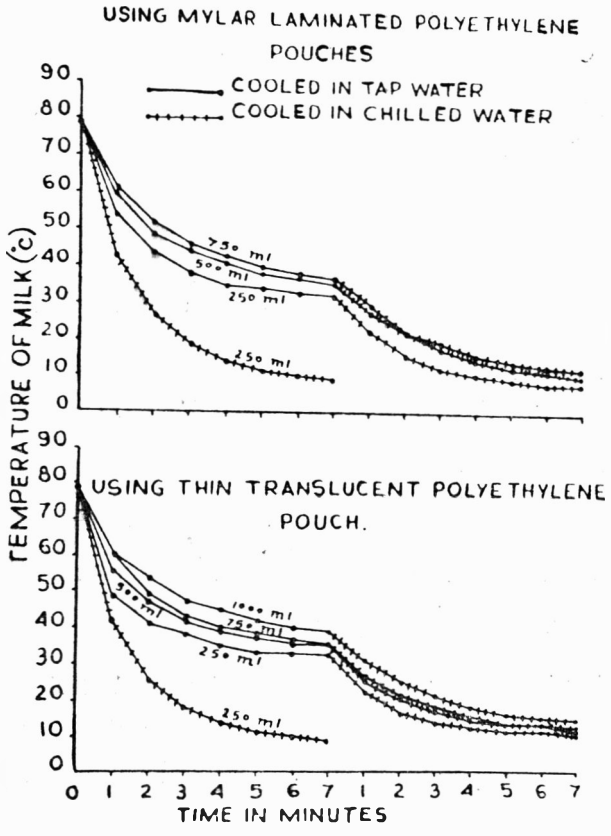


Fig. 7. Cooling curves—influence of quantity of milk in the container on the rate of heat transfer from milk through pouches of different capacities.

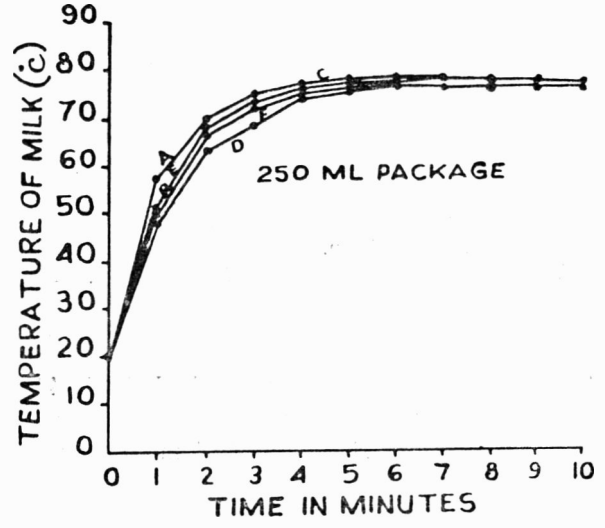


Fig. 8. Heating curves—influence of type of pouches on the rate of heat transfer in milk through the pouches of 250 ml capacities.

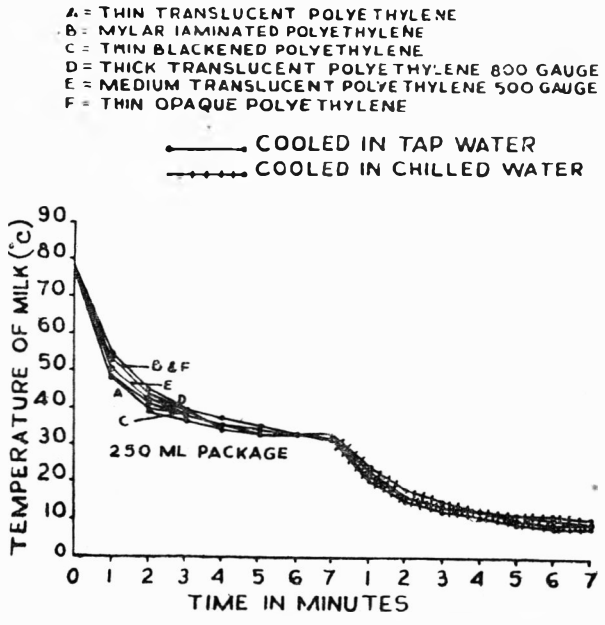


Fig. 9. Cooling curves—influence of type of pouches on the rate of heat transfer from milk through the pouches of 250 ml capacities.

water was observed (Fig. 9). It means the pouches studied did not differ from each other significantly in heat penetration.

Summary: It has been concluded from the above studies on the heat transfer through milk in plastic pouches, that the temperature of heating medium should be 1.0°C and 1.5°C higher than the desired temperature of milk for heating between 70 and 75°C and between 80 and 90°C respectively. As it took 7-9 min with different temperatures of water bath and sizes of packages to reach the maximum temperature of milk in the water bath with abundant quantity of hot water, 10 min coming-up time should be sufficient for in-pack processing, as the ratio in quantities of milk and heating medium may be narrower under the commercial practice.

A minimum of 5-7 min cooling with chilled water is required, depending upon the quantity of cooling medium and size of packages. Larger sizes of 750 and 1000 ml packages would require longer period for heat transfer than the smaller sizes of 250 and 500 ml. Cooling directly with chilled water for 7-10 min period was sufficient.

If the intitial temperature of raw milk is low enough, a higher temperature of heating medium would be necessary. Different types of packages used for processing of milk showed no effect on the coming-up time or the cooling time of milk.

Acknowledgement

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Investigations on Fumigation of Walnuts with Methyl Bromide

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Commercial scale disinfestation trials with methyl bromide was conducted on the export quality walnut. It was observed that a dosage of 2.8 kg 1000 cum. methyl bromide for 16 hr at normal atmospheric pressure and 3 hr under 28 in. vacuum condition gave 100% mortality of test insect, *Tribolium castaneum*. The effect of repeated fumigation of walnut with methyl bromide indicated that the second and subsequent treatments at the prescribed dose affected their quality. The need to determine tolerance limit of bromide level in walnut kernel has also been emphasised which will facilitate in exporting quality walnut.

The annual production of walnut in India is 42,000 tonnes and nearly 40 per cent of this is exported. (Srinath, D. *Plant Prot. Bull* in press). Some of the import-countries have made walnut fumigation a pre-requisite.

Inspite of systematic fumigation and technical supervision, few countries reported about the presence of insects in some of the walnut consignments at the port of entry. Therefore, investigations were conducted

on (*a*) the insect pest complex infesting stored walnuts in India; (*b*) efficacy of recommended dosage of methyl bromide on insect mortality; (*c*) effect of repeated fumigation on the quality of walnuts; and (*d*) bromide residue in walnut consignments subject to repeated fumigation.

A report on the pest infestation of stored walnuts based on an All-India market survey has been published by Srinath and Gill[1]. They recovered 10 species of stored product insects from walnuts, the major ones being *Tribolium castaneum*, *Oryzaephilus* spp. and *Cadra* (*Ephestia*) *cautella*. Investigations on other aspects as indicated above were subsequently undertaken and results thus obtained have been presented in this paper.

Materials and Methods

Screening of fumigants was conducted in the fumigation chambers of recognised fumigators under commercial conditions. These trials were conducted under normal atmospheric pressure (NAP) in 4 chambers, the capacity of which ranged from 27.8 to 83.5 cu m. Vacuum fumigation was conducted in a chamber of 18.6 cu m capacity.

Adults of *T. castaneum* (15-day old) were used as test insects. 20 adults were released in test tubes with the open ends covered by muslin cloth held in position by rubber band. Depending on the chamber size, 15-20 such tubes were distributed in different parts of the chamber by embedding each tube in the middle of kernels in selected walnut crates. The adult mortality count was taken immediately after degassing and observations were continued upto 24 hr in the laboratory before taking the final reading.

Methyl bromide was used at a dosage of 2.8 kg/100 cu m for 16 hr under NAP and the same concentration was screened under a vacuum condition of 28 hr by exposing walnuts for 3 hr.

Organoleptic evaluation was conducted with a panel of 10 members by drawing the samples of walnut after each treatment and comparing the same with that of unfumigated samples.

Samples of walnuts (approx. 500 g) both fumigated and non-fumigated were drawn from all the trials of the residue analysis and post fumigation observations were made to confirm the efficacy of treatment on the immature stages of insects. These samples were incubated for over 6 months at a temperature of 29±1°C. Observations were made at 15 days interval to confirm the presence/re-appearance of infestation.

Walnuts intended for residue analysis were subjected to repeated fumigation with methyl bromide @ 2.8 kg/100 cu m for 16 hr under NAP 3 times at an interval of 15 days. Analysis of inorganic bromide was carried out in walnut samples drawn after each fumigation by ashing method[2].

TABLE 1. EFFECT OF METHYL BROMIDE FUMIGATION ON THE QUALITY OF WALNUT

Treatment	Shelled walnut	walnut-in-shell
First	Flavour and taste comparable with the unfumigated samples	Flavour and taste comparable with unfumigated samples
Second	Off flavour and perceptible affected taste	Off flavour and affected taste not apparent
Third	Off flavour, tongue and throat irritation Tainting of kernels Material tending towards rancidity.	Mild off flavour and perceptible affected taste

Results and Discussions

Effect on insect mortality:[1] Observations made immediately after degassing and 24 hr later, indicated 100 per cent mortality of test insects. It was further observed that even after 6 months of fumigation with methyl bromide, walnut samples were free from infestation whereas in some of the unfumigated samples of the same batch, insect species like *Oryzaphilus* spp., *Stegobium paniceum* and *Carpophylus* spp. were recovered within a period of 30-45 days.

Effect on the quality of walnuts: The observations on the organoleptic evaluation is summarised in Table 1.

Bromine residue in walnut kernels: The bromide level in the unfumigated walnut samples was 4 ppm. The residue levels in these walnut kernels after first and second fumigation under vacuum condition were higher (65 and 77 ppm) compared to the samples which were fumigated under NAP (51 and 54 ppm); whereas after the 3rd fumigation, the residue level in fumigated samples under atmospheric pressure were higher than that of samples from vacuum fumigated batch (Table 2).

TABLE 2. BROMINE RESIDUE IN WALNUT CONSIGNMENTS SUBJECTED TO REPEATED FUMIGATION WITH METHYL BROMIDE

No. of treatment	*Bromine level (as inorganic bromide in ppm)*	
	Under NAP	Vacuum under
First	51.1	65.0
Second	54.0	77.0
Third	123.8	109.6
Control (unfumigated)	4.0	—

TABLE 3. FREQUENCY DISTRIBUTION OF WALNUT SAMPLES FUMIGATED WITH METHYL BROMIDE UNDER DIFFERENT RANGE OF BROMINE RESIDUE

No. of walnut samples	*Bromine level (as inorganic bromide in ppm)*
28	25–50
24	51–100
6	101–150
2	151–200
2	200

Samples of walnut fumigated under commercial conditions were drawn for residue analysis and the range of bromine level in these samples is indicated in Table 3.

The effect of prescribed concentration and dosage of methyl bromide for walnut fumigation indicated that the same is adequate to get 100 per cent mortality of the storage pests, if any, in walnut consignment. Based on the post-fumigation incubation tests, it can be inferred that the present schedule of walnut fumigation is effective in killing immature stages of insects infesting stored walnuts.

The surveys conducted at the premises where fumigated walnuts were stacked during transhipment before loading into the ship and the shipload itself in some of the foreign ships revealed the presence of residual infestation[1]. Thus, this residual population can act as a nucleus foci for fresh infestation by stored product pests. Thus it calls for: (*i*) disinfestation of the possible sources of cross-infestation: (*ii*) provision to keep the fumigated consignment segregated from the untreated cargoes in the shiphold; and (*iii*) use of packing material impervious to insect penetration.

It was observed that walnut kernels can be fumigated once without affecting the quality but the second and subsequent treatments to the same consignment positively affect the taste, colour, etc. In case of in-shell walnuts, two treatments can be given, which apparently do not affect either taste or colour. The adverse effect is perceptible only in the third fumigation done in succession. Hence, the existing procedure of subjecting both in-shell and shelled walnuts to repeated fumigation with methyl bromide, if the consignment is not exported within the stipulated period of 15 days, requires to be suitably amended. Therefore, it has to be ensured that the consignment not exported within 15 days from the date of fumigation may not be allowed to be exported unless it is re-inspected and found free from infestation. Second prophylactic treatment may be given even if fumigation is not required. However, if such a consignment is found to be re-infested, it may be re-fumigated and prophylactic treatment given again. Consignment not exported within 15 days from the date of second fumigation and prophylactic treatment may likewise be re-inspected and allowed to be exported only if found free from infestation. Under no circumstances, the walnuts should be allowed to be exported if found re-infested after second fumigation.

When walnuts are fumigated with methyl bromide (2.8 kg/100 cu m) the average residue will normally be about 50 ppm after first fumigation. The probable reason for the excess bromide residue recovery in some of the samples is that some of the consignments which are stored either at Delhi or Jammu & Kashmir for considerable length of time are fumigated as a precautionary measure before despatching to Bombay. In few samples, the residue level exceeded 100 ppm wherein the consignment has either received high dose of methyl bromide or has received more than two treatments.

The above results clearly bring out the fact that the practice of following the tolerance limits suggested by various institutions to repeatedly subject the same walnut consignment for fumigation is not desirable under tropical conditions. There is an urgent need to determine the tolerance limit of bromine level in walnut kernels keeping in view the effect of repeated fumigation with methyl bromide on the export quality walnuts. This will facilitate in maintenance of quality of walnut being exported from our country.

Acknowledgement

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Effect of Heating Grape Musts on pH and Acidity of Musts and Colour and Tannin Content of Red Wines

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Musts of eight grape varieties were heated to 70°C for 30 min and the must composition of heated and unheated musts and colour and tannin content of wines made from heated and unheated musts were compared. It was found that, with most varieties, the heated musts had slightly higher pH and acidity. The increase in acidity was more with the slip skin varieties. With regard to colour, variation between the varieties was observed due to heat treatment. Wines made from heat treated musts had higher tannin content.

The extraction of sufficient colour from many red grape varieties, except in a few varieties, has always been a problem. This is due to the fact that the anthocyanin pigments present in the epidermal cells are surrounded by semi-permeable membranes. In order to extract maximum colour, the semi-permeable membrane of the skin has to be damaged or destroyed. In the traditional method of fermentation, this is achieved by intermittent mixing of skin with the fermenting juice. The alcohol and CO_2 produced during fermentation increases the permeability of the membrane and thus help the release of pigments into the juice. Another effective method by which the pigments can be extracted is by heating the skin along with juice. Though heating grapes to extract more colour is known for long time, only recently it is gaining importance due to availability of suitable equipments and techniques. Studies carried out in different countries indicate that the colour of red wines made from heat treated grapes was stable and intense[1-5]. Coffelt and Berg[2] observed that different grape varieties reacted differently to heat treatment and also the same variety in different region reacted differently. In this study, musts from eight grape varieties were heat treated and the must composition of heated and unheated musts and colour and tannin content of wines made from heated and unheated musts were compared.

Materials and Methods

Vinification procedure: Eight varieties of grapes namely 'Cabernet Sauvignon', 'Buffalo', 'Malbec', 'Red Malaga', 'Bangalore Blue', 'Delaware', 'Gamsa' and 'Black Champa' grown in Experimental Farm of Indian Institute of Horticultural Research, Hessaraghatta were used. They were harvested during February-March, 1977. Since the brix of these grape varieties were low, they were ameliorated with cane sugar to about 22° Brix. The grapes after crushing were divided into two batches. One batch was fermented by traditional fermentation using either *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe* depending on the initial acidity of musts and processed as described earlier[6]. The other batch was heated to 70°C for 30 min[5]. After cooling, pectinase (a crude enzyme preparation from Central Food Technological Research Institute, Mysore) was added at the rate of 0.25 per cent. After 12 hr, the musts were pressed and the deep coloured juices were inoculated with either *S. cerevisiae* or *Sch. pombe* depending on the acidity. The wines after racking were clarified with bentonite and analysed.

Chemical analysis: The titratable acidity of musts and colour and tannin content of wines were determined by the methods described by Amerine and Ough[7].

TABLE 1. PH AND TITRATABLE ACIDITY OF *UNHEATED* AND *HEATED* GRAPE MUSTS

Variety	*Unheated* pH	*Unheated* Acidity (as g/tartaric acid/100 ml.)	*Heated* pH	*Heated* Acidity (as g/tartaric acid/100 ml.)
Cabernet Sauvignon	3.15	0.93	3.3	1.00
Buffalo	3.15	0.93	3.4	1.36
Malbec	3.20	1.42	3.3	1.42
Red Malaga	3.20	0.93	3.1	1.35
Bangalore Blue	3.10	1.07	3.2	1.35
Delaware	3.20	0.86	3.2	1.21
Gamsa	3.50	0.61	3.6	0.64
Black Champa	3.70	0.68	3.8	0.64

Contribution No. 653 of the Indian Institute of Horticultural Research, Bangalore-560 006.

TABLE 2. COLOUR (1:10 DILUTION) AND TANNIN CONTENT OF WINES MADE FROM *UNHEATED* AND *HEATED* GRAPE MUSTS

Variety	Colour				Tannin	
	Unheated		*Heated*		*Unheated*	*Heated*
	Brightness*	Hue**	Brightness	Hue	(mg/l)	(mg/l)
Cabernet Sauvignon	0.451	0.96	0.651	1.29	1360	1820
Buffalo	0.581	1.38	0.475	1.21	1260	2640
Malbec	0.830	1.08	0.955	1.21	1520	2720
Red Malaga	0.402	1.15	0.390	1.42	840	2540
Bangalore Blue	0.318	1.32	0.370	1.39	660	1520
Delaware	0.245	1.27	0.326	1.61	840	1360
Gamsa	0.195	1.56	0.253	1.75	700	1100
Black Champa	0.423	1.03	0.329	1.12	1020	1440

*Sum of absorbance at 420 and 520 nm

**Ratio of absorbance at 420 and 520 nm.

Relative colour of the wines was determined by diluting it to 1:10 with water and measuring absorbance at 420 and 520 nm with the help of 'Spectrochem' spectrophotometer (Associated Instruments Manufacturers (I) Pvt. Ltd., New Delhi). The pH was measured with Elico pH meter (Model LI-10).

Results and Discussion

Effect of heating on pH and titratable acidity of musts: Except 'Red Malaga' and 'Delaware', the heat treated musts from other varieties showed a slight increase in pH (Table 1). Rankine[4] indicated that heat treatment gives a higher pH and anthocyanin content in wines. Similar to pH, the titratable acidity of heat treated musts showed an increase in most varieties, This increase in acidity may be due to increase in tartaric acid content as suggested by Martiniere and Ribereau-Gayon.[5] This increase in acidity was more with the slipskin varieties like 'Buffalo', 'Red Malaga, Bangalore Blue and Delaware.' The increase in acidity should have resulted in decrease in pH. Contrary to this, the musts from heat treated grapes of some varieties showed an increase in both pH and titratable acidity. Neither Rankine[4] nor Martiniere and Ribereau-Gayon[5] could measure both pH and acidity of heated musts simultaneously. The results of Flora[8] showed that the juice of hot pressed whole grapes had higher titratable acidity than the cold pressed. However, the pH either increased or decreased or did not change due to higher acidity. This observation was similar to our findings.

Effect of heating on colour and tannin content of wines: The wines made from heat treated musts of 'Cabernet Sauvignon', 'Malbec', 'Bangalore Blue', 'Delaware' and 'Gamsa' and the wines made by traditional fermentation from 'Buffalo', 'Red Malaga' and 'Black Champa' had higher intensity of colour (Table 2). This shows the varietal response to heat treatment. Except the wine from 'Buffalo', the wines made from heat treated musts of other varieties had higher hue changes (Ratio 420/520). Though heat treatment increased the intensity of colour, the visual appearance of the wines showed brownish tinge. This brownish colour may be responsible for higher hue changes. Coffelt and Berg[2] classified the grape varieties on the basis of their response to heat treatment into three groups. The group I varieties showed normal reaction, group II varieties showed unfavourable effect and group III varieties showed favourable response to heat treatment. They also observed that the same variety grown in two climatic regions differed in their response to heat treatment. Our results also indicate that 'Cabernet Sauvignon', 'Malbec', 'Bangalore Blue', 'Delaware' and 'Gamsa' showed favourable effect while 'Buffalo', 'Red Malaga' and 'Black Champa' showed unfavourable effect.

The wines made from heat treated musts of all the varieties had higher tannin content than wines from unheated musts (Table 2). This is contradictory to the observation of Rankine[4] that wines made from heat treated grapes had lower tannin content. However, the results of Flora[8] indicate that the juice of hot pressed grapes had higher tannin content than cold pressed.

Organoleptic analysis of the wines showed that the quality of the wines were not affected by heat treatment. The judges rated the heat treated wines better than the unheated.

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Adjuncts in Brewing I. Bajra and Sorghum

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The possibility of using bajra and sorghum as malt adjuncts in brewing has been examined. Analysis of wort and beer thus prepared, suggests the possibility of using sorghum to the extent of 50% and bajra is upto 35% only. The alcohol content of the beers prepared, using varying concentrations of malt adjuncts, was in general, comparable to control. The beer samples prepared, using 35% sorghum and 25% bajra as adjuncts were comparable both analytically and organoleptically to the commercial beer.

Bajra (*Pennisetum typhoides*) and sorghum (*Sorghum vulgare*) are two major summer crops of Haryana State. At present these are mostly used as fodder crops. In recent years with emphasis on greater grain production, attempts for developing or modifying the brewing processes that would yield a quality beer without the use of traditional quality malt, have been made. With the increasing demand for the limited quantity of barley malt for other purposes, it was desired to evalutae these surplus grains for brewing purposes. Earlier work[1] showed that bajra malt alone cannot be used for brewing. Sorghum after malting, used for the production of Bantu beer makes the process relatively expensive[2]. Hence, this paper examines the use of Bajra and Sorghum as malt adjuncts in brewing.

Materials and Methods

Raw materials: Barley, variety 'C-164' and bajra, variety 'HB-3' used in the present investigation are commercially grown varieties and were obtained from the Department of Plant Breeding, Haryana Agricultural University, Hissar. Sorghum (CVS-3') was supplied by Regional Research Station of I.A.R.I., Rajendra Nagar (Hyderabad). These grains were analysed for various ingredients by the methods described by AOAC[3], The starch content was determined by the method of Hassid and Neufeld[4].

Preparation of malt: Barley malt was prepared according to the procedure described by Meredith *et al*[5]. Selected grains (500 g) were washed and steeped at 17°C for 48 hr in 2 l. capacity flasks. The seeds were then allowed to germinate on wet absorbant cotton in trays covered with ordinary filter paper at 17°C in a BOD incubator for 72 hr. Grains showing optimum germination (acrospire 1/2-3/4 of the length of grain) were sorted out manually and kilned at 45°C for 24 hr and then at 55°C for 8 hr. The cured grains were screened to remove the root-lets, cooled and stored in a cool dry place (25°C). The malted grains were coarsely ground in a coffee grinder and analysed by the method of AOAC[3].

Yeast culture: Saccharomyces carlsbergensis NCYC 1324, a bottom yeast strain obtained from the Department of Microbiology, Haryana Agricultural University, Hissar, was used in the present studies. It was maintained on yeast extract, peptone, dextrose (YEPD) slants.

Inoculum: Inoculum required for fermentation was prepared in yeast extract dextrose broth (YEPD broth) of the following composition. Yeast extract, 1.0 g; peptone, 2.0 g; dextrose, 2.0 g; and distilled water, 100 ml. Sterilized broth (100 ml) was inoculated with 24 hr old yeast slant culture and incubated for 48 hr at 30°C on a rotary shaker (210 rpm). This was transferred to 1 l. fresh sterilized YEPD broth in a 2 l. flask and incubated on rotary shaker for 48 hr at 30°C. Flasks were then kept at 25°C under stationary conditions to allow the yeast to settle. The clear broth was decanted and the yeast cells were centrifuged at 10,000 rpm at 20°C for 10 min (International Refrigerated Centrifuge). Cells were washed twice with sterile distilled water and once with sterile phosphoric acid (pH 2.00) to avoid the possibility of bacterial contamination.

About 3 g fresh weight of yeast was used as inoculum per litre of wort. The wort was fermented at 17°C till the fermentation was complete (12-15 days).

Hops: Hops was a gift from Haryana Breweries, (Sonepat) and was added at the rate of 1.75 g/l. of wort.

Preparation of wort: Different combinations of malt and each adjunct (Table 1) were used to prepare the wort keeping the raw material concentration at 15 per cent for a final brew of 2.5 l. The raw materials were coarsely ground in a coffee grinder prior to mixing.

The mashing procedure (Ethiraj S. personal communication) Harayana Agricultural University, Hissar involved gelatinisation of the adjuncts in 2 l. of water at 15 lb pressure for 10 min prior to mixing them with ground malt and diluted to 3.2 to 3.5 l with distilled water depending upon the type and proportion of the adjunct combined with the ground malt (Table 1).

A single stage decoction process of mashing was followed[5,6]. The temperature of proteolysis and saccharification were 47°C and 65°C, respectively. The decoction was conducted during the saccharification period. After mashing, the worts were filtered through an ordinary two fold filter paper covered with cheese cloth. This filterate called 'sweet wort' was mixed with hops and was autoclaved at 5 lb pressure for 30 min to get a clear wort. The hot wort was filtered into a sterile container, cooled and analysed.

Beer fermentation: The cooled worts were inoculated with bottom yeast cells and allowed to ferment at 17°C till the fermentation was complete (12-15 days). The 'green beer' was siphoned off to sterile containers lagered at 0°C for 15 days and clarified with 200 ppm of bentonite agar mixture (2:1)[7]. The clear beer was siphoned off, pasteurized at 60°C for 3 min[8], bottled in sterile bottles and subjected to analysis. The beer was tested organoleptically after storage for 15 days at 10°C.

Analysis of wort and beer: Analysis of wort and beer was carried out as described by Weissler.[8] Specific gravity was determined using boot type pycnometer (50 ml capacity) at 20°C. Extract of wort and beer was calculated from the plato tables of the American Society of Brewing Chemists (ASBC)[9] and related to specific gravity of wort and beer. Real extract was determined according to ASBC procedure. Colour was determined by the spectrophotometric method[8]. The pH was determined using an Elico pH meter Model LT-10 and total acidity as per cent lactic acid by weight according to the procedure described by Hortwitz[9]. Protein content of wort and beer was estimated by Kjeldahl method[10]. Reducing sugars were determined in terms of maltose by dinitro salicylic acid method[11]. Alcohol in beer was estimated by spectrophotometric method[12]. For organoleptic analysis of beer two panels consisting of two and four judges constituted the taste panel. A commercial beer sample (Rozy Pelican of Haryana Breweries, Murthal) was also included among the samples for tasting.

TABLE 1. COMPOSITION OF RAW MATERIALS AND INITIAL TOTAL VOLUME FOR WORT PREPARATION

Treatments	Malt+adjunct (g.)	Initial total volume (lit)
Malt (Control)	375+	3.2
85% M+15% B	320+55	3.2
75% M+25% B	280+95	3.2
65% M+35% B	245+130	3.3
50% M+50% B	188+187	3.4
85% M+15% S	320+55	3.2
75% M+25% S	280+95	3.4
65% M+35% S	245+130	3.4
50% M+50% S	188+187	3.5

M – Barley Malt.
B – Bajra.
S – Sorghum.

Results and Discussion

Chemical composition: Grains used for brewing must have a low fat and protein content but should be rich in starch. The chemical composition of the raw materials used in the present studies is given in Table 2. The starch content which varies from 56.28 to 61.8 per cent is quite adequate for use in brewing. The protein and fat (ether extract) contents in barley and sorghum

TABLE 2. CHEMICAL COMPOSITION OF RAW MATERIALS (DRY WEIGHT BASIS)

Raw material	Moisture (%)	Starch (%)	Protein (N×6.25) (%)	Ether extract (%) Dry weight basis	Red. sugar (as % maltose)
Barley (C-164)	10.9	56.3	8.6	2.25	0.18
Barley malt	8.1	48.5	9.0	2.35	2.08
Bajra (HB-3)	10.8	61.8	13.8	6.08	0.55
Sorghum (cvs-3)	9.4	56.2	9.9	3.20	0.94

TABLE 3. ANALYSIS OF WORTS PREPARED USING BAJRA AS MALT ADJUNCT

Treatment (%)	sp. gr.	Extract (Oplato)	Colour	Red. sugars (% maltose by wt.)	Protein (% by wt.)	pH	Total acidity (% by wt. as lactic acid)
85 M+15B	1.03458	8.68	10.15	6.26	0.38	5.80	0.07
75 M+25B	1.03154	7.93	10.45	6.16	0.38	5.75	0.08
65 M+35B	1.03136	7.88	11.71	6.08	0.38	5.80	0.08
50 M+50B	1.02722	6.86	12.17	5.51	0.31	5.50	0.08
All malt (Control)	1.03581	8.98	9.85	7.72	0.50	5.75	0.10
Av. American	1.04755–	11.80–	3.00–	7.00–	0.38–	5.20–	0.11–
wort.	1.04965	12.30	5.00	8.50	0.50	5.80	0.12

M, Barley malt; B, Bajra.

TABLE 4. ANALYSIS OF WORTS PREPARED USING SORGHUM AS MALT ADJUNCT

Treatment (%)	Sp. gr.	Extract (Oplato)	Colour	Red. sugars (% maltose by wt.)	Protein (% by wt.)	pH	Total acidity (% by wt. as lactic acid)
85 M+15S	1.03428	8.60	9.60	8.27	0.44	5.45	0.09
75 M+25S	1.03378	8.48	8.70	8.19	0.38	5.45	0.09
65 M+35S	1.03330	8.36	7.16	7.66	0.31	5.45	0.09
50 M+50S	1.03332	8.37	5.80	7.66	0.25	5.50	0.10
All malt (Control)	1.03581	8.98	9.85	7.72	0.50	5.75	0.10
Av. American	1.04755–	11.80–	3.00–	7.00–	0.38–	5.20–	0.11–
wort	1.04965	12.30	5.00	8.50	0.50	5.80	0.12

M, Barley malt; S, Sorghum.

TABLE 5. ANALYSIS OF BEERS PREPARED USING BAJRA AS MALT ADJUNCT

Treatment %	Sp. gr.	Apparent extract (Oplato)	Real extract	Colour	Red. sugars (% maltose by wt.)	Protein (% by wt.)	pH	Total acidity (% by wt. as lactic acid).	Alcohol (% by wt.)
85 M+15 B	1.00638	1.64	2.10	8.23	0.99	0.31	5.10	0.10	2.17
75 M+25 B	1.00746	1.91	2.36	8.08	0.93	0.31	5.10	0.10	2.17
65 M+35 B	1.00812	2.08	2.64	8.96	1.07	0.31	4.95	0.10	2.17
50 M+50 B	1.00914	2.34	2.80	9.99	0.99	0.25	5.00	0.09	2.08
All malt (Control)	1.00432	1.10	2.05	7.84	0.76	0.31	5.20	0.10	2.75
Av. American beer	1.01071–1.01410	2.73–3.60	4.08–5.45	2.50–3.50	0.90–1.55	0.24–0.38	4.10–4.50	0.13–0.17	3.10–3.90
ISI Specification	—	—	—	—	—	—	—	—	2.00–10.00

M, Barley malt,; B, Bajra.

TABLE 6. ANALYSIS OF BEERS PREPARED USING SORGHUM AS MALT ADJUNCT

Treatment (%)	Sp. gr.	Apparent extract (Op.ato)	Real extract	Colour	Red. sugars (% maltose by wt.)	Protein (% by wt.)	pH	Total acidity (% by wt. as lactic acid)	Alcohol (% by wt.)
85 M+15 S	1.00440	1.13	2.20	7.05	0.89	0.31	5.15	0.09	2.86
75 M+25 S	1.00448	1.16	2.19	6.18	0.89	0.25	5.15	0.09	2.77
65 M+35 S	1.00472	1.21	2.19	4.86	0.94	0.19	5.10	0.08	2.47
50 M+50 S	1.00492	1.27	2.20	3.31	0.76	0.19	4.70	0.09	2.47
All malt (Control)	1.00432	1.10	2.05	7.84	0.76	0.31	5.20	0.10	2.75
Av. American beer	1.01071–1.01410	2.73–3.60	4.08–5.45	2.50–3.50	0.90–1.55	0.24–0.38	4.10–4.50	0.13–0.17	3.10–3.90
ISI Specification	—	—	—	—	—	—	—	—	2.00–10.00

M, Barley malt; S, Sorghum.

are quite comparable but higher in case of bajra. Cereals with higher fat content are, generally, considered unsuitable for brewing as they have a detrimental effect on the flavour and quality of head retention of beer.

Wort analysis: The major parameters which finally reflect the quality of beer are fermentable sugars, the total protein content ($\propto$ amino N), pH, total acidity and colour of the wort. Tables 3 and 4 show the analysis of worts prepared from bajra and sorghum respectively as adjuncts.

The extract of worts when bajra was used as adjunct decreases with the increase in bajra concentration whereas, it is comparable to control wort in case of sorghum. The variations are perhaps due to the type of adjunct used and the variation in the concentration of various enzymes in different malt adjunct combinations. The colour of bajra adjunct worts was darker than the control whereas sorghum adjunct worts were lighter, indicating the effect of individual adjunct and the level used. Reducing sugars which play an important role in establishing the quality and identity of beer are lower in bajra adjunct worts than the control. The reducing sugars are higher or comparable to control in case of sorghum worts and are within the range of American wort[6].

The protein content of all the adjunct worts is lower than control but within the range of average American wort[6]. This suggests that a major portion of protein in wort is contributed by malt only. The pH and total acidity of all adjunct worts is comparable to that of control and lie within the average American range[6] and slight variations observed are apparently due to the type and level of adjunct used.

Analysis of beer: Tables 5 and 6 show the analysis of beers prepared using bajra and sorghum, respectively as adjuncts. The apparent and real extract for various beers, so prepared were higher than that of control but lower than average American standard[7]. However the variations among the different beers prepared from the different combinations of malt and adjuncts were very small. It may perhaps be a reflection of the individual adjunct used and the presence of higher amounts of dextrins in these beers. The bajra adjunct beers were darker and that of sorghum adjunct beers were lighter than control. All the beers including control were darker than the average American standard[6]. This difference is due to the difference in the colour of malt, adjuncts used and the conditions employed for the production of these worts or beers.

Beer prepared using adjuncts had slightly higher reducing sugar content than control but is within the range prescribed for average American beer[6]. The slightly higher content of left over reducing sugars in adjunct beers may be due to the presence of reducing activity of dextrins which are in plenty. The values for protein content of the beers prepared using bajra as adjunct were comparable to that of control and within the range of average American standard[6]. However, protein contents of sorghum adjunct beers were slightly lower than control which may be as a result of decrease in amount of malt in different malt-adjunct combinations. Thus, indicating that most of the proteins of wort or beer are contributed by malt and slight differences are a reflection of the adjuncts used. The pH values of different adjuncts beers were slightly less, or more or less comparable to that of control but higher than average Americal standard[6]. Total acids in the beers derived from different treatments were comparable to the control, however, slightly less than average American beer[6]. Hence, suggesting that adjuncts do not have much influence on pH and total acidity. The low alcohol in beers prepared using bajra may be the result of relatively lower amount of fermentable sugars present in the corresponding worts. The alcohol content of beer

produced when sorghum was used to the extent of 25 per cent was comparable to that of control. The alcohol content of all the beers lie within the limits prescribed by Indian standards Institution (2.0 to 10 per cent).

The beers after storage for 15 days were subjected to organoleptic analysis. It was found that the adjunct beers were better than control (except where 50 per cent bajra was as adjunct). The beers prepared using sorghum up to 35 per cent and bajra up to 25 per cent were comparable to commercial beer. The beer prepared from 25 per cent bajra as an adjunct was adjudged to be the best of all the beers produced from adjuncts.

From the above studies, it is suggested that beer produced by the utilization of adjuncts are comparable both analytically and organoleptically with the beer prepared from all malt. However, further scaling up of this process is desired.

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Incidence of Drug Resistant Coliforms in Some Ready to Serve Foods

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Vegetarian and nonvegetarian preparations from kitchens were sampled for their coliform content. From each item containing coliforms about 20% of colonies were picked from violet red bile agar plates and biochemically characterised. Coliforms were recovered from 64% of samples. Salads had the highest coliforms, followed by vegetable curries.

***E. coli* from foods, water and human sources, *Klebsiella* and *Enterobacter* from foods and non-lactose fermenters (NLF) from foods and human sources were examined for resistance to twelve antibiotics. Most of the organisms were resistant to ampicillin, penicillin, chloramphenicol, erythromycin and sulphadimidine. *E. coli* from foods (1 strain) and human sources (1 strain) were sensitive to ampicillin, pencillin and chloramphenicol.**

Presence of coliforms, particularly *E. coli* in foods is regarded as indicative of faecal contamination, recent or remote.

The phenomenon of transferable drug resistance which was first demonstrated by Japanese workers has now been shown to operate in all the enterobacteria including non pathogenic *E.coli*[1]. The widespread emergence of antibiotic resistance among disease causing bacteria has been reported widely[2-6]. Transfer of resistance factors (R-factors) has been observed *in vivo* in subjects under chemotherapy[7,8] and also between *E.coli* and *Salmonella* in the rumen of sheep under starvation[9]. However, reports of antibiotic resistant *E. coli* with R factors from foods are meagre[10].

The present study deals with isolation of drug resistant coliforms from various foods. Coliforms from water and human subjects consuming diets comprised

of foods similar to those included in the investigation have also been studied for purposes of comparison.

Materials and Methods

Media were prepared and total plate counts and coliforms were determined according to standard methods[11]. Dextrose tryptone agar (DTA) and Violet Red Bille Agar (VRBA) media were used for total mesophilic (TPC) counts and coliforms respectively. Identification of the genera of the coliforms group was done according to Edwards and Ewing[12]. Eosin methylene blue agar (EMB agar) was used as the initial step in the identification of the above genera and MacConkey agar for isolation from faecal specimens. Tetrathionate broth was used for *Salmonella* enrichment. All cultures were stored as stab cultures in semisolid agar medium (0.2-0.3 per cent agar) at 25-27°C.

Foods: All food samples except the cakes were drawn from two Defence establishment kitchens. Semisolid or gravy containing foods such as curries and dals were collected in sterile 250 ml glass beakers covered with aluminium foil and dry foods such as rice, chapaties, etc. in U.V. sterilized polythene pouches. Specimens of cakes and pastries obtained from the local market were also screened.

Isolation from foods: Food samples were collected within an hour of their preparation and analysed within 2 hr of collection. Ten gram of samples were macerated in sterile glass mortars and suspended in 90 ml of 0.1 per cent peptone water. The mixtures were incubated for 2 hr at room temperature (25-28°C) as a resuscitation measure. Serial dilutions were prepared in quarter strength Ringers solution[11] and 1 ml portions were pour-plated in DTA and VRBA for TPC and coliforms respectively. The plates were incubated at 35°C overnight. Macerated food samples (1-3g) were also inolculated into 10 ml tetrathionate broth and incubated at 37°C for 48 hr for the enrichment of *Salmonella*.

Isolation from faecal specimens: Human gut coliforms were isolated from adult faecal specimens sent to the two local hospitals for routine analysis. One or two loopfuls of the emulsified specimens were streaked on MacConkey agar plates and the latter incubated at 37°C for 16 hr. Later they were kept at 4-6°C in a refrigerator in the hospital until collection. The plates were collected twice a week and further processed in our laboratory.

Identification: Five to ten colonies from VRBA and MacConkey plates of every food and faecal specimen were picked and tested on EMB agar. Colonies (*a*) showing distinct greenish metallic sheen; (*b*) which were mucoid; and (*c*) which were light pink to deep red were characterised by the Indole (37° and 44.5°C), Methyl red, Voges-Proskauer and Citrate (IMVIC) tests into *E.coli* biotype I, *Klebsiella* and *Enterobacter*. Tetrathionate broth enriched samples were streaked on MacConkey agar and the nonlactose fermenting (NLF) organisms were picked. These were not further characterised.

Water isolates: Seventeen *E. coli* strains from five water samples were obtained from the local hospital for drug resistance studies.

Standard strains: Fourteen strains of enteropathogenic *E. coli* (E. Ec) of the Staten Serum Institut, Copenhagen, collection were obtained from St. Johns Medical College, Bangalore. These were included in the drug resistance studies for comparative purposes.

Only those coliform isolates which were completely identified were included in drug resistance studies.

Antibiotic sensitivity: Antibiotic sensitivity of isolates was tested in concentrations recommended[13] by the paper disc method. Overnight broth cultures (0.3 ml) transferred directly from the stabs stored in semisolid agar (10^{8-9} or ml/g) were used as inoculum which was brushed over well dried agar plates by sterile cotton swabs. Sterile antibiotic solutions were made from products commercially available as injectable solutions, tablets or capsules as the case may be. Antibiotic impregnated discs (6 mm dia, punched out from Whatman No. 1 paper) carrying ampicillin (A, 10 μg), tetracycline (T, 30 μg), septran or supristol (Se, 25 μg, containing trimethoprim+sulphamozaxole), penicillin G (P, 10μg), streptomycin (St, 10 μg), chloramphenical (Ch, 30 μg), neomycin (N, 30 μg), erythromycin (E, 15 μg), sulphadimidine (Su, 300 μg), kanamycin (K, 30 μg), gentamycin or garamycin (G, 10 μg) and doxycycline an analogue of tetracycline (D, 10 μg) were prepared by soaking them in solutions of appropriate concentrations and freeze drying them. They were stored under silica gel desiccant in tightly capped vials in the refrigerator until required.

The composition of solid medium employed for sensitivity tests was the same as that of the oxoid Isosensitest agar C471 made with Bacto-agar (Difco) and triple distilled water which gave reproducible results in trials employing various agar media.

Results and Discussion

Foods not subjected to time-temperature abuse were microbiologically satisfactory as indicated by the data given in Table 1. Cooked foods had total counts showing no more than 10^4 org/g, 21 per cent had about 10^2 org/g and 34 per cent contained 10 org/g. Coliforms were absent in 36 per cent of the samples. Coliforms when present contributed to a major proportion of the total microbial load and this was not noticed with fish curries. Coliforms are heat labile and

TABLE 1. TPC AND COLIFORM COUNTS OF VARIOUS FOODS SAMPLES FROM SERVICE KITCHENS AND THE OCCURRENCE OF DRUG RESISTANCE AMONG COLIFORMS

Food sample	Numbers tested	TPC/g	Coliforms /g	**Drug resistant coliforms
Salads	5	10^7	10^4–10^5* (4)	+ ●
Vegetable curry	20	10^2–10^4	10 –10^2 (10)	+
Rice	5	10^2–10^3	10 –10^2 (3)	+
Dals	4	10 –10^2	0 –10 (1)	+
Chapatis	12	10 –10^3	0 –10 (4)	+
Mutton curry	4	10 –10^2	0 –10^2 (2)	+
Fish curry	3	10^3–10^4	0	—
Cakes & Pastries	8	10^2–10^3	0 –10 (2)	not done

*Figures in parenthesis indicate the number of samples that showed the higher counts of coliforms

**Resistant to one or more of the twelve antibiotics tested

● Drug resistant coliforms present in samples.

their recovery from foods indicates either insufficient or improper cooking or post cooking contamination from handlers and equipment. Inadequate cleaning of raw materials could also lead to salads and vegetable curries of microbiologically poor quality. In foods such as chapaties there may have been inadequate heat penetration into the raw material whereby the coliforms have survived. The rather low count of coliforms in meat curries may possibly be due to the longer cooking time involved.

A total of 45 *E. coli* biotype I strains, 23 *Klebsiella* and 25 *Enterobacter* out of 180 isolates were identified from the various foods from which coliforms were recovered (Table 2). Sixteen non-lactose fermenting (NLF) organisms were also found in foods. Numerous isolates from coliform plates could be allocated to only the groups intermediate between *E. coli* and *Enterobacter*.

The maximum number of *E. coli* biotype I were obtained from salads, vegetable curries and rice. The same three foods also contributed to most of the *Klebsiella*, *Enterobacter* and non-lactose fermenters.

Normal human gut coliforms as revealed by analysis of samples obtained from hospital were mostly *E. coli*. A few NLF organisms were found occasionally. Eighty

TABLE 2. DISTRIBUTION OF DRUG RESISTANT GENERA ISOLATED FROM DIFFERENT FOODS

Food	*E. coli*	*Klebsiella*	*Enterobacter*	NLF*
Salads	19	9	4	5
Vegetable curry	16	10	16	6
Rice	8	3	3	0
Dal	0	1	0	2
Mutton curry	0	0	1	3
Chapatis	2	0	1	0

*NLF—Nonlactose fermenting isolates. These were not further characterised.

two isolates from 30 faecal samples were analysed for drug resistance.

The resistance patterns of all organisms tested against different antibiotics are given in Table 3. All 82 human isolates (75 *E. coli* and 7 NLF) presented the first three resistance patterns viz. ATSePStCh, ATSePCh and ATPStCh, they being resistant to ampicillin, penicillin, tetracycline and chloramphenicol in the concentrations tested. The same patterns of resistance were also prevalent among most of the isolates from foods. Four strains of enteropathogenic *E. coli* (E.Ec) and some of the *E. coli* from water also showed these patterns. Resistance to only three antibiotics were shown by 15 food isolates, 11 of them being *E. coli*, 3 water isolates and 5 E.Ec However, 68 per cent of the isolates were resitant to a combination of at least five antibiotics as represented by patterns 1, 2 and 3.

Basically all genera were resistant to ampicillin, penicillin, chloramphenicol and sulphadimidine at the concentrations employed (Table 4). Except for a few they were also resistant to erythromycin and doxycycline. Neomycin resistance was shown by at least 40 per cent of organisms in each category. Resistance to tetracycline was noticed in more than 60 per cent of *E. coli* from different sources and resistance to Septran was shown by more than 35 per cent in all genera. Streptomycin and gentamycin reistance was exhibited to a lesser degree by all strains from most sources except in case of *Enterobacter* from foods. NLF from foods showed uniformly high resistance to all drugs including kanamycin and gentamycin. However, none of the NLF from human gut were resistant to those two drugs.

E. coli present in cooked sausages have been found to be resistant to some antibiotics[10]. In a study of *E. coli* with resistance factors and its correlation with diet it was found that the percentage of such *E. coli* were higher in the vegetarian group than in the meat eating group[14]. In the present study more coliforms were detected in salads and vegetable curries and all isolates

TABLE 3. COMPARATIVE RESISTANCE PATTERNS OR ORGANISMS ISOLATED FROM DIFFERENT SOURCES

Resistance to a combination of	Human *E. coli* + NLF	Enteropathogenic *E. coli* serotypes	Water *E. coli*	Foods			
				E. coli	*Klebsiella*	*Enterobacter*	NLF
*A T Se P St Ch	14	0	1	13**	5	5	5
A T Se P Ch	21	0	3	4	4	4	0
A T P St Ch	47	4	1	7	5	7	2
A P Ch	0	5	3	11	2	1	1
A Se P Ch	0	1	2	4	1	5	3
A Se P St Ch	0	1	0	0	0	0	0
A T P Ch	0	3	7	5	6	0	5
A P St Ch	0	0	0	0	0	3	0

*A – Ampicillin, T – Tetracycline, Se – Septran, P – Penicillin, St – Streptomycin, Ch – Chloramphenicol.

**A single *E. coli* strain each from foods and human sources was sensitive to both penicillin, ampicillin and chloramphenicol.

were resistant to some antibiotics. As shorter cooking time and frequent use of raw ingredients is involved in vegetarian preparations, more organisms are likely to escape being subjected to heat. Consequently more live organisms are ingested by vegetarians which could perhaps explain why vegetarians may have more drug resistant *E. coli*. The possibility of ingesting drug resistant *E. coli* from water should also be considered as much of the population drink water straight from the source without further filtering or boiling.

The experimental results show that three of the drug resistance patterns are common to isolates from foods and human sources. Since the human isolates were derived from subjects presumably not under chemotherapy at the time of investigation, it is indicative that at least a section of the population already has antibiotic resistant enteric coliforms. Linton and coworkers[15] have observed that a high percentage of the tetracycline resistant *E. coli* they studied transferred resistance to a standard *E. coli*$^{R-}$ recipient strain regardless of their source of primary isolation. We may expect the possibility of at least some of the coliforms from different sources in the present study to have such potentialities. The genera of Enterobacteriaceae included in our study though forming the normal gut flora are essentially opportunistic. *E. coli* and *Klebsiella* can cause infections under certain circumstances and in different organs. They may be an important source of R-factors and conjugal transfer of resistance to more dangerous pathogens cannot be ruled out. The presence of multiple drug resistant NLF in foods along with other genera is also noteworthy.

The detection of a number of drug resistant coliforms in the present study in humans, foods and water clearly points to an indiscriminate antibiotic usage because resistance and its spread results from selection pressure of antibiotics. Besides, antibiotics may also act[16] indirectly by interfering with the gram positive or gram negative anaerobic flora which may normally suppress R-factor transfer. Recourse to newer antibiotics does not offer any permanent solution as our studies show that already a large number of coliforms are resistant to the relatively new drug septran. Resistant bacteria are transmitted among people and resistance is transmitted

TABLE 4. RESISTANCE OF COLIFORMS TO 12 ANTIBIOTICS (EXPRESSED AS % OF THE TOTAL NUMBERS)

Organisms/source	Amp	Tetra	Septran	Pen	Strep	Chloro	Neo	Erythro	Sulpha	Kana	Genta	Doxy
E. coli (foods)	97.8	62.2	37.8	97.8	40	97.8	46.7	97.8	100	13.3	8.9	77.8
E. coli (water)	100	70.6	35.3	100	11.8	100	82.4	100	100	50	11.8	76.5
E. coli (human gut)	97.3	100	41.3	98.7	78.7	98.7	46.7	98.7	100	17.3	9.8	100
Klebsiella (foods)	100	82.6	53.2	100	43.5	100	69.6	100	100	17.4	8.7	100
Enterobacter (foods)	100	56.0	72.0	100	52.0	100	48.0	100	100	8.0	0	72
NLF (foods)	100	75.0	50	100	37.5	100	43.8	100	100	25.0	12.5	100

among bacteria[17]. One example of an organism resistant to a front line antibiotic like chloramphenicol and causing concern all over the world[18,19] is that of *Salmonella typhi* and *S. typhimurium*. Significance and hazard of faecal contamination of vegetable foods and food stuffs with reference to salmonellosis was shown as early as 1967[20]. The WHO[21] Consultation Meeting on Public Health aspects of antibiotic resistant bacteria in the environment rightly recommends that for surveillance of such bacteria the possible role of food and animal feeds in the dissemination of R^+ bacteria be considered among other disseminants.

The present studies indicate that food and water are vectors of drug resistant bacteria. Drug resistance is rather prevalent in our environment and due precautions must be taken to prevent misuse of antibiotics.

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Direct Assessment of the Toxicity of Organic Insecticide Residues in Sugar Liquid Baits with the Adult Housefly, *Musca domestica nebulo (Fabr.)*

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The toxicity of organic insecticides was assessed by feeding insecticide-sugar baits dissolved in distilled water to house flies *ad libitum* from a test container with a lid. A cotton wick placed in a glass micropippete fixed in the centre of the lid avoided unnecessary contact of other parts of the body during feeding. Flies were held in a cylindrical mesh cage, which provided free access to air and avoided a build up of insectidal vapour, if any, during the operation.

The toxicity of DDT, Malathion, Fenitrothion and Sevin deposits was assessed in terms of mortality rate. Potentiation of toxicity of DDT, Malathion, Fenitrothion and Sevin to house flies in the presence of sucrose and glucose was revealed.

The methods for tests with liquid stomach poisons on house flies *Musca domestica* L., are used either to determine the toxicity of insecticides,[1-3] as a function of a single pure insecticide, or to estimate the quantity of toxic residues to a degree of sensitivity unattainable by chemical methods[4-6].

Current methods for oral administration of insecticides have certain disadvantages. One of these is that insects not only feed on the liquid poisons, but they also remain in contact with the poison-bait. The mortality recorded is the total effect of oral and contact toxicity, although the sensitivity of the oral assay is somewhat less than that of the contact assay[7,8]. Therefore, the adoption of such a combination of trials will definitely cause discrepancy in the results and the ultimate toxicity picture obtained will not be due strictly to oral toxicity.

Existing physical and chemical methods to detect and determine the residues of pesticides only indicate the presence of an active chemical in an experimental sample irrespective of their "toxicity potential". If at all any method is used to determine toxicity potential it suffers from certain disadvantages. One of these is, that the liquid posion-bait has to be regulated in the feeding tube at every 4-6 hr interval[7]. Therefore, to overcome these drawbacks and to study directly the influence of the constituents of the substrate on the toxicity of the pesticidal compound a suitable oral feeding method has been developed by using the housefly (*M. d. nebulo* Fabr.) as the test insect. The results obtained with this method are reported here.

Materials and Methods

In the present study an apparatus was designed to accommodate 25 flies at a time. This apparatus has the same principles as described by Bailey *et al*[9]. who used treated sugar placed in a plastic petridish to feed flies through the gauze on the sugar, while here liquid poison-bait was provided through a cotton wick placed in a micropipette with negligible contact of the flies during feeding. The apparatus under description (Fig. 1) consists of two parts:

(1) A cylindrical testing cage (B) made of 45 gauge mesh 10×15 cm dimension.

(2) A test container with lid (C) consisting of a glass container (E) of 2×2 cm dimension with a lid (F). This lid is provided with a cotton wick (G) placed in a glass micropippete (J) of 0.1×0.1 cm dimension fixed centrally in the lid.

Flies were grouped each consisting of 25 individuals

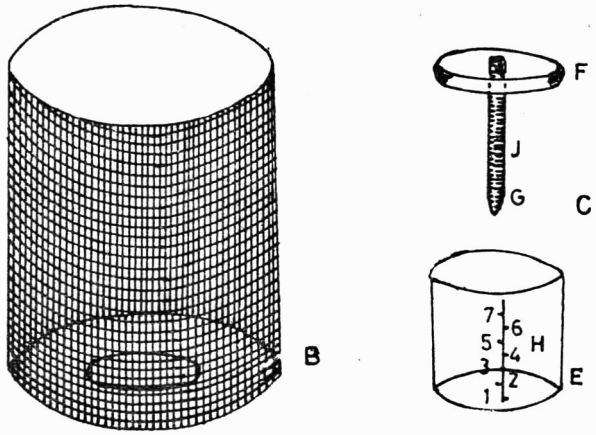

Fig. 1. An apparatus to administer orally insecticide—sugar bait to house-flies.

B: A cylindrical testing cage; C: A test container with lids E: A glass container; F: A lid; G: A cotton wick; J: A glas; micropipette.

TABLE 1. LD_{50} VALUES OBTAINED IN EXPERIMENTS ON ORAL FEEDING OF DDT IN SUGAR AND ACETONE WATER BAITS TO HOUSEFLY FOR 24 HOURS

Substratum	Regression equation	LD^*_{50} μg/ml	S.E. of Log LD_{50}	Relative toxicity
Sucrose	$y = -0.630+3.317\times$	49.89	1.698 ± 0.025	2.83
Distilled water	$y = -0.519+2.566\times$	141.60	2.151 ± 0.037	1.00

*Read from regression line.

and released in metallic testing cages. This holding cage in an inverted position was placed in such a way that it could hold the liquid-bait container in its mouth as shown in Fig. 1. There were four replicates for each treatment. The quantities of liquid-baits consumed by flies *ad libitum* in 24 hr were read from the scale (H) shown on the wall of the container and mortality data were recorded.

This method had the following advantages: (*i*) sufficient space was provided to accommodate 25 flies to feed insecticide-sugar liquid-bait without body contact and (*ii*) vapour toxicity caused by fumes was negated to a large extent.

LD_{50} of DDT, Malathion, Fenitrothion and Sevin with sugar liquid-baits: The susceptible strain of house flies (*M. d. nebulo* Fabr.) was raised at a temperature 28 ± 2°C and R. H. of 65 to 75 per cent. The experiments were carried out under the same conditions. Three to five days old female house flies were selected, belonging to the same progeny in a set of experiments. Known quantities of pp'-DDT (100 per cent pure, supplied by the Pesticide Repository of Pesticides Research Laboratory, Perrine Florida 33157, U.S.A.), Malathion (97 per cent premium grade supplied by Cynamide Industries Ltd., Bombay, India), Fenitrothion (technical grade, supplied by Tata Fison Industries Ltd., Bombay, India) and Sevin (Union Carbide India Ltd., Bombay, India) in acetone were applied to granulated sucrose or glucose powder weighing 5 g, which was then evaporated. DDT, Malathion, Fenitrothion and Sevin were thus tested in the range of 25-180, 9-30, 1-5 and 80-550 μg/g sugar respectively. The insecticide-coated sugar was dissolved in distilled water to make the volume to 25 ml to obtain 20 per cent sugar liquid-bait. For check, parallel trials were carried out only with sugar-acetone-water solution. Mortality data obtained from a plot of the log-dosage and probit kill were subjected to probit analysis,[10] and the LD_{50} values calculated are shown in Table 1.

Generally the rate of consumption of insecticide-sugar liquid-bait per 100 flies was 2.5-3.0 ml in 24 hr. A reduction in the consumption rate from 3 to 1.8 ml

TABLE 2. REPRESENTATIVE DATA COLLECTED AFTER ALLOWING 3 TO 5-DAY OLD FEMALE HOUSE FLIES TO FEED DIRECTLY ON DDT, MALATHION, FENITROTHION AND SEVIN SUGAR AND ACETONE-WATER BAITS FOR 24 HOURS

Insecticide	Substratum	Regression equation	$LD^*_{50}\mu$g/ml	S.E. of Log LD_{50}	Toxicity Unit (T)	% increase decrease in toxicity
DDT	Sucrose	$y = -0.630+3.317\times$	49.89	1.698 ± 0.025	1.65	+64.77
	Glucose	$y = 1.808+1.666\times$	82.41	1.916 ± 0.056	1.42	+41.81
	Distilled water	$y = -0.519+2.566\times$	141.60	2.151 ± 0.037	1.00	—
Malathion	Sucrose	$y = 2.205+2.450\times$	13.80	1.140 ± 0.058	1.76	+75.70
	Glucose	$y = 1.695+1.000\times$	20.18	1.305 ± 0.016	1.64	+64.43
	Distilled water	$y = 10.621+8.896\times$	56.75	1.754 ± 0.009	1.00	—
Fenitrothion	Sucrose	$y = 2.086+8.075\times$	2.291	0.360 ± 0.011	1.64	+63.69
	Glucose	$y = -0.962+2.352\times$	2.170	0.336 ± 0.039	1.66	+65.61
	Distilled water	$y = 0.207+5.916\times$	6.310	0.800 ± 0.017	1.00	—
Sevin	Sucrose	$y = -0.220+1.314\times$	93.97	1.973 ± 0.016	1.69	+69.13
	Glucose	$y = -2.780+1.758\times$	266.70	2.426 ± 0.013	1.12	+12.43
	Distilled	$y = 2.920+0.430\times$	304.70	2.484 ± 0.022	1.00	—

* Read from regression line

+Sign indicates an increase in toxicity.

was observed when flies were provided lethal doses of insecticides in place of sublethal doses.

To study the effect of different sugars on the toxicity of DDT, Malathion, Fenitrothion and Sevin residues further experiments were carried cut. The sugars were thoroughly treated with insecticide-acetone solution in ether as mentioned earlier and the LD_{50} values were estimated are shown in Table 2. Parallel control trials were run for comparison as described earlier. Mortality data were recorded after 24 hr of feeding on insecticide-sugar liquid-bait and each dosage was calculated by subjecting it to probit analysis.

Computation of toxicity unit: LD_{50} values presented (Table 1) were calculated from their respective regression lines. The relative toxicity units for different sugars were calculated by taking LD_{50} value of insecticide-distilled water-bait as 1.00 in the respective trials. On the basis of these values, per cent increase or decrease in the toxicity of DDT, Malathion, Fenitrothion and Sevin was calculated (Table 2) respectively.

Results and Discussion

To determine whether the results could be duplicated with the apparatus, tests were conducted with house flies at 24 hr feeding period. Each test was carried out by providing insecticide-acetone-water to house-flies. Data obtained are shown in (Table 1). From LD_{50} values it is clear that DDT-sugar liquid-bait was more toxic to flies than DDT-water-bait.

The study of the influence of sugar substrates on the toxicity of DDT, Malathion, Fenitrothion and Sevin residues was carried out by feeding the known doses of the insecticide-sugar liquid-bait in comparison to insecticide-water-bait as mentioned earlier. The data obtained for the LD_{50} are shown in (Table 2).

In these experiments, sucrose and glucose have shown the potentiation of toxicity of all the four insecticides to house flies as compared to insecticide-water-bait. The results of these experiments have shown that toxicity unit (T) could be used as a measure of the comparative toxicities of DDT, Malathion, Fenitrothion and Sevin as influenced by the substrate factors. Thus, these data have indicated that the toxicity of a particular insecticide is modified profoundly by the substrate composition.

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

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

The method described is sensitive to as low as 50 ppm of DDT, 14 ppm of Malathion, 2 ppm of Fenitrothion and 94 ppm of Sevin residues in sugars. It is also possible to estimate the residues of the insecticides used from the treated sugars by running parallel control trials with known amounts of pesticides without involving any extraction and clean up steps.

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Factors Contributing to Mashiness of Canned Prawn*

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Mashiness of canned prawn in brine during storage is not enzymatic but physico-chemical in nature. This phenomenon is species specific. Of the commercial important species of prawn *M. dobsoni* is highly resistant while *M. affines* is very susceptible to mashiness. The probable reasons have also been discussed. Icing freezing, icing followed by freezing and thawing show only an apparent improvement in the texture of canned prawn initially over the standard pack but have no significant improvement on prolonged storage. Susceptibility of canned prawn to mashiness was found to be related to the lipid content of connective tissues of prawn. Higher the lipid content of connective tissue, more resistant is the species to mashiness. The erratic behaviour of small size of prawn (*Thilli*) to mashiness has been explained.

Canned prawn is one of the established fishery industries in India and it is entirely export oriented. The export earning through canned prawn is declining gradually from 11.1 per cent in 1970 to 0.6 per cent in 1975. However, on an average India exported 1915 tonnes of canned prawn during the period of eight years from 1966 to 1973.

Indian processors use all varieties of prawn for canning depending upon their availability. However, all the canned prawn under identical conditions of processing and storage do not behave similarly and finally softening of tissue takes place which is known as mashiness or 'sloughening'. Nothing is specifically known about the factors responsible for sloughening. Attempts have therefore been made to find out the factors responsible for mashiness of canned prawn.

Materials and Methods

Marine species of prawns used in the study were obtained from landing places in and around Cochin and backish water species from the adjoining backish water. Material after collection was brought to the laboratory in ice.

Time lag between collection and processing was kept within 24 hr particularly in cases whose storage life with reference to mashiness was judged. Otherwise prawns collected from local processing factories were used during the investigation. Prawns were canned according to the procedure of Chaudhuri and Balachandran[1] in 310×206 cans which were processed at 0.7 kg/sq cm steam pressure for 18 min. The incubated cans (at 37°C) were subsequently analysed at regular intervals.

The study is based particularly on textural changes of canned prawn during incubation. Due to the lack of texture measuring instrument, subjective method using a five point scale was applied. The frozen and subsequently thawed prawn when used as raw material for canning using over-blanching technique (by doubling the blanching time) gives very hard texture, with a score point of 5. Texture of canned prawn when processed under standard conditions keeping the time lag between collection and processing within 24 hr becomes soft but firm and was given 3 as the score point. The texture which becomes soft and disintegrates easily on pressing was given 1 as the score point. The intermediate textural hardness comes in between these points. The texture of the canned prawn was judged by an expert panel, consisting of 5 members drawn from the Institute and the sample having the score point below 2 is considered as unsuitable for consumption with reference to texture. The score points are as follows:

	Score point
Very hard	5
Moderately hard	4
Soft but firm	3
Slightly soft	2
Soft and pasty	1

However, subjective tests were supplemented with some objective tests by estimating the amount of protein and free α-amino acids leached out in filled brine during incubation.

Soluble protein in filled brine was precipitated with 20 per cent trichloroacetic acid (TCA) and the residue was estimated by microkjeldahl method and the filtrate for non-protein nitrogen (NPN) was estimated according to AOAC method[2]. Free α-amino acids were determined by the method of Pope and Stevens[3]. The

*The work was carried out at C.I.F.T., Cochin.

results are expressed as the amount present in whole liquor/100 g of the sample. Volatile sulphides were estimated by the method of Piggot and Stansby[4]. Hydroxyproline as an index of connective tissue in muscle, was estimated according to Wierbicki and Deatherage[5].

Two grams of devitaminised pure casein (E. Merk) was processed separately in 100 ml of sodium chloride solution in concentration of 3, 5, 7, 10 and 15 per cent. The contents were processed like that of prawn and the respective solubilities at different concentrations were studied after incubation at 37°C.

Connective tissue (CT) obtained after alkali extraction method of Lowry *et al*[6]. was dried for 6 hr at 105°C. The dried meat was subsequently extracted with petroleum ether (B.P. 40-60°C) for estimating its lipid content.

The residual dopa oxidase and phenolase activity of canned prawn was studied according to the method of Bailey *et al*[7] and Smith and Stoltz[8] respectively, using 3:4 dihydroxy phenylalanine and 2:6 dichloro phenol indophenol as substrates respectively.

The residual cathepsin activity of canned prawn was studied indirectly by adding 10^{-4}, 10^{-5}M, 10^{-6}M cobalt according to Siebert[9] to the extent of 1 ml/20 g of meat and then the solubility of protein was studied with different incubation periods. In the same way the effect of addition of ethylene diamine tetra acetate (EDTA) as chelating agent and para chloro mercury benzoate (PCMB) as a specific blocking agent of -SH group in the concentration range of 0.1, 0.2, 03 per cent added to the extent of 1 ml processed can, was studied.

Initially prawns were thermally processed under standard conditions in concial flasks using cotton plug. Replacement of cotton plug with sterlized rubber cork was carried out asceptically after the addition of chemicals/inhibitors and was then incubated at 37°C for solubility studies.

Results and Discussion

Mashiness of prawn may be due to (*i*) physical, (*ii*) chemical, and (*iii*) enzymatic factors.

Physical: The behaviour of pure protein under identical conditions of processing and incubation of canned prawn was studied. The results indicate the solubility of casein changes with the concentration of salt used for packing. The solubility is maximum (54 mg per cent) at 3 per cent salt solution which however, decreased with further increase in concentration of salt.

The effect of concentrations of salt used for packing prawn meat (*M.affinis*) on solubility is shown in Fig. 1. The maximum solubility of prawn tissue was observed at 7 per cent salt concentration but with further increase in concentration of salt the solubility gradually decreased.

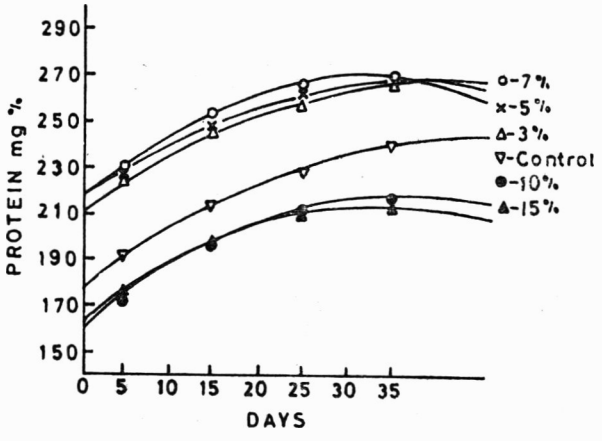

Fig. 1. Solubility of canned prawn meat in salt solution.

It may be concluded that probably connective tissue or similar factors responsible for holding the muscle tissue intact are affected or get disintegrated during incubation thereby destroying their textural integrity and leading to increase in solubility of muscle tissue in filled brine by leaching. This phenomenon is totally absent in pure casein and therefore, its solubility remains unchanged during the incubation period.

Chemical: Degradation of canned muscle tissue takes place during incubation. Biochemical changes leading to progressive release of ammonia, volatile sulphide non-protein nitrogen and free α-amino acid (Table 1) in the system indicate the formation of compounds either through thermal processing or through biochemical degradation which are then leached out partially or completely in filled brine or by both during incubation.

It has been observed that the free α-amino acid content and volatile sulphides of canned prawn system increase with increase in processing time (Table 2)

TABLE 1. PROGRESSIVE RELEASE OF SOME COMPOUNDS FROM CANNED MEAT AND BRINE WITH INCUBATION

Incubation period (days)	Ammonia mg/100 g	Non-protein N (mg/100g)	Free α-amino N (mg/100g)	Volatile sulphides as H_2S (μ g)/100g
0	30.1	482.5	124.8	220.0
15	37.0	—	—	238.5
30	—	556.9	127.4	—
40	45.3	—	—	245.7
60	52.8	602.3	130.2	276.9
100	—	604.4	132.7	—

TABLE 2. RATE OF RELEASE OF COMPOUNDS FROM MEAT AND BRINE WITH CHANGE IN PROCESSING CONDITION

Processing time* (min)	Free ∝-amino N (mg/100g)	Volatile sulphides (as H_2S (μg/100g
15	—	412.5
20	52.7	226.9
30	—	824.9
40	54.8	1100.0
50	58.4	—
60	59.8	—

*Pressure applied in all cases was 10 lb except in case of 15 min when 15 lb pressure was applied.

and temperature of processing. From this it may be concluded that even during thermal processing also same or some chemical changes do take place which may explain the changes during the process of incubation.

Enzymatic: Mashiness of tissue may be explained provided the proteolytic enzymes usually associated with raw meat are active or regenerated even after commercial sterilization. Reddi *et al*[10], Guyer and Holmquist[11] and Farkas *et al*[12]. showed that under some processing condition the amount of regenerated enzyme can cause quality deterioration in the processed products and that the degree of regeneration depends on the inactivating process and the condition of incubation.

Tests were performed to detect the residual Dopase and phenolase activities of commercially canned prawn but no activity could be detected when tested according to the methods of Bailey *et al*[7]. and Smith and Stoltz[8] respectively. The fresh prawn *M. affinis*, *P. indicus* and *M. dobsoni* showed relative Dopase activity in the range 650-700, 370-430 and 70-90 respectively.

Another batch of experiments were performed where the residual enzyme activity was measured indirectly through protein solubility and the results were compared with control where inhibitors, chelating agents (EDTA), etc were not added. PCMB was added to the processed can for blocking the active -SH groups of enzyme and cobalt salts were added as inhibitors of cathepsin[3]. The addition of chemicals did not either retard mashiness or prevent the relative degree of leaching in filled brine and the results are comparable to control samples (Table 3).

Experiment was also performed by increasing the processing temperature in the range of 115.2° to 122°C by keeping the processing time constant to kill the residual enzyme. The results also do not show any indication (Table 3) of extension of storage life of canned prawn over the control indicating mashiness to be non-enzymatic in nature.

Species specificity: Mashiness of prawn may be a characteristic phenomenon to a particular species. Experimental results indicate that it is common to all species but their relative resistance to mashiness are different and in a generalised form may be represented as follows:

Species	Local name	Days needed to develop mashiness at 37°C (Score point <2)
M. affinis	Kazhanthan	20–30
P. stylifera	Karikadi	25–40
P. indicus	Naran	20–40
M. dobsoni	Poovalan	40–60

The characteristic behaviour of different species of prawn to mashiness may probably be explained by the relative percentage of CT (Table 4) present in the muscle. Factors governing cohesiveness of muscle tissue are not fully understood but probably CT content of muscle tissue plays prominent role according to Hughes[13].

TABLE 3. EFFECT OF DIFFERENT ENZYME CONTROLLING AGENTS ON SOLUBILITY

Incubation period (days)	Std. pack	EDTA* added in filled brine			PCMB* added in filled brine			Cobalt salt* added in filled brine			*Processing temp* (°C)			
		0.1%	0.2%	0.3%	0.1%	0.2%	0.3%	10^{-4}M	10^{-5}M	10^{-6}M	115.2	117.6	119.9	122
0	163.1	160.5	166.7	160.2	165.5	164.5	160.2	168.2	167.3	169.5	115.2	125.1	175.2	275.2
5	172.2	170.3	175.1	174.5	170.2	169.9	170.5	172.5	175.4	175.8	125.3	130.2	205.4	301.5
10	178.2	175.2	180.5	179.8	174.5	178.5	175.5	176.5	174.6	177.3	140.4	145.5	225.3	320.4
20	192.0	190.7	192.5	195.3	190.7	195.2	194.5	195.2	198.5	199.5	149.5	155.3	220.4	330.3
25	197.5	195.3	196.4	198.2	195.3	196.3	196.3	198.3	196.5	198.7	165.4	175.2	250.2	345.4

*Species used *M. dobsoni*

**Species used *P. indicus*

TABLE 4. EFFECT OF SIZE, SOURCE AND SPECIES ON SOLUBILITY OF PROTEIN

Incubation period (days)	*P. indicus* obtained from river				*P. indicus* from Sea		*M. affinis* of size group			
	Tiny		Medium		Medium		Large		Medium	
	Control	Std. pack	Control	Std. pack	Control	Std. pack	Control	Std. pack	Control	Std. pack
0	100.4	125.2	135.5	156.2	82.2	136.2	143.5	168.2	150.2	171.5
20	105.2	165.2	150.2	180.5	140.1	190.5	160.8	177.2	165.3	213.5
35	117.3	195.5	167.3	202.7	195.4	210.2	198.5	206.3	206.1	241.5
56	227.5	205.1	248.2	230.5	241.8	248.5	269.3	276.5	271.9	277.9

Count: Tiny = No. 64-102/100 g
Medium = No. 23-36/100 g
Large = No. 14-22/100 g

Analysis of canned prawn meat before and after incubation indicates 30 to 50 per cent degradation of CT as evident from its hydroxy prolein content during an incubation period of 1-2 months. This in turn may affect the cohesiveness of muscle tissue leading to progressive increase in its solubility with storage.

Source, Size and Maturity: Same species of prawn obtained from different sources also behave differently under identical conditions of processing and storage. For example, *P. indicus* obtained from brakish water is more prone to mashiness than that obtained from sea (Table 4) indicating that the species differ either in biochemical composition or some other micro constituents which control the specific character of the species to mashiness.

P. indicus	Days needed to develop mashines at 37°C (Score point <2)
Sea *Naran*	30–40
River *Naran*	20–25

It is known that *P. indicus* during its life cycle migrates from estuary to deep sea and obviously the species generally obtained from sea is more mature than that obtained from the river. So by the relative proportion of CT content of the tissue it may be possible to explain the specific phenomenon of the species. However, study on *M. dobsoni* reveals that its smaller size group (*Thilli*) is comparatively more resistant than its corresponding bigger size groups (*Poovalan*) inspite of its low CT content. So it may be concluded that CT content of the tissue is not the only factor by which mashiness may be explained.

Surface area: M. dobsoni (*Thilli*) and *P. indicus* (River *naran*) having comparatively larger surface area per unit body weight compared to the corresponding bigger size groups of *Poovalan* and Sea *naran*, are comparatively resistant and susceptible respectively to mashiness under identical conditions of processing and incubation. However, when the bigger size of prawn is made smaller by cutting the individual prawn into two or three pieces (thereby increasing surface area) the cut pieces show greater susceptibility to mashiness (Table 5). Artificial reduction in size may affect the textural integrity of muscle either through disturbance of CT or disturbing the arrangement of muscle fibre pattern thereby the physcial action of leaching becomes more effective. However, greater surface area does not necessarily increase the susceptability of the species to mashiness.

Processing condition and raw material quality: The texture of canned prawn prepared either from iced/iced followed by freezing and thawing or from fresh prawn itself can be made harder by overblanching (by doubling the standard blanching time). The initial harder textures of the former canned meat samples do not however, show an appreciable extension of storage life over both standard blanched or overblanched control samples. Initial protein solubilities of these former samples after thermal processing are obviously less (119.2 mg per cent +107.2 mg per cent) compared to the overblanched controlled samples (143.2 mg per cent) prepared from fresh prawn. TCA precipitable protein in filled brine

TABLE 5. EFFECT OF INCREASED SURFACE AREA ON MASHINESS AS INDICATED BY SOLUBILITY

Incubation period (days)	Whole prawn		Cut into 3 pieces	
	Control	Std. pack	Control	Std. pack
0	485.5	460.5	576.0	602.1
15	565.4	648.3	624.2	676.2
30	585.9	725.1	712.5	724.3
45	617.5	735.5	750.3	852.1

Species: *P. indicus* Size: Jumbo

TABLE 6. LIPID CONTENT OF CONNECTIVE TISSUE OF PRAWN IN DIFFERENT SPECIES

Species	No. of Observations	Length P+D form (cm)	Fat in connective tissue (dry wt. basis) (%)	Connective tissue content (dry wt. basis) (%)
1. *M. dobsoni*	12	3–6.20	2–77–9–70	2.84–4.05
(*b*) *Thilli*	15	1.5–2.5	3.59–10.62	2.74–3.85
2. *M. affinis*	15	6.8–9.0	2.08–4.23	3.24–4.09
3. *P. indicus*	15	7.5–10.8	2.49–4.06	2.44–4.55
(*b*) River *Naran*	12	4.8–8.0	2.02–2.86	1.72–2.29
4. *P. carinatus* (*Kara*)	10	7.5–16.5	2. 5–7.94	3. 0–5.02
5. *P. stylifera*	15	4.0–5.5	2.69–7.56	2.56–4.28
6. *M. monoceros* (Coodan)	12	5.0–7.0	2.01–4.52	2.25–3.5

content of the incubated samples prepared from iced prawn is comparatively less (253.8 mg per cent) compared to iced and then subsequently frozen and thawed prawn (249.7 mg per cent) while controlled cans prepared from over blanched and standard blanched fresh prawn show a solubility of 315 mg per cent and 297.5 mg per cent respectively after an incubation period of 90 days.

Lipid content of CT: Lipid content of CT was found to be inversely related to the mashiness i.e. higher the percentage of lipid in CT, less susceptible is the species to mashiness. The lipid contents of CT of susceptible species (*M. affinis*, *P. indicus*) of prawn are relatively less compared to the resistant species (*M. dobsoni*). Moreover, the erratic behaviour of the smaller *Thilli* compared to bigger *M. dobsoni* (*Poovalan*) to mashiness can also be explained by the relative higher percentage of lipid in its CT content (Table 6). Similar is the behaviour of *P. indicus* obtained from different sources. However, it has been shown by Gopakumar[14] that the nature of lipid content and its relative distribution in the muscle tissue of commercially important species of prawn are different which may also probably be responsible for their specific character to mashiness.

Saltless packing medium: Though the rate of solubility of protein was found to be less in samples which were blanched in water and subsequently packed in pure water (control), compared to the standard blanched prawn packed in brine solution, but mashiness was found to be quicker in the former samples.

Control of mashiness: Various hardening agents like $CaCl_2$, $MgCl_2$ and alum were tried either alone or in combinations upto 0.3 per cent in the filled brine. Prior dipping in 10 per cent brine solution containing the chemicals for 30 min followed by blanching and subsequently packing without the addition of chemicals or *vice versa*, maintaining their level within 0.3 per cent (PFA Act 1954)[15] were tried but none of them were found effective in controlling mashiness.

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RESEARCH NOTES

POLYPHENOLOXIDASE OF *CAPSICUM FRUTESCENS* VAR. GROSSA SENDT

The properties of a polyphenoloxidase from green chillies (*Capsicum frutescens*) have been studied. The enzyme has a pH optimum of 7. The enzyme is comparatively heat stable at 60°C. It is inhibited by cysteine, sodium diethyldithiocarbamate and sodium metabisulphite. Pyrogallol acts as a competitive inhibitor in the presence of which the K_m for catechol, is increased from 3.65 to 16.67 $_m$M.

Green chillies (*Capsicum frutescens*), like many other fruits and vegetables, discolour when bruised or cut presumably due to the action of polyphenoloxidase (PPO). The latter are known to act on phenolic constituents present in these tissues and convert them to brown pigments known as 'Melanins'[1]. Except for a single report on the enzymic browning in seeds of sweet pepper during cold storage, there are no other reports with respect to chillies on this aspect[2]. The present note deals with the PPO activity of green chillies.

The enzyme was extracted from the pulp of 100 g green chillies (*Capsicum frutescens* var. *grossa* Sendt—Simla variety) by homogenizing in a waring blender with chilled phosphate buffer (5 mM, pH 7.0) for 3 min. The supernatant obtained after centrifuging at 7,500 rpm for 30 min was used as the source of enzyme in various experiments. All these operations were carried out at 4-5°C.

Enzyme assay was carried out at 25°C, an assay system containing 2 ml of 18.2 mM catechol, 2.5 ml of phosphate buffer (5 mM, pH 7.0) and 0.5 ml of enzyme solution. Exactly 2 min after mixing, the colour developed was measured in a Klett colorimeter with a 420 filter. One enzyme unit was defined as the amount of enzyme bringing about a change of ten Klett readings under the conditions of assay. Protein content was estimated by the method of Lowry[3].

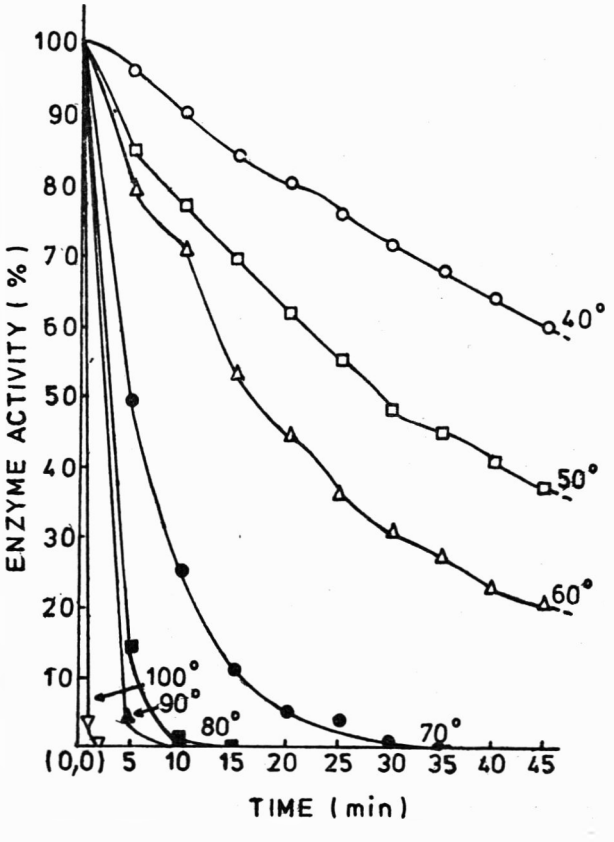
The crude enzyme from green chillies showed a pH optimum of 7.0, temperature 37°C and a substrate concentration of 36.4 mM. A single pH optimum is observed with the chilli enzyme as in the case of PPO from several other fruits[4]. The mushroom enzyme is reported to exhibit two pH optima[5].

PPO from other sources have been known to exhibit broad substrate specificity[6]. In the present case also, maximum enzyme activity of 4.3 units was obtained with D-catechin whereas with catechol, L-Dopa, pyrogallol the activities obtained were 1.0, 0.8 and 0.6 respectively. Monohydroxy and para or meta-dihydroxy aromatic phenolic compounds however, did not serve as substrate for the enzyme.

Though dipping of fruits and vegetables in salt solution prior to canning is practised for minimising browning[4], in the present case sodium chloride at 85 mM concentration could cause only 18 per cent inhibition. With other known inhibitors, viz. cysteine, sodium metabisulphite and sodium diethyl dithio carbamate, the inhibition obtained was 65 per cent (1 mM), 36 per cent (0.1 mM) and 45 per cent (0.1 mM) respectively.

Heat inactivation of PPO in chillies was not brought about at temperatures 40-60°C even after 45 min (Fig. 1). However, 90 per cent inhibition was observed at 90°C and 100°C. In this regard, it appears to be more heat resistant than the PPO of strawberry, black currant, sour cherry and prune which get inactivated within 2.5-3 min at 70-80°C[7].

The chilli enzyme was found to be comparitively inhibited by pyrogallol. The Line-weaver Burk plots with catechol as substrate in presence of pyrogallol (7.94 mM) indicated a change from 3.60 to 16.65 mM indicating that the substrates are apparently being acted upon by the same enzyme and therefore competing also for the same active site.



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REMOVAL OF CARBARYL RESIDUES FROM ONION

The application of 0.1 and 0.2 per cent carbaryl suspension at the rate of 450 l/ha on onion resulted in the deposits of 45.3 and 119.7 ppm which dissipated below the tolerance limit of 10 ppm in 2 and 3 weeks respectively. Washing the treated leaves for 1-5 min removed the residues below the tolerance limit. The amount of carbaryl residues in onion bulbs was also below the detectable level.

The onion bulb and leaves are eaten raw as well as cooked vegetable. It is severely attacked by thrips which lower the yield upto 50 per cent, if not controlled at proper time[1]. For the control of onion thrips, carbaryl was found as one of the most effective insecticides under agro-climatic conditions of Udaipur, which persisted for 35 days on onion leaves. In view of this, it was thought desirable to investigate the way by which the residue can be lowered down so that by simple processing method the onion and onion leaves can be consumed without health hazards.

The treatments of 0.1 and 0.2 per cent carbaryl suspension @ 450 l/ha were done on 70 days transplanted onion crop. Each treatment was replicated thrice. The samples of onion bulbs and leaves were collected at weekly intervals upto one month. The samples were also washed under tap water for 1, 3 and 5 min.

A sample of chopped onion leaves/bulbs (100 g) was extracted with 300 ml of distilled methylene chloride by blending in a 1 litre Bajaj senior mixer for 2 min. The extract was filtered under vacuum through a Whatman filter paper no. 1 over which a thin layer of Hyflo-supercel and anhydrous sodium sulphate was placed. A slight modification which involved (*i*) increasing the contact time between coagulating solution and plant extract from 10 to 20 min and (*ii*) filtering the supernatant coagulated extract through Whatman filter paper no. 42 was done in the method of Benson and Finocchiaro[2] used for the determination of carbaryl residues (Gangwar[3]). It was possible to detect as low as 0.013 μ g/g of the carbaryl with this method.

The application of 0.1 per cent carbaryl suspension resulted in the deposits of 45.3 ppm on onion leaves which dissipated to 18.7, 5.4 and 2.1 ppm in 1,2 and 3

TABLE 1. EFFECT OF WASHING ON THE REMOVAL OF RESIDUES FROM CARRARYL TREATED ONION LEAVES

Weeks after treatment	0.1 % carbaryl				0.2 % carbaryl			
	Av. residue before washing (ppm)	Av. residue (ppm) after washing			Av. residue before washing (ppm)	Av. residue (ppm) after washing		
		1 min	3 min	5 min		1 min	3 min	5 min
0	45.3	5.2 (88.5)	1.1 (97.6)	BDL (100.0)	119.8	12.5 (89.6)	4.3 (96.4)	0.8 (99.4)
1	18.7	4.5 (75.4)	0.6 (96.6)	0.3 (98.3)	56.9	7.1 (87.5)	1.4 (97.6)	0.3 (100.0)
2	5.4	3.1 (42.6)	0.1 (96.1)	0.2 (96.3)	15.7	4.5 (71.0)	0.7 (95.4)	0.1 (99.3)
3	2.1	BDL (100.0)	BDL (100.0)	BDL (1000.0)	6.5	1.0 (85.4)	BDL (100.0)	BDL (100.0)
4	BDL	—	—	—	1.1	BDL (100.0)	—	—

BDL: Below Detectable Level Figures in parenthesis represent percentage reduction.

weeks of treatment (Table 1). The removal of deposit by simple washing for 1,3 and 5 min was to the extent of 88.5, 97.6 and 100.0 per cent respectively. The residues of 18.7 ppm obtained after a week on leaves removed by 75.4, 96.6 and 98.3 per cent due to 1,3 and 5 min washing. Similar trend of removal of carbaryl residues from leaves was also noted at other time interval. After a lapse of time, due to absorption, some quantity of residues which might have penetrated, could not be removed even by 5 min washing at 2 weeks interval.

In case of 0.2 per cent spray, the deposits of 119.7 ppm of carbaryl could be washed out by 89.6 to 99.4 per cent. After a week the residues to the same extent was removed by 1-5 min washing. However, after 2 weeks one minute's washing could remove the residue by 71.0 per cent while 3 and 5 min washing gave the reduction to more or less same extent as described above, In this case washing under tap water also had significant effect on the removal of carbaryl deposit/residue from onion leaves. Similar type of observations were also recorded by Singh *et al*[4]. who reported that carbaryl was not a systemic insecticide as it could not penetrate beyond the bark of sponge gourd. The washing removed the residues by 77.7 per cent from treated bhindi fruits[5] and 66.1 to 69.5 per cent from vegetables[6]. The washing of treated onion removed the residues below the prescribed tolerance limit of 10 ppm at each time interval. These results are in agreement with the findings of Dewan *et al*[7]. who reported that washing of leaves and head of cauliflower brought the residues well below the tolerance limit. The residue detected in onion bulbs was below the detectable level at each time interval which indicates the non-systemic activity in onion.

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BOOK REVIEWS

Textured Protein Products by Marcia H. Gutcho, Noyes Data Corporation, Park Ridge, New Jersey, USA, 1977, pp. 355. Price 39.

This book covers the US Patents literature since early 1960's in the area of textured protein products. The author has compiled from the various patents, details of the texturizing processes and the factors, chemical and technical, which affect the profile of the products.

The material has been divided into 6 sections (1) spun protein fibres; (2) protein fibres without spinning; (3) expanded textured proteins; (4) protein texturizing techniques; (5) Preparation of simulated meat products; (6) Other textured Food Products; (7) Simulated Flavouring agents for meat and meat based products. The book includes (*a*) Company index; (*b*) inventor index and (*c*) U.S. Patent number index.

The process of spinning proteins into fibres for food purposes developed originally from the technique used in the preparation of artificial textile fibres and even now resembles it in many ways. Bayer was the first to use this approach for preparing textured proteins. The spun filaments are bound together with edible binders, compressed into tows and cut into suitable lengths. Apparently this technique needs sophisticated machinery.

Protein fibres can also be obtained without spinning. The protein slurry is heated by conducting it through a heat exchanger under pressure. Afterwards it is either cooled or pumped into a collecting zone when texturization occurs. The exact mechanism of texturization is not known. Perhaps elongated multimolecular protein polymers are formed. Another simple method of texturizing proteins is extrusion cooking under heat and pressure. Although fibres are not formed, fibrous particulates with good mouth feel and chewiness are obtained. Another simple technique of texturizing prteins is the chewy gel approach. In this method protein slurry of optimum concentration is heated at appropriate pH and temperature when it sets into a gel. It is then dried and chopped.

For acceptability the textured proteins are flavoured with meat-like flavours. Patents covering the production of meat flavour, poultry flavour and smoke flavour are described. There is also an account of various simulated meat products.

The book describes in direct and clear style the various patents for texturization and the equipment used. However, the book does not contain any description of methods for the preparation of traditional textured proteins such as Tempeh.

In Western countries where meat is consumed on a large scale, simulated meat products based on textured proteins can be of commercial and economic interest. It is estimated that in the USA by 1985 meat analogues would form about 10 per cent all the "meats" consumed in the country. The scope for such products in developing countries appears to be limited. In this context description of methods for the preparation of products such as Tempeh would have been useful. The book should be of interest to technologists engaged in the area of vegetable proteins and foods based on them.

G. RAMANATHAM
M. S. NARASINGA RAO
CFTRI, MYSORE

Journal of Environmental Science & Health, Part C, Environmental Health Sciences, Vol. C 13, No. 1, 1978. Edited by Robert J. Rubin, Marcel Dekker, Inc., P.O. Box 11305, Church Street Station, New York 10249. Annual subscription $ 49.40 per year.

This journal publishes papers on biological effects of both natural and synthetic chemicals (xenobiotics) consumed from the environment. Emphasis is laid on toxicology, carcinogenesis, mutagenesis and teratogenesis and the scope of publications will range from whole animals to microorganisms at cellular and subcellular levels.

The first issue has detailed papers on hexachlorophene, sulphuric acid, endosulfan, methyl mercury poisoning and haemolytic activity of peroxidised microsomes from different corners of the globe.

The editor, Dr. R. J. Rubin is assisted by a strong editorial board and promises a ten week period for appearance of a publication by the photo offset method following final editorial approval. Contributions to this journal are published free of charge.

I recommend this quarterly journal for institutions and laboratories wherein physiological, biochemical and pathological studies are undertaken with various types of pesticides and other xenobiotics.

R. RADHAKRISHNAMURTY
CFTRI, MYSORE

Rice Processing: a Check List of Commercially Available Machinery, edited by Mr. P. A. Clarke, Tropical Products Institute 56/62 Gray's Inn Road, London, Ministry of Overseas Development, January 1978, pp. 21 Price $ 0.60.

The report gives the list of manufacturers and their addresses in U.K. and other overseas countries including U.S.A., India, Japan, Philippines, Italy, Switzerland, Federal Republic of Germany and Spain dealing in all aspects of rice processing viz. Threshing and winnowing, Drying, Cleaning, Parboiling, Hulling, Whitening, Grading, Finishing and Integrated mills. However, the list does not include names of some of the leading manufacturers in Japan specialised in rice milling, for example, Yamamoto Manufacturing company Ltd., Tendo-ko, Tendo-Shi, Yamagate-Ken 994, Japan is the most reputed manufacturer of paddy storage cum driers. Likewise Fuji Noki K. K. Kawasakicity 210 and Sanriku Koeki K. K., Bunkyo-ku, Tokyo-113, Japan are famous for being the pioneers in manufacturing centrifugal paddy shellers. M/s Binny Ltd. India is recently manufacturing integrated mills of 500 Kg. per hour capacity besides other products mentioned in the list.

The information given in the report is fairly exhaustive and useful for the R & D and industry personnel. The report may be a worthwhile addition to libraries.

PRABIR KUMAR CHANDRA
CFTRI, MYSORE

ASSOCIATION NEWS

Proceedings of the Annual General Body Meeting held on June 25, 1978 at C.F.T.R.I., Mysore.

The Annual General Body Meeting (AGBM) of the Association was held on June 25, 1978 at the Central Food Technological Research Institute, Mysore. Shri C. P. Natarajan, President of the Association presided over the meeting. Welcoming the members to the AGBM the President said that the Association has got now 1600 active members in the list. The office bearers during the course of the year have striven hard to achieve its objectives by arranging group discussions, workshops, seminars and symposia and by publishing the scientific Journal.

The minutes of the last AGBM was presented by Shri A. M. Nanjundaswamy, Honorary Executive Secretary, and was unanimously adopted. Then the Secretary presented the report for the year 1977–78, which was also unanimously adopted. This was followed by the presentation of reports by the Secretaries of various zones and chapters of the Association.

The Treasurer Dr. Richard Joseph presented the audited statement of accounts for the year 1977 and the budget proposal for 1978. During the discussion, suggestions were made to put the surplus funds as endowment fund and to earmark some amount for the building fund. The various zones and chapters, it was pointed out, should henceforth get their accounts audited and present the same in AGBM. The audited report was adopted and the budget proposal was approved. Shri A. K. Krishnamurthy was approved as the auditor for 1978 also.

Prof. V. Subrahmanyan Industrial Achievement Award for the year 1977 was presented to Dr. G. S. Siddappa, Food Technologist and Chief production Manager, Coorg Fruit Products Ltd, Gonikoppal, S. Kodagu. The award consists of a Plaque and a cash amount of Rs, 1,000/-.

The *Gardners Award*, for the best Research Paper published in Journal of Food Science and Technology, during 1976, went to G. P. Kalle, S. Y. Deshpande, and B. Z. Lashkari, for their paper entitled "Fermentative Production of Cheese-like Flavour Concentrate by *Candida Lipolytica*".

The *Suman Food Consultants Travel Award*, for the year 1977 was presented to Shri Ashok Kumar Jain, M.Sc. Student, G. B. Pant University of Agriculture and Technology, Pantnagar.

Following is the list of Office-bearers of the Association for the year 1978, as per the results of the election conducted. The President of the Association announced the names of these office-bearers:

President

Dr. B. P. Baliga

President-Elect

Mr. Daya Nand

Vice-Presidents

Dr. P. B. Rama Rao (Headquarters)
Dr. A. G. Mathew (Southern Zone)
Dr. J. S. Pruthi (Northern Zone)
Mr. B. N. Srimani (Eastern Zone)
Dr. G. B. Nadkarni (Western Zone)

Hon. Exec. Secretary

Dr. J. V. Prabhakar (Headquarters)

Hon. Joint Secretary

Mr. J. D. Patel (Headquarters)

Hon. Treasurer

Mr. A. Ramesh (Headquarters)

Councillors

Nominations from all zones/chapters were not received and hence it was agreed to circulate after the receipt of all nominations.

These were approved by the General Body.

The President in his concluding remarks said that, there was no opportunity till now to for a get together of all scientists and technologists from different disciplines of food science and technology, and it was therefore felt that the time has come to provide such an opportunity by arranging a Convention of Food Scientists and Technologists. So as a new approach, this year, the Association was able to organise the First Indian Convention of Food Scientists and Technologists in a successful way. He hoped the Association would be able to organise such conventions once in two or three years in future. While thanking the members of the Association for their help and cooperation during the previous year, he requested them to extend similar help and cooperation to the new committee and also wished all success to the incoming Executive Committee.

Some other suggestions made during the course of AGBM included, arranging of special lectures during AGBM, providing funds for the Silver Jubilee Year,

circulation of bio-data of candidates contesting for various offices of the Association.

Dr. B. P. Baliga, President elect for 1977, was inducted as President for 1978 by the outgoing President Shri C. P. Natarajan. The President thanked the members for electing him to that August Post. He also spelt out the programme for 1978 and requested utmost cooperation from all members.

The meeting ended with a Vote of Thanks by the Hon. Joint. Secretary, Dr. J. V. Prabhakar, to all members and specially the E. C. members and the Secretariat Staff for the help and cooperation.

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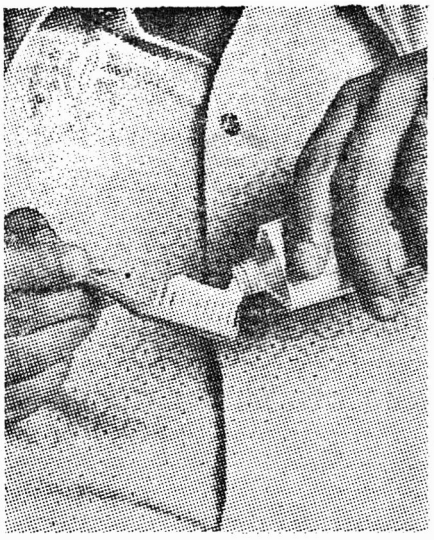

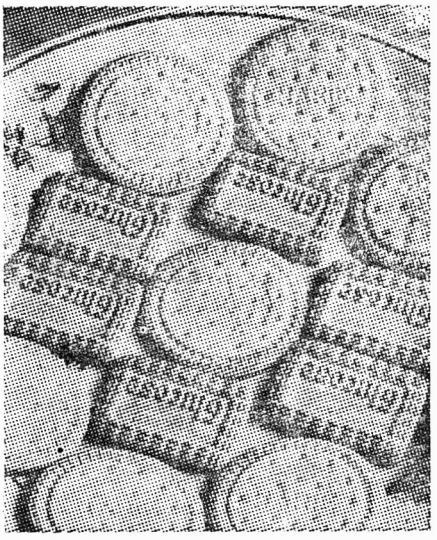

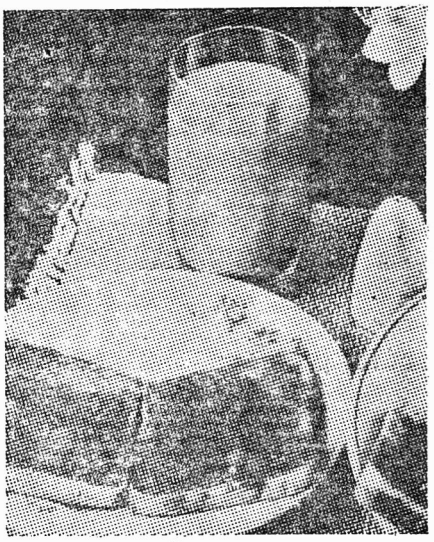

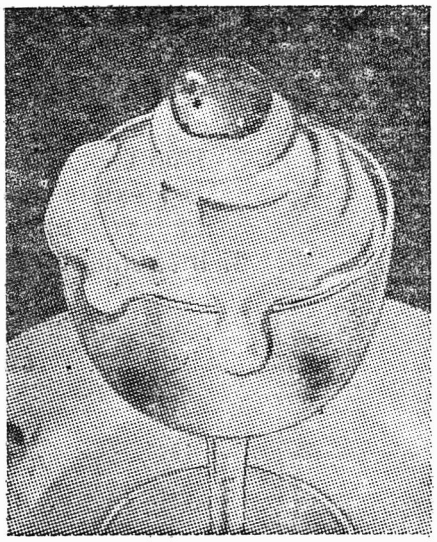

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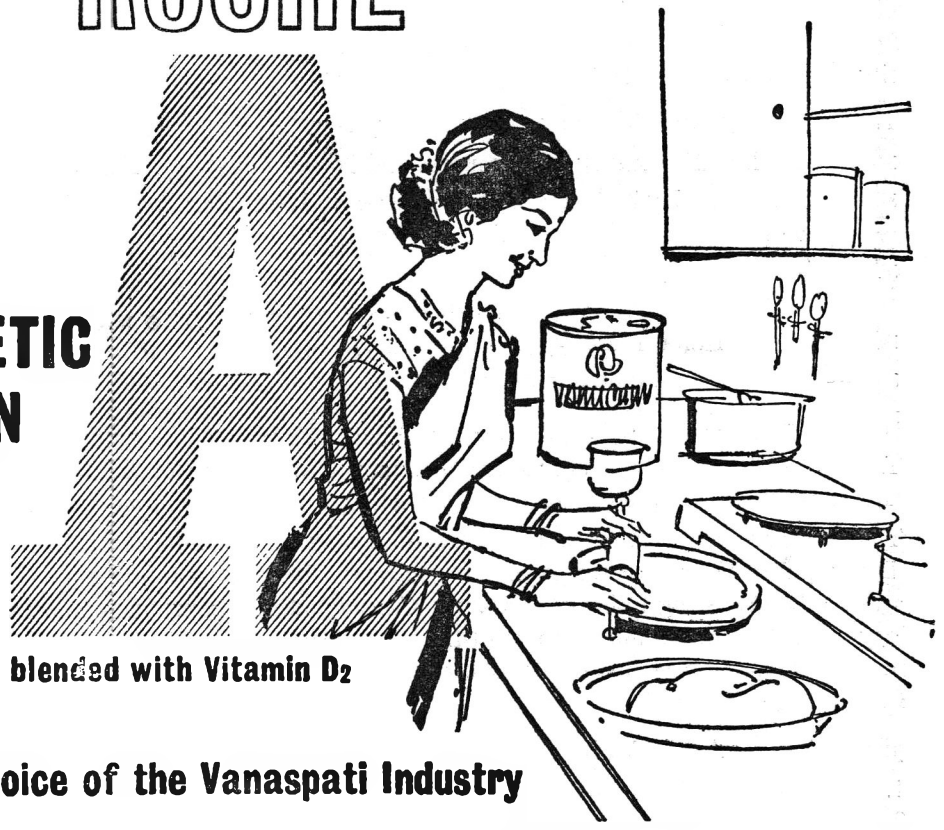

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Fats and Oils in relation to Food Products and their preparations

held at CFTRI, Mysore–13, 3–4 June 1976

(Demy quarto, Paper, back)

Price : **India** *Rs.* **25/–** ; **Abroad :** *Seamail* **$ 8/–** *Surfacemail* **$ 10/–**

This two-day symposium on the uses of fats and oils in foods was organised jointly by the Association of Food Scientists and Technologists (India), the Oil Technologists' Association of India (SRB), and the Central Food Technological Research Institute, Mysore. It belongs among the series of quasi-annual symposia organised on an all-India basis at the C.F.T.R.I., since the last ten years or so. Most of the leading research workers in the field of oils and fats have participated in it. They come from leading National Laboratories, as well as from University Departments and Industries.

The Proceedings consist mainly of 42 papers presented in Six sessions; viz.,

SESSION I—Raw Material Survey of Resources and Newer Sources of Fats & Oils .....(6 papers)

SESSION II—Processing, Hydrogenation, Emulsification, Inter-esterification, Refining and Modification of Fats and Oils .....(7 papers)

SESSION III—Fat-based Food Products-Indian Confectionery, Deep-fat fried products, Margarine, Pickles, Salad cream, Mayonnaise, Butter, Cheese and Ghee .....(8 papers)

SESSION IV—Nutrition and Toxicity .....(7 papers)

SESSION Z—Autoxidation, Antioxidants and Storage characteristics of fats and oils .....(8 papers)

SESSION VI—Chemistry, Analytical Techniques, Adulteration and Quality control .....(6 papers)

The topics covered in the special lectures are: "Mechanism of Blood-Cholesterol lowering effects of unsaturated fatty acids" and "Producing and Marketing a low-cost, volume food".

Thus a very wide range of subjects have been covered. Though comprehensiveness cannot be expected from a Demy-quarto Volume of 155 pages, the reader will find a lot of information that would help to modernize the fats and oils processing Industry in India. Considered as a compact report on the state of oils and fats technology in India at the commencement of the last quarter of the twentieth century, this publication must be considered as indispensable for all scientists and technologists, as well as institutions that work in the field of edible fats and oils.

Other publications of A.F.S.T. (I)

1. Utilisation of Groundnut and other Oilseeds for Edible Purposes (Out of print).
2. Alcoholic Beverage Industry in India (Out of print)
3. Development and Prospects of Spice Industry in India: (Price: India Rs. 10/-, Abroad: 5 $ by Surfacemail and 8 $ by Airmail).
4. Fish Processing Industry in India: (Price: India Rs. 10/-, Abroad: 5 $ by Surfacemail and 8 $ by Airmail).
5. Souvenir of Indian Convention of Food Scientists and Technologists: (Price: India Rs. 10/-, Abroad: 5 $ by Surfacemail and 8 $ by Airmail).
6. Reviews in Food Technology—Vol. 1 to 4—published (Between 1958-62): Price—On request.

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY (Bi-monthly), now running in the 15th year of publication.

Subscription (for Calender Year): India: Rs. 80/-, Abroad: 20 $ by Surfacemail and 28 $ by Airmail.

(a few back volumes are available).

Mail enquiries/orders to The Honorary Secretary, Association of Food Scientists and Technologists (India), C.F.T.R.I. Campus, Mysore-575 013 (India).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Vol. 15 No. 6 **Contents of forthcoming issue** **Nov.-Dec. 1978**

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