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# ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS

(INDIA)

The Association is a professional and educational organization  
of Food Scientists and Technologists

Affiliated to the Institute of Food Technologists, USA

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1. To stimulate research on various aspects of Food Science and Technology.
  2. To provide a forum for the exchange, discussion and dissemination of current developments in the field of Food Science and Technology.
  3. To promote the profession of Food Science and Technology.
- The ultimate object is to serve humanity through better food.

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2. Arranging lectures and seminars for the benefit of members.
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## EDITORIAL

The editorship of the Journal of Food Science and Technology (JFST) has been entrusted to me by the Central Executive Committee (CEC) of the Association of Food Scientists and Technologists (India), Mysore with effect from July 1, 1992. My duties as editor, however, began from late 1991 to process the manuscripts for the issues commencing from No. 4 of the Volume 29.

I am extremely happy to place the first issue in your hands. You may notice the clarity and brevity in each published paper/note. On this occasion, I wish to make the following few points:

1. My illustrious predecessors have nurtured and brought up the journal to the present stature. My endeavour will be to strive hard and further improve the quality of the journal. The areas of Food Science and Food Technology are multi-disciplinary and adequate justice to manuscripts is possible only with the involvement of appropriate experts. Consequently, the editorial board is expanded to cover almost all the areas. Further, I have also been able to enlist the cooperation of a number of excellent referees, who can give authoritative comments on the manuscripts and valuable suggestions for improvements. By depending more on prompt referees, it has become possible to reduce the processing time and to take decisions on pending papers. I look forward to the cooperation from all to further improve and make JFST more or less a rapid publication.
2. To begin with, a section has been introduced under the head "Rapid Communications" in order to give importance to impact-making, innovative, newer research findings (Please refer "Instructions to Authors" appearing in this issue). Such papers will be published within 5 months from the date of receipt of manuscript. I do hope that this will serve the interest of researchers and attract quality publications.
3. A few changes are made in the format, while "The Instructions to Authors" is made explicit and definitive. Further changes are contemplated to make cover page more attractive from January/February 1993 issue.
4. The editor and editorial board alone cannot improve the quality of Journal; but we are willing to strive hard. The efforts from the authors, reviewers and all those concerned with JFST and AFST can go a long way to improve the situation. In this regard, I wholeheartedly invite the national and international scientists and food technologists not only to contribute quality papers to this journal but also to make every effort to either individually subscribe and to recommend the journal to their institutions. A specimen copy of the journal is available on request.
5. The cost of publication of the journal is becoming expensive year by year and it has become difficult to manage it with the existing resources. One easiest way available was to cut down the number of pages; but it is against the very vital objective of the publication of the JFST, i.e. to disseminate the food science technology and nutrition information among the researchers, development scientists and food industry from both developed and developing countries. Therefore, we have resorted to a few changes in the format and policies. The sections on research paper and research note are now printed continuously to avoid wastage of paper and printing cost. The style of titles for research papers and research notes is now same and this makes the research notes meaningful.
6. As an additional measure of economy, the practice of supplying 25 gratis reprints has been discontinued from September/October 1992 issue (Vol. 29, No.5). The authors can purchase the reprints in a lot of 25 at nominal cost. Moreover, they can take photo copies of their paper without seeking permission from the Editor.
7. It has also been decided to accept advertisements in the JFST and the present issue carries two advertisements. Our subscribers include institutions, industry as well as individuals working in R&D organisations and universities. The circulation of the journal has exceeded 2000 and is gaining momentum gradually. A decision is also taken not to restrict the new members for opting the

companion journal "Indian Food Industry". Moreover, those members who are getting Indian Food Industry now can also opt for JFST if they so desire.

8. I and the editorial board are pained to note that some of the authors prepare the manuscript without any regard to the style/format of JFST. Some of our referees have even opined that the authors aspiring to publish their data in JFST do not even care to follow the format. A vital need exists to follow the format *in toto* to avoid wastage of our time in pointing out these lacunae, shunting of the manuscript between editor to authors and subsequent delays in processing. I, therefore, would like to request all the authors to follow the "Instructions to Authors" (as published in this issue and all future issues) strictly.

It is our fond hope to improve the standard and quality of the JFST and we, in this effort are determined not to leave any stone unturned. Our endeavours will bear fruits only when the cooperation is coming forth from all concerned. I and the editorial board have just made the beginning at our end and we await you to join hands with us in this noble cause.

**B. K. LONSANE**  
EDITOR

## ANNOUNCEMENT

**IF CON 1993**

**REVISED DATE 7<sup>th</sup> – 11<sup>th</sup> SEPTEMBER 1993**

**(Instead of 5<sup>th</sup> – 9<sup>th</sup> July 1993)**





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## Determination of Thermal Process Schedules For Canned Drumstick, Okra, Elephant Yam and Potato

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Thermal process schedules have been evolved for canned drumstick, okra, elephant yam and potato using *Clostridium sporogenes* as the test organism. The values of thermal resistance (D) found using  $\sim 10^7$  spores (B) were  $1.75 \pm 0.21$  times the values found using  $\sim 10^4$  spores (A). The sterilisation values ( $F_0$ ) corresponding to 3.0 min (Botulinum cook), 5D of A, or a value found by integrating D values of A and B concentration of spores did not prevent spoilage of inoculated packs.  $F_0$  of 5.2 and 5.4 min (equal to 5D of B and experimental z) are considered adequate for commercial processing of drumstick and elephant yam respectively. Okra is best canned in acidified form. Potato requires an  $F_0$  of 9.7 min to overcome facultative thermophilic spoilage. A procedure for interconversion of F value corresponding to experimental z value in terms of  $F_0$  value is given.

India is the second largest producer of vegetables in the world, next only to China, the annual production being 49.6 million tonnes in 1989<sup>1</sup>. Thermal process schedules followed for canned vegetables are those recommended in books of western origin, or slightly higher, and for indigenous vegetable, essentially empirical, based on experience gained in processing similar products.

Detection of *Clostridium botulinum* in commercially canned low acid foods (pH > 4.6) which produces potent exotoxin, has increased the attention to the technology of thermal processing in recent years<sup>2</sup>. A pH of 4.6 or less inhibits the germination of *Clostridium botulinum* spores<sup>3</sup>. *C. sporogenes* which is more heat resistant than *C. botulinum* is a potent mesophilic spoilage organism in canned low acid foods, and is generally used as the test organism in evolving thermal process schedule<sup>4</sup>. The heat resistance of spores decreases with pH from 6.0<sup>5</sup>.

Weaknesses exist in the assumption that spore inactivation is logarithmic<sup>6</sup>. Berry and Bradshaw<sup>7</sup> found more than three-fold increase in the decimal reduction time (D) of *Bacillus stearothermophilus* in mushroom as the spore population decreased to  $10^3$  per ml and lower, and hence suggested two segmented approach to estimate the process.

Formula methods for (i) a single critical point (generally used for ensuring microbiological safety) and (ii) mass average survivor determination, and shortcomings in the methodology which, in turn, affect the F value have been reviewed by Cleland and Robertson<sup>8</sup>. Smith and Tung<sup>9</sup> have

made comparative study of the accuracy of the formula method of Ball<sup>9</sup> and its modification for single critical point in conduction heating of low-acid foods.

The literature cited reflects the complexities in evolving thermal process schedules. In the present studies, effect of spore concentration, procedures used for calculation of D value of the test organism and of process time have been investigated in the determination of safe thermal process for drumstick (*Moringa olifera*), Okra (lady's finger) (*Abelmoschus esculentus*), elephant yam (*Amorphophallus campanulatus*) and potato (*Solanum tuberosum*) which are canned in brine for export besides indigenous requirements.

### Materials and Methods

#### Determination of D Value

The spore crop of *C. sporogenes* (PA 3679) used as the test organism was produced in liver broth under anaerobic conditions<sup>9,10</sup>. Spores after harvest were suspended in 0.9 per cent saline, counted by the most probable number technique using five tubes<sup>6</sup> and diluted with the brine from the processed material so that 2 ml contained  $\sim 10^4$  spores (designated as "A" hereafter) or  $\sim 10^3$  spores (designated as "B" hereafter). Similar suspensions were prepared in Sorensen's phosphate buffer (pH 7.0).

D value was determined by the thermal death time (TDT) tube technique<sup>4</sup> using a temperature controlled oil bath. Spore suspension in sealed tubes were activated by heating at 100°C for 15 min. Three timings at each temperature and

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6 tubes at each timing were used. The suspension after heating was subcultured in pork infusion agar and incubated at 37°C for one month or longer to determine the growth.

The D values were calculated by the fraction negative methods of Stumbo<sup>11</sup> (termed D1 hereafter) and Stumbo *et al.*,<sup>12</sup> (termed D2 hereafter).

Correction for the heating lag during come up time (Cut) was determined by the graphical method of Nath and Ranganna<sup>13</sup> and by the formula method using the expression:  $BB = fh (\log jI - \log g)$  in which  $g$  was assumed to be 0.1.<sup>14,15</sup>  $fh/U$  values corresponding to  $g$  of 0.1 and experimental  $z$  from uncorrected thermal resistance curves were found from  $fh/U:g$  tables<sup>10</sup>. Corrected heating time (Cht) was equal to  $Uht - tc$ , where  $tc = BB - U$  in which  $U = fh/(fh/U)$ .

The thermal resistance curves were constructed by the method of least square using Hp 97 programmable calculator, and the significance of correlation coefficient found from Table of Fisher and Yates<sup>10</sup>.

**Heat penetration studies:** Fresh vegetables purchased from Bulsar market were used. Tender drumstick was scraped to remove the outer peel, edges trimmed and cut into pieces of 7 to 10 cm length; stem end of tender small sized okra was removed; potato was peeled in an abrasive peeler and cut into halves; yam was peeled and cut into small pieces. The prepared vegetables were blanched in boiling water for 3 min, filled (230 g) into Al Tall (301x411) cans, covered with brine, exhausted, sealed, processed at 115.6°C, and cooled in water.

Heat penetration studies were carried out in a factory during regular production runs using Ecklund thermocouple. The mode of heating was by convection. Six cans fitted with the thermocouples at the cold point with the tips embedded in the plant material were distributed in two crates at different locations amidst other cans and placed in a vertical still retort of 600 cans capacity per batch. Temperature changes during heating and cooling were recorded at intervals of 1 min using Leeds and Northrup potentiometer. The cans showing the slowest rate of heat penetration was used for process calculation.

**Calculation of process time:** Improved graphical and formula methods were used for process calculation<sup>4,10</sup>. The BB corresponding to desired F value in the graphical method was found from the plot of F vs time. In the formula method, calculations were made by the programmes given by Vinters *et al.*,<sup>16</sup> Steele *et al.*,<sup>17</sup> and Kao *et al.*,<sup>18</sup> using Hp 97 calculator to find which procedure gives values similar to those of graphical method. For comparison and application, actual processing time (Pt) exclusive of the retort come-up time is more meaningful, and was calculated using the expression,  $Pt = BB - \text{Cut}$  in the graphical method, and  $Pt = BB - (0.42 \times \text{Cut})$  in the formula method.

**Inoculated pack studies:** One ml of inoculum containing  $\sim 10^3$  or  $\sim 10^4$  spores cf *C. sporogenes* was added to each can after exhausting, sealed and processed. For each Pt,

30 cans were used. Heat penetration data during processing and cooling were recorded and  $F_0$  value calculated. The cans were incubated at room temperature (28-30°C), 35°C and 55°C. During storage, cans were examined as and when swelling was observed, and the flat cans after 8 months of storage<sup>10</sup>.

## Results and Discussion

The pH of the fresh drumstick, okra, yam and potato were 5.1, 6.4, 5.6 and 5.5 respectively, but reduced on canning to 4.8, 5.9, 5.2 and 5.3 respectively.

### D value of *C. sporogenes* in phosphate buffer and plant material

**Correction for heating lag:** Table 1 shows the data for applying correction for heating lag in the determination of D value in drumstick. The corrected heating time (Cht) calculated by the graphical method differed from the formula method by less than  $8.2 \pm 5.9$  sec, and hence, the latter method was used in other vegetables and buffer. The heating lag period in the vegetables studied ranged between 2.2 and 3.1 min ( $2.52 \pm 0.25$  min) of which  $30.5 \pm 8$  per cent had lethal effect. The actual value found at each temperature was used for correction.

**Thermal resistance (i) in buffer:** Spore concentration being the same, corrected D value of *C. sporogenes* in neutral phosphate buffer calculated by the method of Stumbo *et al.*,<sup>12</sup> (i.e., D2) were higher by 0.6 sec than the values found by the method of Stumbo<sup>11</sup> (i.e., D1). Fig.1 shows thermal resistance curves.

The classical D and F values of *C. botulinum* at 121.1°C in neutral phosphate buffer are 0.204 and 2.45 min respectively with a  $z$  value of 10°C<sup>4</sup>. Five D of *C. sporogenes* is generally considered as equivalent to 12 D of *C. botulinum*<sup>4</sup>. The experimental  $D_{121.1}$  of *Clostridium sporogenes* found using 9,200 spores (i.e., A) is 0.52 min with a  $z$  value of 8.98°C (Table 2). Five D corresponds to  $F_{121.1}^{8.98} = 2.6$  min which is equal to BB of 20.75 min which, in turn, corresponds to  $F_0$  of 3.0 min (see Fig. 3 drumstick). In terms of the heat resistance *C. botulinum* (i.e., 5/12 value), the D was 0.25 min indicating that the test organism was more heat resistant. Similarly, the experimental value of  $D_{121.1}$  of 0.75 min and  $z$

TABLE 1. DETERMINATION OF D VALUE OF *C. SPOROGENES* IN BRINE FROM CANNED DRUMSTICK: CORRECTION FOR HEATING LAG

Temp. (°C)	Formula method						Graphical method	
	IT (°C)	fh (min)	jh	Uht (min)	Cut (min)	Tc (min)	Cht (min)	Cht (min)
118.0	38.0	1.23	1.15	41.0	2.43	1.80	39.20	39.51
112.8	26.7	1.15	1.23	23.0	2.92	1.75	21.25	21.18
115.6	26.7	1.18	1.86	13.5	3.10	1.62	11.88	11.77
118.3	28.9	8.88	1.00	7.5	2.30	2.28	6.22	6.14
121.1	23.3	1.07	1.14	3.5	2.42	1.64	1.86	1.97

See nomenclature for abbreviations

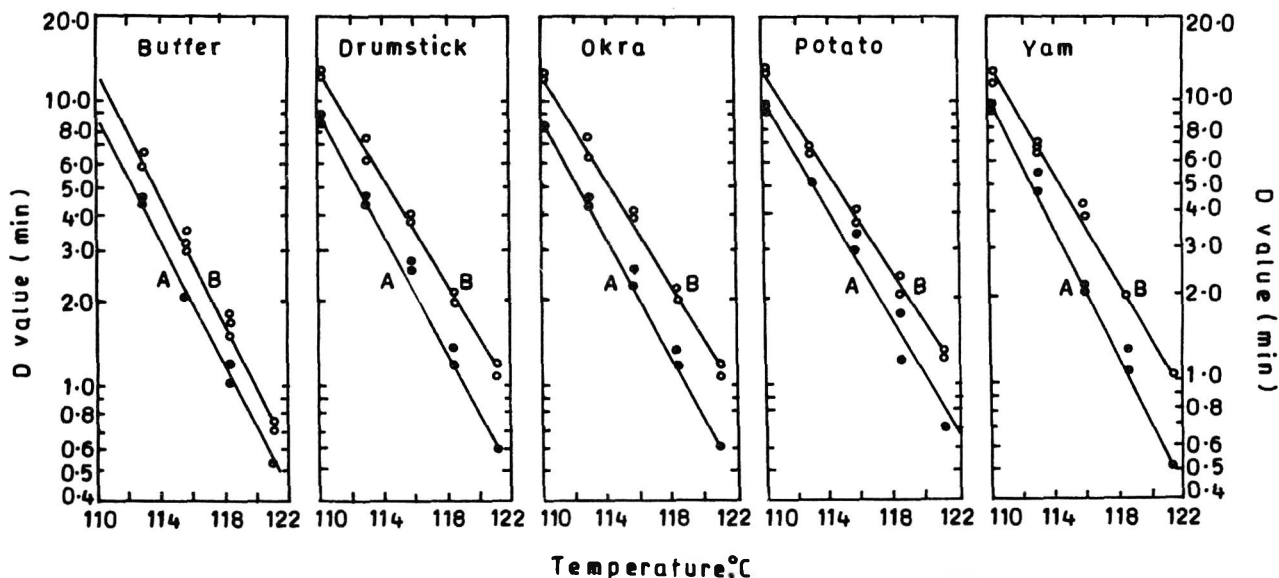


Fig.1. Thermal resistance curves of *C. sporogenes* A: using  $\sim 10^4$  spores B: using  $\sim 10^3$  spores

TABLE 2. CORRECTED D AT 121.1°C AND z OF *C. SPOROGENES*

Medium	Spores/ tube	Thermal resistance data					
		D1 at 121.1°C (min)	z (°C)	D2 at 121.1°C (min)	z (°C)	r of D2	
Buffer	A	9200	0.51	8.94	0.52	8.98	0.9980
	B	820	0.74	9.1	0.75	8.90	0.9986
Drumstick	A	11288	0.58	9.49	0.63	9.72	0.9989
	B	1380	1.10	10.8	1.18	10.9	0.9968
Okra	A	11200	0.60	9.7	0.63	9.8	0.9946
	B	1380	0.91	10.1	0.95	10.2	0.9963
Potato	A	11288	0.76	10.2	0.81	10.2	0.9933
	B	1185	1.27	11.4	1.29	11.3	0.9985
Yam	A	11280	0.50	8.9	0.53	8.9	0.9985
	B	820	0.99	10.0	1.07	10.3	0.9822

r: Significant at 1% level

A and B represent spore concentration per tube

See under nomenclature for abbreviations

of 8.90°C found using 820 spores (i.e., B) was equal to 0.3 min which was 1.5 times higher. Procedures for interconversion of F corresponding to one z to another z and for integration of F values corresponding to 2 different z are given in Table 3.

*In vegetable preparations:* Thermal resistance is affected by the environmental factors prevailing at the time of heating<sup>9,10</sup>. Some vegetables contain factors having inhibitory effect on the growth of bacteria<sup>14</sup>. The D2 values of *C. sporogenes* in the four vegetables using  $\sim 10^4$  spores (A) at 121.1°C ranged from 0.53 to 0.81 min, and using  $\sim 10^3$  spores per tube (B) from 0.95 to 1.29 min respectively (Table 2). The extent of increase in the D value was  $1.75 \pm 0.21$  times higher as against three-fold increase in *B. stearothermophilus* at spore concentration of  $\sim 10^3$  or lower reported by Berry and Bradshaw<sup>7</sup>.

TABLE 3. PROCEDURE FOR CALCULATION OF  $F_0$  VALUE FROM THE EXPERIMENTAL D2 VALUES AT 121°C AND z BY SINGLE AND TWO SEGMENTS PROCEDURES

Heat resistance data of <i>C. sporogenes</i> in drumstick (see Table 2)		
Number of spores per tube -	A: 11,280	B: 1,380
D2 value at 121°C	0.63	1.18
z value in °C	9.72	10.90

By graphical method

Inter conversion of  $F_0$  from graphical interpolation curves for different z values (see Fig 3, drumstick)

Single segment approach:

$$F = 5D_2 \text{ of A} = F_{121.1}^{9.72} \text{ of } 3.15 = \text{BB at } 115.6^\circ\text{C of } 21.5 \text{ min} = F_0 \text{ of } 3.3$$

Two segment approach

$$F = 1D_2 \text{ of A} + 3.5D_2 \text{ of B} \\ = F_{121.1}^{9.72} \text{ of } 0.63 + F_{121.1}^{10.9} \text{ of } 4.13 \\ = F_{121.1}^{10.9} \text{ of } 4.13 = \text{BB of } 22.5 \text{ min} = F_{121.1}^{9.72} \text{ of } 3.40 \\ = F_{121.1}^{9.72} \text{ of } 0.63 + 3.40 = 4.03 = \text{BB of } 25.0 \text{ min} = F_0 \text{ of } 4.2$$

By formula method

In single or two segment approach, procedure being similar to the graphical method, interconversions were made using the programme of Kao *et al.*<sup>18</sup> and Hp 97 calculator

*z values:* The z values found using  $\sim 10^3$  spores (B) were higher by  $1.02 \pm 0.35^\circ\text{C}$  than the values found using  $\sim 10^4$  spores (A) (Table 2). The z value increased with D value as reported by Pflug and Odlaug<sup>19</sup>.

*F value for process calculation:* The thermal resistance studies resulted in two D values and two z values for each vegetable (Table 2). Considering the findings of Berry and Bradshaw<sup>7</sup>,  $F_0$  and Pt values equivalent to (i) 5D of A, (ii) 5D of B, or (iii) a value in between integrating A and B were calculated using the experimental D and z values.



*Process calculation by graphical method using D2 and experimental z value:* Fig 2 shows the heat penetration pattern into the canned drumstick and the lethality curve corresponding to experimental z value of 9.72°C (A). Similar lethality curves were drawn corresponding to experimental z value of 10.9°C (B) and the classical z value of 10°C. Fig 3 shows the graphical interpolation curves to find BB corresponding to F value and *vice versa* for drumstick and other vegetables. Table 3 gives the procedure for conversion of F value corresponding to experimental z value to  $F_0$  value, and the procedure used in the two segment approach for the integration of the experimental D and z values corresponding to A and B concentration of spores.

(i) *Single segment approach:* The Pt required for Al Tall cans of drumstick at 115.6°C corresponding to an  $F_0$  of 3.0 min, the statutory requirement<sup>15</sup>, was the lowest (Sl.No.1 in Table 4). Five D2 of A (i.e.,  $F_{121.1}^{9.72} = 3.15$ ) was equal to an  $F_0$  of 3.3 min and Pt of 13.5 min (Sl.No.2). Five D2 of B (i.e.,  $F_{121.1}^{10.9} = 5.9$ ) was equal to an  $F_0$  of 5.2 min and Pt of 20.3 (Sl.No.7). These represent the minimum and maximum requirements.

(ii) *Two segment approach vs single segment approach for process calculation:*  $F_0$  and Pt values by the two segment approach of Berry and Bradshaw<sup>7</sup> (Sl. No. 3 in Table 4) were slightly higher than 5D of A. Two segment procedures in Sl.No. 4 to 6 intended to reduce the spore concentration to less than one, yielded  $F_0$  and Pt values higher than the values found at 3, but lower than those corresponding to 5D2 of B.

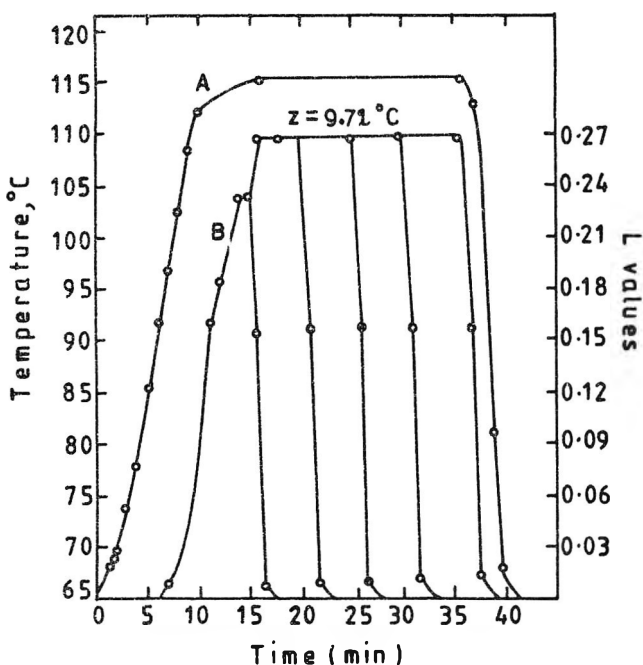


Fig.2. Heat penetration curve into Al Tall (301x411) cans of drumstick in brine processed at 115.6°C and the lethality curve corresponding to z of 9.72°C

(iii) *Considering z as 10°C in place of experimental z value:* In drumstick  $F_0$  corresponding to 5D2 A (i.e.,  $F_{121.1}^{9.72} = 3.15$  min) was equal to  $F_0$  3.3 min (Sl.No. 2), but would be 3.1 min, if the calculations were to be made assuming z as 10°C, and Pt would decrease from 13.5 to 13.0 min. The  $F_0$  corresponding to 5D2 of B and experimental z of 10.9°C was 5.2 min but would be 5.9 min and Pt would increase from 20.3 to 22.5 min if the calculations were made assuming z as 10°C (Table 4). Hence, the use of classical z of 10°C in calculation adds to the safety of the processed product only when the experimental z value is more than 10°C but not when less.

*Using D1 values:* The D1 values as compared to D2 values were lower by  $2.46 \pm 1.71$  sec and the z values by  $0.51 \pm 0.1^\circ\text{C}$  (Table 2).  $F_0$  values corresponding to 5D1 and experimental z as compared to D2 and z differed by 0.1 to 0.4 min which is considered negligible<sup>15</sup>, and so also the Pt by 0.5 to 1.3 min (Table 4).

The pattern of results for canned okra, potato and yam was similar.

*Process calculation by formula method:* Semi-log plot of heat penetration data except during the initial lag, yielded a straight line. The heat penetration parameters (fh, jh and jc) found from statistical fit curves slightly differed from the eyefit curves, and showed a high degree of correlation (Table 5).

Graphical method of calculation of  $F_0$  and Pt is accurate as no assumptions are involved, but can be used only when processing conditions are identical to the heat penetration studies. Ball<sup>9</sup> in developing the formula method, assumed fh and fc to be equal, and jc as 1.41. Vinters *et al.*,<sup>16</sup> developed a programme for calculation using computer when z was 10°C (Procedure C). Steele *et al.*<sup>17</sup> revised the fh/U:g values as the values given by Ball reduced the safety factors of thermal processing, and gave revised coefficients for calculation by Vinters *et al.*<sup>16</sup> (Procedure D.). Stumbo<sup>9</sup> found that fh/U:g values were virtually independent of the process parameters such as IT, RT and jh but depended on j of the cooling curves (i.e, jc), and z which Smith and Tung<sup>8</sup> found to result in more accurate results for conduction heating foods. Kao *et al.*,<sup>18</sup> have given algorithm for calculation which makes use of these values (Procedure A). Procedure-B is same as A except that the heat penetration parameters found from linear fit semilog plot were made use of.

$F_0$  and Pt calculated by procedures A and B of formula method were almost the same (Table 4). The values were similar by Procedures-C and D in drumstick and other vegetables, unlike the conduction heating foods, where in only the Procedure A was found best suited<sup>8</sup>. Compared to the values calculated by the graphical procedure, in the formula method, the  $F_0$  values corresponding to experimental z values in single and two segment procedure(s) were higher by 0.1 to 0.4 min and Pt by 0.2 to 1.9 min.

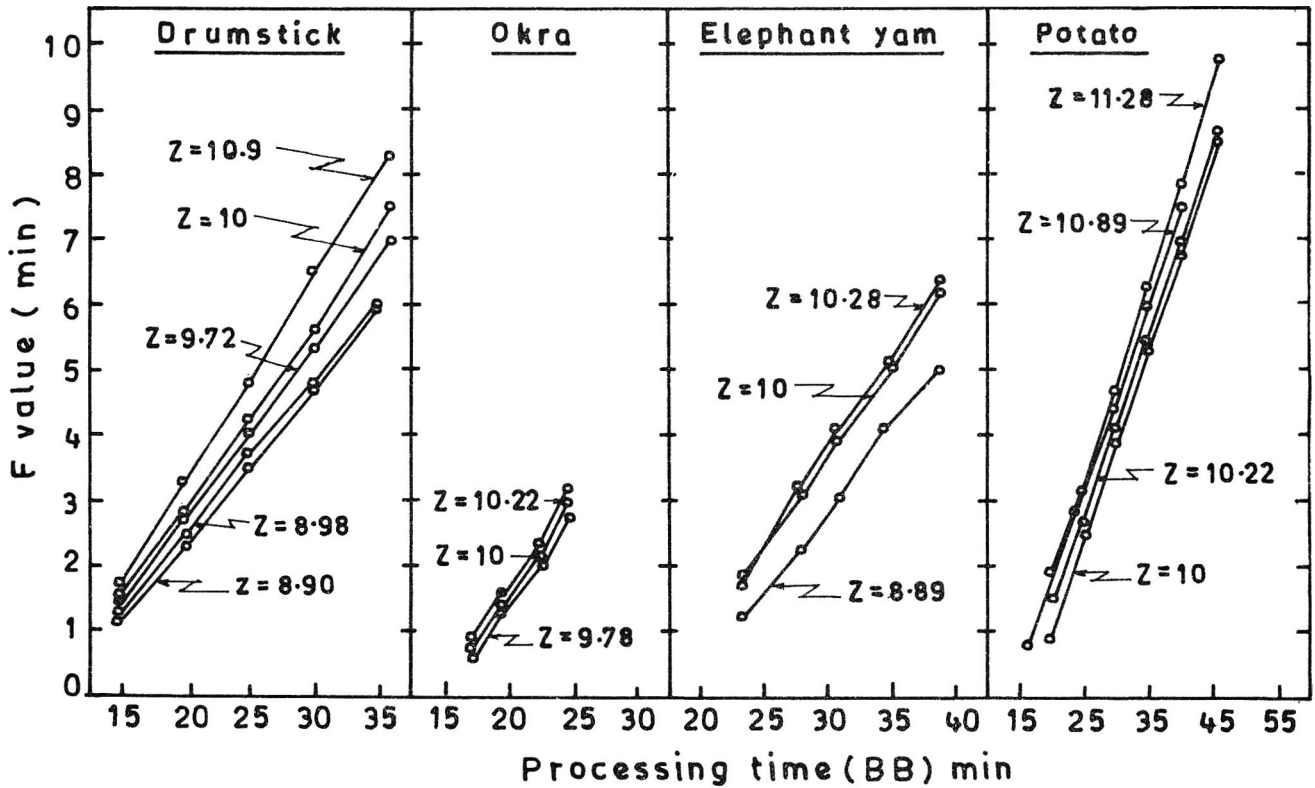


Fig.3. Graphical interpolation curves to find BB and F value for different values of z

TABLE 4.  $F_0$  AND Pt BY GRAPHICAL AND FORMULA METHODS FOR SINGLE AND TWO SEGMENT PROCEDURES FOR 301x411 CANS OF DRUMSTICK IN BRINE PROCESSED AT 115.6°C

F value calculation procedure <sup>c</sup>	IGM method						Formula method <sup>b</sup>			
	Using D1		Using D2				Using D2 and exptl z			
	Exptl z		Exptl z		Assuming z as 10°C		Eye fit Proc. C		Linear fit Proc. B	
	F (min)	Pt (min)	F (min)	Pt (min)	F (min)	Pt (min)	F (min)	Pt (min)	F (min)	Pt (min)
1. $F_0 = 3.0^a$	—	—	—	—	3.0	12.8	3.0	13.5	3.0	13.6
2. $F = 5D$ of A	3.1	13.0	3.3	13.5	3.1	13.0	3.3	14.5	3.3	14.6
3. $F = D$ of A (log a-3.0)+ D of B(3-log bx)	3.5	14.5	3.5	13.0	3.5	14.5	3.2	14.4	3.2	14.5
4. $F = 1D$ of A+3.5D of B	4.0	16.0	4.2	17.0	4.8	18.8	4.4	18.4	4.4	18.5
5. $F = D$ of D(log a-log ax) +D of B(log ax-log l)	4.2	17.0	4.6	18.3	5.2	20.3	4.7	19.6	4.7	19.7
6. $F = 1D$ of A+4D of B	4.6	18.0	4.7	18.5	5.4	21.0	4.9	20.2	4.9	20.3
7. $F = 5D$ of B	5.0	19.5	5.2	20.3	5.9	22.5	5.3	21.6	5.3	21.7

<sup>a</sup>Statutory requirement for low-acid canned foods

<sup>b</sup>See Table 5 for heat penetration parameters

<sup>c</sup>See Table 3 for conversion of F corresponding to experimental z to  $F_0$  and particulars of calculation by two segment procedure.

**Inoculated pack studies:** The calculated process time (Pt) at 115.6°C for drumstick canned in A1 Tall cans was 12.8 min corresponding to experimental 5 D1 of A and z of 9.5°C and 22.5 min corresponding to 5 D2 of B and classical z of 10°C, while the values found by 2 segment procedure were in-

between (Table 4). Swelling occurred in the inoculated canned product receiving Pt of 10 and 15 min but not of 20 and 25 min indicating that  $F_0$  of 3.0 min (i.e., botulinum cook) or 3.2 to 3.4 min would not render the inoculated cans microbiologically safe. The cans processed for 20 min equal

TABLE 5. THERMAL PROCESS DATA FOR 301x411 CANS OF VEGETABLES IN BRINE PROCESSED AT 115.6°C

Product	Curve fitting	Heat penetration data									
		RT (°C)	IT (°C)	Cut (min)	jh	fh (min)	jc	CWT (°C)	r of heating	F <sub>0</sub> (min)	Pt (min)
Drum stick	Eye fit	116	64.4	8.0	1.09	4.70	1.23	30		5.3	21.6
	Linear fit	116	64.4	8.0	1.05	4.83	1.20	30	0.9701	5.3	21.7
Okra	Eye fit	116	58.9	9.0	2.75	6.52	1.10	30		4.6	24.2
	Linear fit	116	58.9	9.0	2.79	6.61	1.08	30	9.9940	4.6	24.4
Potato	Eye fit	116	62.8	6.0	1.05	9.50	1.05	26		5.4a	29.3a
	Linear fit	116	62.8	6.0	1.13	9.12	1.00	26	0.9940	5.4	29.1
Yam	Eye fit	116	58.9	9.0	1.03	10.00	1.10	29		5.2	28.1
	Linear fit	116	58.9	9.0	1.02	10.23	1.01	29	0.9983	5.2	28.4

F<sub>0</sub> and Pt values corresponding to D found using 10<sup>3</sup> spores (i.e., B) and experimental z

See recommendations for potato to overcome facultative spoilage

to F<sub>0</sub> of 5.2 min [corresponding to 5 D<sub>2</sub> of B and experimental z (Table 4)], and above remained normal over a storage period of 8 months at 35 and 55°C. Subculturing of normal cans showed no growth under aerobic or anaerobic conditions.

Swelling occurred in canned potato processed for 25 min or less when stored at 37 and 55°C and in cans processed for 30 min at 55°C indicating thermophilic spoilage which should be controlled by good manufacturing practices. From the canned product containing unpeeled and unwashed potatoes, spore forming short rods capable of growing at 45°C and 55°C were isolated, the D values of which at 121.1°C using ~10<sup>4</sup> and ~10<sup>3</sup> spores were 2.34 min and 3.37 min respectively with a z value of 11°C. Though this represents an extreme condition, considering the high ambient temperatures prevailing during summer in many parts of India, processing at 115.6°C for 45 min is recommended.

The heating lag in the determination of D value of *Clostridium sporogenes* in drumstick, okra, elephant yam and potato was 2.67 ± 0.075 min of which 32.42 ± 1.08 per cent had lethal effect. The graphical and formula methods of determining the extent of lethality of the come-up time made no significant difference. In terms of z value of 10°C, the D values found using 10<sup>3</sup> spores (B) were 1.54 ± 0.14 times higher than the values found using 10<sup>4</sup> spores (A). F<sub>0</sub> corresponding to 3.0 min or 5D<sub>2</sub> of A and experimental z value were inadequate to prevent the spoilage of inoculated packs. Under commercial processing conditions, F<sub>0</sub> values of 5.2, 5.4 and 9.7 min are recommended for canned drumstick, yam and potato respectively (Table 5). Pt corresponding to F<sub>0</sub> of even 3.0 min rendered the canned okra mushy, and is best canned in acidified form. Different modifications of Ball's formula method made no significant difference in the calculated values.

#### Nomenclature

a number of spores (A) per tube × number of tubes used for determining D

ax	number of spores (B) per tube × number of tubes used for determining D
A	10 <sup>4</sup> spores per tube
b	Spores surviving after heating a
bx	spores surviving after heating ax
B	10 <sup>3</sup> spores per tube
BB	process time in min from the time the retort reaches processing temperature (RT) until steam is turned off
Cht	corrected heating time in determination of D
Cut	come up time in the determination of D or retort come up time during processing
CWT	cooling water temperature
D	time required to reduce the bacterial population by 90% at a given temperature
D1	D calculated by the method of Stumbo <sup>11</sup>
D2	D calculated by the method of Stumbo <i>et al</i> <sup>12</sup> .
F	time in min required to destroy the microorganism or its spores at a given temperature and z value
F <sub>0</sub>	F value when the temperature is 121.1°C and z is 10°C
fh <sup>o</sup>	time in min required for the straight line portion of the heating curve to traverse one log cycle
fc	time in min. required for the straight line portion of the cooling curve to traverse one log cycle
g	time in min. required for the straight line portion of the cooling curve to traverse one log cycle difference between the RT and the maximum temperature reached at the cold point
I	RT-IT
IT	actual initial temperature at the start of processing
IGM	improved graphical or general method
jc	cooling lag factor
jh	heating lag factor
Pt	actual process time
RT	retort temperature

tc	correction factor
ThIT	theoretical IT
U	time in min required to destroy the organism at RT
Uht	uncorrected heating time in the determination of D
z	°C required for the thermal resistance curve to traverse one log cycle
~	approximately

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## Effect of Spices and Salt on the Storage Stability of Precooked Dehydrated Rice

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Effect of nineteen spices (1%), sodium chloride (0.5-2%) and transition metal ions ( $\text{Fe}^{++}$ ,  $\text{Cu}^{++}$ ,  $\text{Co}^{++}$ ,  $\text{Ni}^{++}$  each at 5, 50 and 500 p.p.m.) on the storage stability of pre-cooked, dehydrated rice processed using refined sunflower oil and vanaspati was investigated. All the spices except *Tejpat* (*Cinnamomum tamala*) exhibited antioxidygenic activity in dehydrated rice as well as pure sunflower oil and vanaspati. Red chilli, clove, mace and nutmeg exhibited maximum anti-oxygenic activity. *Tejpat*, on the other hand, exhibited pro-oxygenic activity in all the systems. In dehydrated rice, sodium chloride exhibited slight pro-oxygenic activity but  $\text{Cu}^{++}$ ,  $\text{Fe}^{++}$  and  $\text{Co}^{++}$  exhibited very strong pro-oxygenic activity. Only at 500 p.p.m. level  $\text{Ni}^{++}$  exhibited marginal anti-oxygenic activity. Nature of the oil used during processing also significantly influenced the stability of dehydrated rice and anti-oxygenic or pro-oxygenic activity of the spices and metal ions.

A minimum shelf life of one year under all weather conditions is an essential requirement laid down by Indian Armed Forces for all operational pack rations. The off-flavours and discolourations resulting from autoxidation of lipids and pigments and non-enzymic browning reactions pose serious limitations to shelf life of pre-cooked dehydrated foods. While rate of lipid autoxidation is influenced by a number of factors like storage temperature, moisture and packaging, the overall shelf life is mainly determined by the ingredients used in the foods. In some of the dehydrated convenience foods, like *pulav*, *khichdi*, *upma*, *insta nutro cereal mix* etc., developed for pack rations, spices and salt form important constituents. Though many studies<sup>1-5</sup> have been reported on the anti-oxygenic effect of spices on cooked and cured meat products, lard and oil-water emulsions, little information is available on the role of spices on the stability of dehydrated foods based on cereals and pulses.

Information on the effect of common salt on autoxidation of lipids in foods is rather confusing. Salt exerts powerful pro-oxygenic effect in frozen meat and anti-oxygenic effect in linoleate emulsions, lard spread on paper or in gel and in dehydrated carrots<sup>6-10</sup>. Results of studies on the role of spices, salt and some transition metal ions in determining the shelf life of pre-cooked dehydrated rice are reported in this paper.

### Materials and Methods

**Preparation of pre-cooked, dehydrated rice:** Commercially available rice ('Bar.gara Sanna' variety) was cleaned and washed with water. One part of rice was cooked with 2.5 parts of water in an autoclave at 1.08 kg/cm<sup>2</sup> steam pressure for 15 min. The cooked rice was spread in trays and dried at 80°C

to a moisture level of 5 per cent in a hot air cabinet dryer. Water containing required quantities of sodium chloride or sulphates of  $\text{Fe}^{++}$ ,  $\text{Cu}^{++}$ ,  $\text{Co}^{++}$  or  $\text{Ni}^{++}$  was used for cooking rice for studying the effect of salt or metal ions on the stability of rice.

**Preparation of spices:** Commercially available good quality spices [red chilli (*Capsicum annum*), clove (*Eugenia caryophyllus*), cumin (*Cuminum cyminum*), turmeric (*Curcuma longa*), pepper (*Piper nigrum*), coriander (*Coriandrum sativum*), mace (*Myristica fragrans*), nutmeg (*Myristica fragrans*), black cardamom (*Amomum subulatum*), fenugreek (*Trigonella foenum-graecum*), dry ginger (*Zingiber officinale*), garlic (*Allium sativum*), onion (*Allium cepa*), Bishop's weed (*Trachyspermum ammi*), aniseed (*Pimpinella anisum*), cardamom (*Elettaria cardamomum*), cinnamon (*Cinnamomum zeylanicum*), *tejpat* (*Cinnamomum tamala*), poppy seed (*Papaver somniferum*)] were cleaned and powdered in an ultracentrifugal mill (Retsch R1, Germany) using 1 mm sieve. Ten g of the powdered spice was suspended in 150 g refined sunflower oil or vanaspati and mixed with 1 kg pre-cooked dehydrated rice in a stainless steel vessel. Control samples were prepared by mixing 1 kg pre-cooked dehydrated rice with 150 g refined sunflower oil or vanaspati.

**Storage and evaluation:** Treated rice samples (100 g) were packed in hermetically sealed polypropylene (75  $\mu$ ) pouches (10x10 cm) and were stored in an incubator maintained at  $37 \pm 1^\circ\text{C}$ . The samples were analysed for peroxide value (PV) initially and at regular intervals. To study the effect of spices on the stability of refined sunflower oil or vanaspati, 100 g samples of each were stored at  $37^\circ\text{C}$  and  $55^\circ\text{C}$ . in 250 ml glass beakers, both with and without 0.1, 0.25 and 0.5 per cent of powdered spices.



## Results and Discussion

**Effect of spices on dehydrated rice and oils:** The pattern of autoxidation expressed as changes in PV in dehydrated rice both with and without spices, practically remained same (Fig. 1). PV of the samples treated with red chilli and clove were, however, considerably lower than the untreated controls indicating anti-oxygenic activity of these spices.

In order to quantitatively estimate the anti-oxygenic or pro-oxygenic activity of the various spices, the ratios of PV of the control to that of spice treated sample were calculated after varying storage periods (30, 45 and 60 days in case of sunflower oil and 45, 60, 90, 120 and 150 days in case of vanaspati treated samples) and the means of these values along with standard deviation are given in Table 1. The values  $> 1$  indicate anti-oxygenic activity, while  $< 1$  indicate pro-oxygenic activity of the spice. Except *tejpat*, all other spices exhibited slight to strong anti-oxygenic activity. Among the 19 spices tested, red chilli, clove, mace and nutmeg exhibited strong anti-oxygenic activity in dehydrated rice. Earlier, Chipault *et al.*<sup>1-3</sup> have reported higher anti-oxygenic activity

TABLE 1. ANTIOXYGENIC ACTIVITY\* OF SPICES (1%) IN DEHYDRATED RICE PROCESSED IN REFINED SUNFLOWER OIL AND VANASPATI AND STORED AT 37°C IN POLYPROPYLENE POUCHES

Spice	Sunflower oil* Mean $\pm$ S.D.	Vanaspati** Mean $\pm$ S.D.
Red chilli	1.99 $\pm$ 0.16	1.73 $\pm$ 0.30
Clove	2.04 $\pm$ 0.12	1.55 $\pm$ 0.33
Cumin	1.28 $\pm$ 0.12	1.26 $\pm$ 0.10
Turmeric	1.23 $\pm$ 0.08	1.15 $\pm$ 0.08
Pepper	1.39 $\pm$ 0.11	1.17 $\pm$ 0.09
Coriander	1.12 $\pm$ 0.04	1.14 $\pm$ 0.04
Mace	2.04 $\pm$ 0.19	1.50 $\pm$ 0.32
Nutmeg	1.73 $\pm$ 0.18	1.46 $\pm$ 0.29
Black cardamom	1.37 $\pm$ 0.14	1.29 $\pm$ 0.08
Fenugreek	1.11 $\pm$ 0.07	1.15 $\pm$ 0.05
Dry ginger	1.10 $\pm$ 0.07	1.20 $\pm$ 0.14
Garlic	1.33 $\pm$ 0.09	1.33 $\pm$ 0.12
Onion	1.20 $\pm$ 0.03	1.16 $\pm$ 0.02
Bishopweed	1.44 $\pm$ 0.02	1.15 $\pm$ 0.04
Aniseed	1.03 $\pm$ 0.02	1.22 $\pm$ 0.12
Cardamom	1.08 $\pm$ 0.05	1.12 $\pm$ 0.02
Cinnamon	1.01 $\pm$ 0.03	1.05 $\pm$ 0.03
Tejpat	0.81 $\pm$ 0.13	0.59 $\pm$ 0.12
Poppy seed	1.11 $\pm$ 0.05	1.17 $\pm$ 0.05

\* Values  $> 1$  indicate anti-oxygenic activity and  $< 1$  indicate pro-oxygenic activity which is the ratio of PV of the control to that of spice treated sample.

\*Mean of 3 values (after 30, 45 and 60 days)

\*\*Mean of 5 values (after 45, 60, 90, 120 and 150 days.)

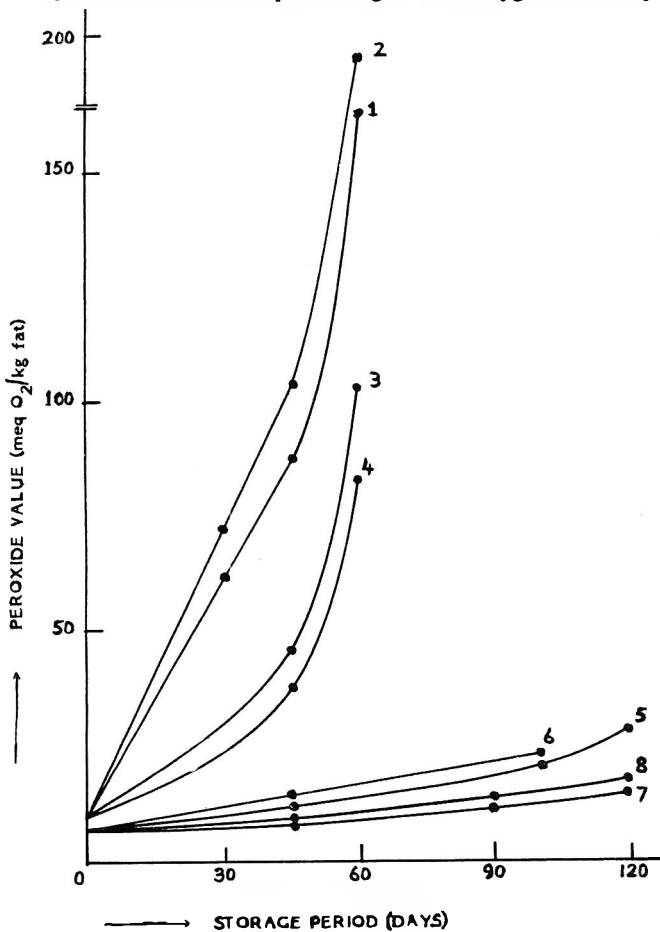


Fig.1. Effect of spices and sodium chloride on peroxide value of dehydrated rice processed using (a) sun-flower oil (1,2,3,4.) and (b) vanaspathi (5,6,7,8) during storage in polypropylene pouches at 37°C. 1 and 5, control samples; 2 and 6, treated with sodium chloride, 3 and 7, treated with red chilli; 4 and 8, treated with clove.

of turmeric, nutmeg and pepper than chilli and clove in lard. However, in the present study, turmeric and pepper exhibited only moderate anti-oxygenic activity in dehydrated rice processed with sunflower oil or vanaspati. Anti-oxygenic activity of cardamom, cinnamon and aniseed was only marginal in dehydrated rice.

*Tejpat* showed consistently pro-oxygenic activity in dehydrated rice, sunflower oil and vanaspati (Tables 1 and 2). Also, anti-oxygenic or pro-oxygenic activity of the spices was relatively more pronounced in dehydrated rice than in sunflower oil or vanaspati. Interestingly, clove exerted very strong anti-oxygenic activity in dehydrated rice, while its action in refined sunflower oil and vanaspati was only marginal (Tables 1 and 2). Red chilli, nutmeg and mace exhibited maximum anti-oxygenic activity both in rice and in oils. Fenugreek, coriander and dry ginger also exhibited relatively higher anti-oxygenic activity in sunflower oil than in vanaspati and dehydrated rice (Tables 1 and 2). Earlier also, clove has been reported to be relatively less effective in lard, but more effective in emulsions<sup>1-3</sup>. This substrate specific anti-oxygenic action of spices has been confirmed in the present study.

In sunflower oil and vanaspati, majority of the spices exhibited maximum anti-oxygenic activity even at 0.1 per cent concentration. Further, increase in concentration upto 0.5 per cent did not enhance anti-oxygenic activity significantly. In

TABLE 2. ANTIOXYGENIC ACTIVITY OF SPICES (0.1%) IN REFINED SUNFLOWER OIL STORED AT 37° AND VANASPATI STORED AT 55°C

Spice	Sunflower oil* Mean ± S.D.	Vanaspati** Mean ± S.D.
Red chilli	1.32 ± 0.01	1.32 ± 0.10
Clove	1.13 ± 0.02	1.22 ± 0.05
Cumin	1.26 ± 0.04	1.24 ± 0.18
Turmeric	1.30 ± 0.01	1.11 ± 0.02
Pepper	1.19 ± 0.01	1.11 ± 0.01
Coriander	1.19 ± 0.02	1.12 ± 0.03
Mace	1.35 ± 0.04	1.25 ± 0.12
Nutmeg	1.34 ± 0.03	1.29 ± 0.11
Black cardamom	1.28 ± 0.02	1.13 ± 0.05
Fenugreek	1.32 ± 0.07	1.22 ± 0.12
Dry ginger	1.27 ± 0.04	1.22 ± 0.11
Garlic	1.01 ± 0.01	1.19 ± 0.07
Onion	1.03 ± 0.02	1.22 ± 0.13
Bishopweed	1.09 ± 0.02	1.20 ± 0.11
Aniseed	0.96 ± 0.02	1.16 ± 0.10
Cardamom	1.00 ± 0.02	1.16 ± 0.08
Cinnamon	1.16 ± 0.02	1.14 ± 0.02
Tejpat	0.87 ± 0.01	0.91 ± 0.05
Poppy seed	1.05 ± 0.03	1.24 ± 0.08

\*Mean of 4 values (after 10, 20, 27 and 35 day)

\*\*Mean of 4 values (after 30, 60, 120 and 150 days)

case of cumin, mace, dry ginger coriander and poppy seed, antioxygenic activity increased slightly with rise in the concentration of spices.

**Effect of transition metal ions and salt:** Effect of  $\text{Cu}^{++}$ ,  $\text{Fe}^{++}$  and  $\text{Ni}^{++}$  at 5 to 50 p.p.m. levels on the rate of autoxidation in dehydrated rice processed in sunflower oil and vanaspathi is shown in Fig. 2. Addition of  $\text{Cu}^{++}$ ,  $\text{Co}^{++}$  and  $\text{Fe}^{++}$  even at 5 p.p.m. level considerably enhanced the rate of lipid peroxidation in dehydrated rice. The catalytic action was dependent on concentration and increased with rise in concentration of metal ions from 5 to 500 p.p.m. Though it has been claimed earlier that at higher concentrations transition metal ions exert anti-oxygenic activity by decomposing peroxides<sup>12</sup>, such effect has not been observed in dehydrated rice even at 500 p.p.m. levels of  $\text{Cu}^{++}$ ,  $\text{Co}^{++}$  and  $\text{Fe}^{++}$ . Incorporation of  $\text{Ni}^{++}$  upto 50 p.p.m. level did not exert significant effect, but at 500 p.p.m. level, it exerted slight anti-oxygenic activity. The effect of transition metal ions was comparatively more pronounced in samples having added sunflower oil than in ones having vanaspathi. Incorporation of salt also slightly enhanced the rate of lipid peroxidation in rice having added sunflower oil and its catalytic action increased with rise in concentration (Fig. 1). In samples having added vanaspathi, the effect of salt was not appreciable.

In conclusion, most of the spices except *tejpat* exert stabilising effect but salt and transition metal ions exert strong pro-oxygenic effect in dehydrated convenience foods. Also, type of fat used during processing considerably influences the stability of the product. The results of present studies have

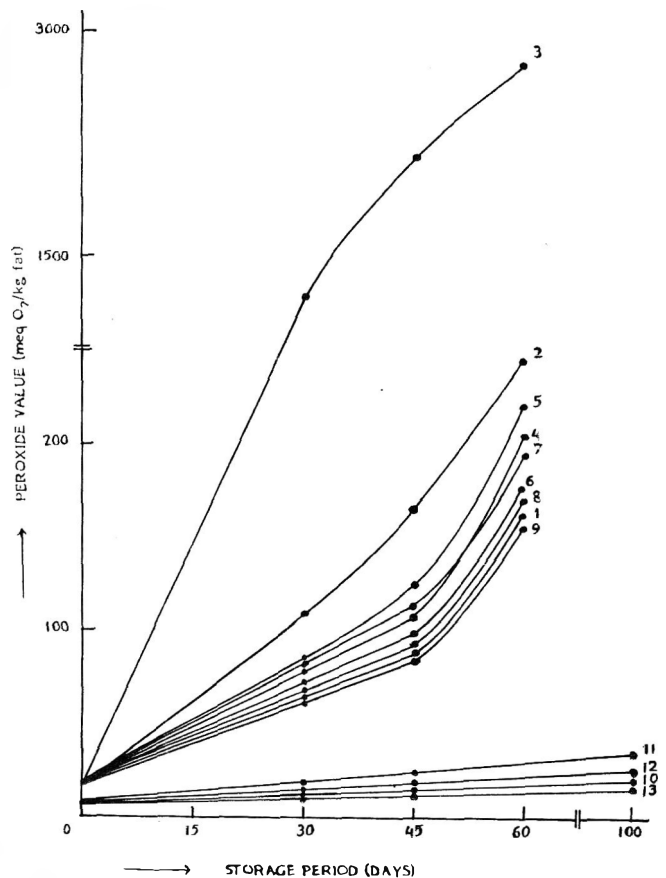


Fig. 2. Effect of transition metal ions on peroxide value of dehydrated rice processed using (a) sun-flower oil (1,2,3,4,5,6,7,8,9) and (b) vanaspathi (10,11,12,13) during storage in polypropylene pouches at 37° C. 1 and 10, control samples; 2 and 3, treated with copper 5 and 50 ppm; 4 and 5, treated with cobalt 5 and 50 ppm; 6 and 7, treated with iron 5 and 50 ppm; 8 and 9, treated with Nickel 5 and 50 ppm; 11, 12 and 13, treated with 50 ppm of Cu, Co and Ni respectively.

highlighted the importance of selection of ingredients and care to be taken during processing operations for preventing metal ion contamination which exert strong pro-oxygenic action during storage.

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## Studies on Fruit Bread

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Studies on fruit bread indicated that minimum levels of 15.5 and 25% sugar, fat and fruit respectively were required to obtain bread with desired taste. The bread thus made had slightly undesirable hard texture which was overcome by increasing the fermentation and proofing times from 120 to 150 and 55 to 70 mins respectively. Fruit bread dough required about 6% less water as compared to that required for a normal bread dough. Fermentation rate was reduced as indicated by the Expansograph characteristics showing longer time to reach the peak (135 min) compared to normal bread dough (65 min). Fruit bread could be stored at ambient condition of 25-28°C and relative humidity of 60-65% after packing in polypropylene pouches (100 gauge) for a longer period of 8 days without mould growth and with minimum of textural changes in contrast to that for 3 days for normal bread.

Bakery industry is one of the major food industries of the country with an annual turnover of over Rs. 2,500 crores. Production and consumption of bakery products in India have been increasing at a fast rate as indicated by two fold increase in the last five years and it is reported to increase further at a rate of 10.2 per cent per annum. Bread occupies an important place and constitutes over 50 per cent to total Indian market<sup>1</sup> for bakery products. Among the several varieties of breads produced in the country, fruit bread forms an important variety next to white pan bread. It is different from other varieties in that it contains fruit pieces in addition to higher amounts of fat and sugar. Literature survey indicates no scientific information of fruit bread and various aspects governing its quality. Hence, the present studies were undertaken to optimise the ingredients and processing conditions for the preparation of fruit bread.

### Materials and Methods

Commercially available *aestivum* hard wheat, procured from the local market, was cleaned, conditioned to

15.5 per cent moisture overnight and milled in a Buhler Laboratory Mill (Model MLU 202). The straight run flour thus obtained was used in the studies. Commercially available (tasty fruity), a candy processed from papaya, normally used in the preparation of fruit bread, was used as a source of fruit. Moisture, ash and gluten of flour were estimated according to AACC<sup>2</sup> procedure. Gas retention and production capacity of doughs were determined using expansograph.

*Rheological characteristics:* Consistency of bread doughs as affected by different levels of fat and sugar was assessed in farinograph. The bread dough equivalent to 50 g flour was mixed in Farinograph till peak consistency was obtained. Dough hardness was determined using the General Food Texturometer<sup>3</sup> (conditions: diameter of the plunger=20 mm; cup=meat cup; attenuator=1; voltage=1. V; clearance=1 mm; plunging speed=low). The dough was sheeted to 1 cm thickness using a rolling pin and cut into 20 mm diameter and placed in the texturometer cup. The curve obtained was evaluated for hardness as per the standard procedure<sup>4</sup>. The rheological parameters of fermenting doughs were measured

by determining the spread ratio according to the method of Hosney *et al.*<sup>3</sup>. Pasting characteristics of flour containing different levels of fat and sugar were determined using Brabender amylograph<sup>2</sup>.

**Bread making trials:** Test baking of bread was carried out using Irvine and McMullan<sup>5</sup> procedure. Baking trials were carried out by varying yeast from 0.5 to 2.5 per cent, fat 2.5 to 10 per cent, sugar 2.5 to 20 per cent and fruits 10 to 25 per cent fermentation time from 1 to 3 hr, proofing time from 55 to 75 min and baking time from 20 to 30 min. Breads were also made by different methods such as sponge and dough method<sup>2</sup> and chemical dough development method<sup>6</sup>. In chemical dough development method, cysteine hydrochloride, potassium bromate and ascorbic acid were used at levels of 35, 25 and 50 p.p.m. respectively.

**Evaluation:** Loaf volume of bread was measured by rape seed displacement method<sup>2</sup>. The crust and crumb characteristics were evaluated by a panel of semi-trained judges using scoring method (maximum score of 10 was given for each parameter).

**Storage studies:** Breads were wrapped in polypropylene pouches (100 gauge) and stored at ambient conditions (25-28°C temperature and relative humidity of 65-70 per cent). The stored breads were observed for mould growth. The texture of stored bread was measured in General Food Texturometer (conditions: plunger=50 mm brass; platform=flat aluminium; clearance=1 mm; voltage=0.5 V.; speed=low). Bread slices of 5 cm square and 1 cm thick were used for the textural studies.

**Statistical analysis:** Statistical analysis of the data was carried out by Duncan's new multiple range test<sup>7</sup>.

## Results and Discussion

The flour used for the preparation of bread had gluten 9.0 per cent, ash 0.49 per cent, sedimentation value 22.5 ml and farinograph water absorption 61.6 per cent (14 per cent moisture basis), suggesting the suitability of flour for bread making.

**Effect of ingredients on the quality of fruit bread:** Effect of various ingredients such as fruits, sugar, fat and yeast on the specific volume of bread is given in Table 1. As expected, specific volume of bread containing 5 per cent fat and 15 per cent sugar decreased when fruit level was increased. Incorporation of fruits upto 15 per cent did not affect the specific volume significantly, but beyond that level it reduced from 4.1 to 3.4 ml.g. However, considering the desired taste and distribution of number of fruit pieces in the crumb, it was found necessary to use minimum of 25 per cent fruits in fruit bread. Further, studies carried out using 25 per cent fruits, 15 per cent sugar and varying levels of fat indicated that incorporation of more than 5 per cent fat did not show much improvement in the specific volume of bread (Table 1). Hence, 5 per cent fat was considered optimum. Sugar

improved the volume upto 10 per cent and further increase in sugar level, reduced the same. The trend is similar to the one reported for white bread<sup>8</sup>. The score for crumb texture decreased slightly beyond 10 per cent level of incorporation. However, minimum of 15 per cent sugar was required to obtain the desired taste and overall quality (Table 1). Increase in the level of dry yeast from 0.5 to 2.5 per cent increased the specific volume from 2.3 to 3.7 ml/g and improved the crumb texture and overall quality of bread. Maximum improvement was observed at 2 per cent yeast level. Higher requirement of yeast as compared to normal plain bread (1 per cent dry yeast) is expected because of retardation of yeast activity due to higher sugar level.

**Water absorption for fruit bread doughs:** Use of 1 per cent fat, 2.5 per cent sugar and water equivalent to farinograph water absorption reduced the dough consistency from 500 to 320-330 B.U. for different types of flour (Table 2). Use of the same water but higher levels of fat (5 per cent) and sugar (15 per cent) as in fruit bread reduced the consistency further to about 100 B.U. and the dough was sticky and had poor handling characteristics. However, to maintain similar

TABLE 1. SENSORY QUALITY AND SPECIFIC VOLUMES OF FRUIT BREAD AS AFFECTED BY DIFFERENT INGREDIENTS

Fat/sugar yeast (%)	Sp vol (ml/g)	Crust colour	Crumb texture	Overall quality
<b>Fat</b>				
0	2.50	7.05 <sup>a</sup>	7.05 <sup>a</sup>	7.90 <sup>a</sup>
2.5	3.10	9.10 <sup>b</sup>	8.90 <sup>b</sup>	9.00 <sup>b</sup>
5.0	3.40	8.68 <sup>c</sup>	9.10 <sup>b</sup>	9.20 <sup>b</sup>
7.5	3.45	7.40 <sup>d</sup>	8.40 <sup>d</sup>	8.92 <sup>bc</sup>
10.0	3.30	7.10 <sup>a</sup>	8.38 <sup>d</sup>	8.82 <sup>c</sup>
SEM		+0.07	+0.05	+0.09
<b>Sugar</b>				
2.5	3.50	7.70 <sup>a</sup>	7.95 <sup>a</sup>	8.05 <sup>a</sup>
5.0	3.60	9.10 <sup>b</sup>	8.50 <sup>b</sup>	8.25 <sup>b</sup>
10.0	3.75	8.50 <sup>c</sup>	8.90 <sup>c</sup>	8.80 <sup>c</sup>
15.0	3.40	8.08 <sup>d</sup>	8.70 <sup>d</sup>	9.15 <sup>d</sup>
20.0	3.20	6.68 <sup>c</sup>	8.30 <sup>c</sup>	9.08 <sup>a</sup>
SEM		+0.06	+0.05	+0.06
<b>Yeast</b>				
0.5	2.3	4.22 <sup>a</sup>	6.15 <sup>a</sup>	6.02 <sup>a</sup>
1.0	2.7	5.18 <sup>b</sup>	7.05 <sup>b</sup>	7.05 <sup>b</sup>
1.5	3.0	7.15 <sup>c</sup>	7.78 <sup>c</sup>	8.50 <sup>c</sup>
2.0	3.4	8.05 <sup>d</sup>	8.68 <sup>d</sup>	9.20 <sup>d</sup>
2.5	3.7	7.90 <sup>d</sup>	8.08 <sup>c</sup>	8.20 <sup>c</sup>
SEM		+0.07	+0.07	+0.06

Means followed by different superscripts in the same column differ significantly according to Duncan's new multiple range test ( $p < 0.05$ ). SEM at 15 df.

TABLE 2. FARINOGRAPH CONSISTENCY OF NORMAL AND FRUIT BREAD DOUGHS

Sample no.	Farinograph water absorption (%)	Farinograph consistency of normal bread dough (B.U.)	Water absorption of fruit bread (%)
1	61.6	320	56.0
2	61.2	320	55.3
3	67.0	330	61.0
4	59.9	325	53.9

consistency of 320-330 B.U., as observed for normal bread dough, about 6 per cent water had to be reduced from the farinograph water absorption for fruit bread dough. This was

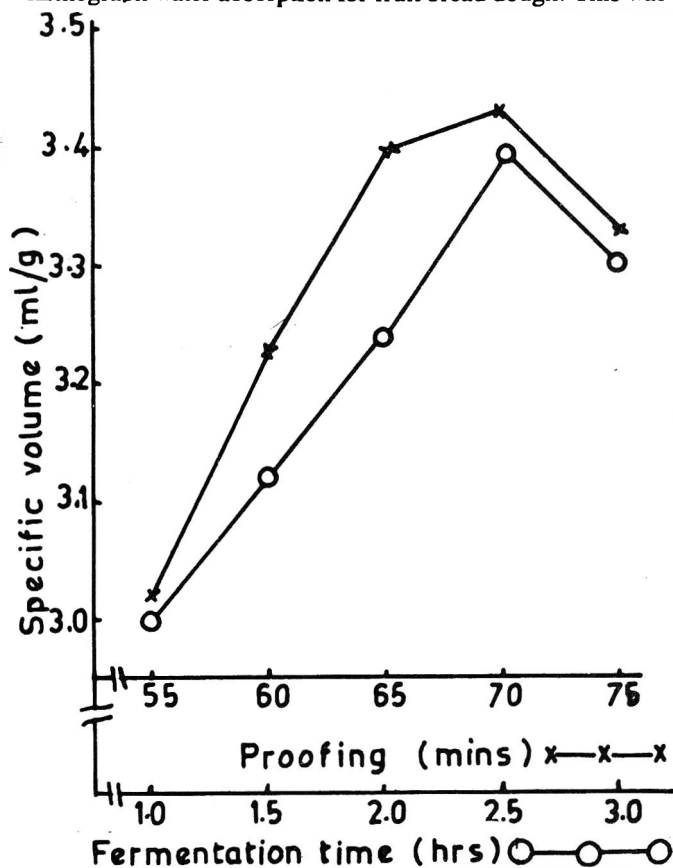


Fig. 1. Effect of proofing and fermentation time on specific volume of fruit bread.

found to be true in four different types of flour having varying water absorption.

*Effect of processing conditions:* Fruit bread dough required longer fermentation time (2½ hr) for getting maximum improvement in volume and texture. While the optimum fermentation time for a normal plain bread dough containing 2.5 per cent sugar and 2 per cent fat was found to be 1½ hr. Similarly, the optimum proofing time ranged from 65 to 70 min in contrast to 50-55 min for normal bread dough (Fig. 1).

*Effect of different bread making methods:* The specific volume and quality of bread made by different quantities of flour (50 to 90 per cent) and varying the sponge fermentation time (1 to 5 hr) indicated an optimum level of 80 per cent flour in the sponge and 4 hr sponge fermentation period. The bread made using the above conditions improved the loaf specific volume from 3.4 to 4.2 ml/g as compared to straight dough method (Table 3). The texture and taste of bread made by sponge and dough method were better than those made by either straight dough method or active dough development method.

*Rheological characteristics of fruit bread dough:* Farinograph characteristics of normal and fruit bread dough (Fig. 2) indicated slight difference in the dough characteristics. Mixing time for fruit bread dough was more by 2 min and stability was less by 4 min as compared to normal bread dough. Hardness values for normal bread dough (2.6 kg/V) made with farinograph flour water absorption was similar to that of fruit bread dough made by reducing about 6 per cent water from farinograph water absorption. This confirms that fruit bread dough requires 5-6 per cent less quantity of water for obtaining desired consistency.

Incorporation of 1 per cent fat did not affect the viscosity, though slight reduction was reported at 5 per cent level<sup>10,11</sup>. However, fat (1 per cent) and sugar (2.5 per cent) when added together, as in a normal bread, increased the peak viscosity to 3660 B.U. Sugar at 2.5 per cent level slightly increased the peak viscosity, but at 15 per cent level the increase was considerable. Addition of both sugar (15 per cent) and fat (5 per cent), as used in fruit bread dough greatly increased the peak viscosity from 3560 to 3900 B.U. (Table 4). Expansograph characteristics of normal and fruit bread doughs (Fig. 3) indicated negligible difference in the gas

TABLE 3. EFFECT OF DIFFERENT BREAD MAKING METHODS ON THE QUALITY OF FRUIT BREAD

Bread making method	Sp vol (ml/g)	Crust			Crumb		Overall quality
		Shape	Colour	Symmetry	Colour	Texture	
Straight dough	3.4	9.85 <sup>a</sup>	9.75 <sup>a</sup>	9.90 <sup>a</sup>	8.18 <sup>a</sup>	5.95 <sup>a</sup>	8.05 <sup>a</sup>
Sponge and dough	4.2	9.00 <sup>b</sup>	9.85 <sup>a</sup>	9.85 <sup>a</sup>	9.82 <sup>b</sup>	8.08 <sup>b</sup>	9.85 <sup>b</sup>
Active dough development	2.8	4.95 <sup>c</sup>	8.20 <sup>b</sup>	3.60 <sup>b</sup>	5.90 <sup>c</sup>	6.20 <sup>c</sup>	5.95 <sup>c</sup>
SEM (9 df)		±0.074	±0.097	±0.13	±0.08	±0.07	±0.08

Means followed by different superscripts in the same column differ significantly according to Duncan's new multiple range test ( $P < 0.05$ ).



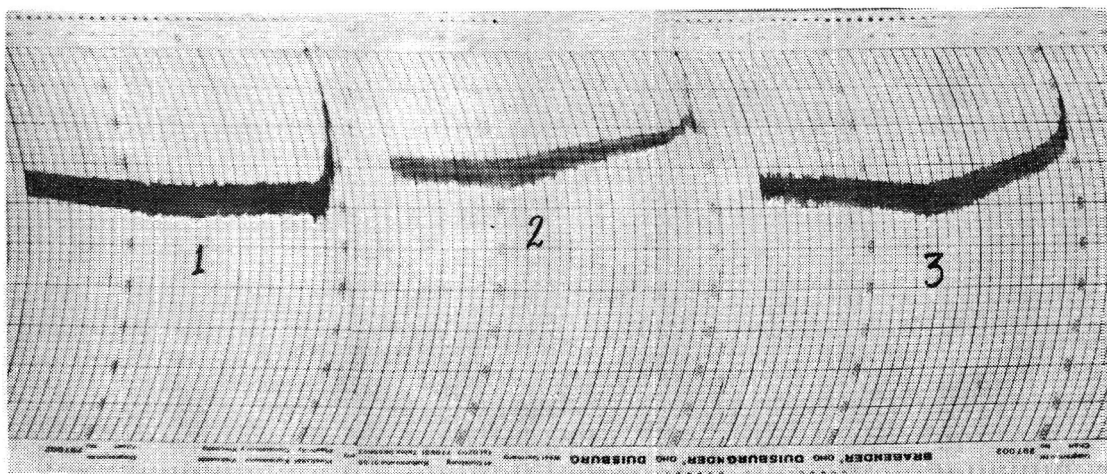


Fig.2. Farinograph characteristics of normal and fruit bread dough.

retention capacity among the doughs. However, the gas production rate was very slow in fruit bread dough as indicated by the longer time taken to reach the peak. Stability of the dough was also less in fruit bread dough.

*Rheological characteristics of fermenting doughs:* Changes in the rheological parameters in the fermenting fruit bread dough are given in Fig. 4. Though the spread ratio increased during fermentation in all the doughs studied, greater changes

were observed in fruit bread dough as compared to normal bread dough. The spread increased from 1.62 to 2.05 in case of fruit bread as compared to the increase from 1.44 to 1.65 observed for normal bread dough. The greater changes could be attributed to the presence of higher quantity of sugar and fat which are known to weaken gluten network, thereby making the dough less cohesive and more flowy<sup>5</sup>.

*Keeping quality of fruit bread:* Studies on storage indicated that fruit bread could be stored without mould growth for about 8 days. The bread made with the similar level of ingredients, but without fruits could also be kept for 8 days without mould growth. However, mould growth appeared in control bread containing 1 per cent fat and 2.5 per cent sugar within 3 days of storage. Delay in mould growth in fruit bread could be attributed to presence of high fat and sugar<sup>12</sup>. The texture of bread (Table 5) showed higher hardness values for fruit bread as compared to bread made without fruits. The greater hardness of fruit bread could be attributed to its lowering gas retention capacity resulting in poor volume. The control bread made with only 1 per cent fat and 2.5 per cent sugar had a hardness value of 7.1 kg/V

TABLE 4. EFFECT OF FAT AND SUGAR ON AMYLOGRAPH PEAK CONSISTENCY OF FLOUR

Fat (%)	Sugar (%)	Peak consistency (B.U.)
0	0	3560
1	0	3540
0	2.5	3590
1	2.5	3660
5	0	3510
0	15	3810
5	15	3900

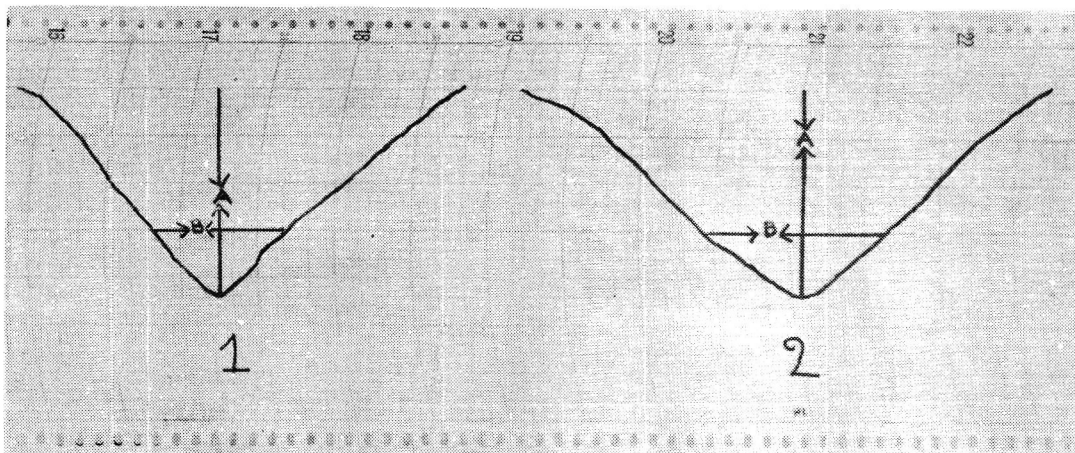


Fig.3. Typical expansograph characteristics of normal and fruit bread dough. 1. Gas retaining ability 2. Stability

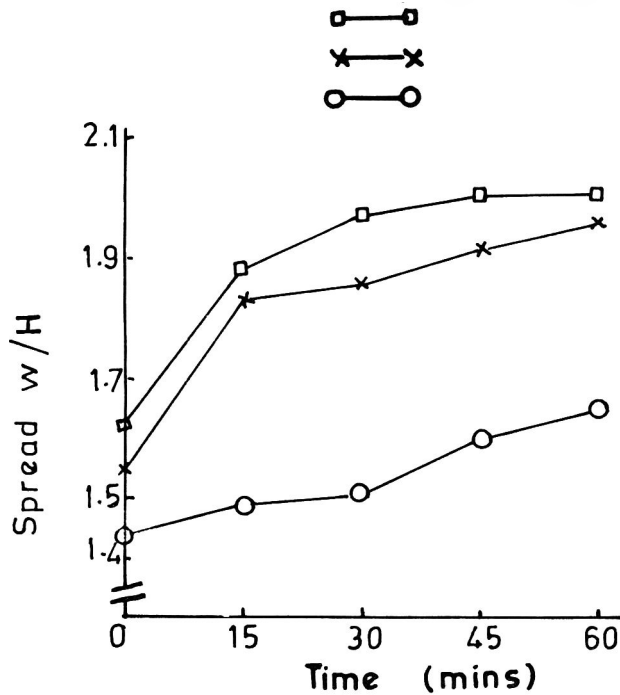


Fig.4. Spread ratio of fermented normal and fruit bread dough.

TABLE 5. CHANGES IN THE TEXTURE OF FRUIT BREAD DURING STORAGE

	Hardness (kg/V) at indicated days of storage			
	Fresh	1	2	3
Normal bread	7.1	7.6	8.3	8.7
Fruit bread	8.6	8.8	9.2	9.3
Fruit bread formula	6.4	6.7	7.0	7.3

in fresh bread which increased to 8.7 after 3 days of storage, whereas in fruit bread, the increase in hardness value was less (8.6 to 9.3 kg/V). This indicated that staling of bread is much slower in fruit bread. Addition of fruits did not considerably affect the staling.

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# Effect of Milling Methods on the Chemical, Rheological and Bread Making Characteristics of Whole Wheat Flour

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Whole wheat flour obtained by milling wheat in hammer, disc, stone and roller mills showed considerable variation in particle size distribution. The damaged starch content and diastatic activity ranged from 10.7 to 21.2% and from 187 to 380 mg of maltose/10g of flour respectively thereby reflecting variation in degree of severity of grinding in different mills. The dough raising capacity was also affected with the values ranging between 53.4 and 79.6%. The dough properties of flours showed considerable variation and the water absorption varied from 64.9 to 72.6%. Bread samples prepared from hammer and roller milled flours were better in quality than those from disc and stone milled flours.

Bakery products are becoming increasingly popular due to convenience, variety of taste and textural profiles. Generally, refined wheat flour (maida) is the main ingredient for the bakery products. Utilization of whole wheat flour in bakery products is potential from the view points of the diversification of its use and typical wholesome wheaty taste. The problems related to the use of whole wheat flour in bread have been reported by several workers<sup>1,5</sup>. The results of studies on the quality of whole wheat flours, milled in different mills, and their bread making characteristics are presented in this paper.

## Materials and Methods

Commercial *aestivum* wheat, procured from local market, was milled in hammer mill (HM), disc mill (DM), stone mill (SM) and roller mill (RM) to obtain the whole wheat flour. In roller flour mill, the bran and shorts obtained were re-ground by reducing the gap between the rolls of the break as well as reduction systems. The ground bran and shorts passing through 80 mesh sieve were mixed with straight run flour to make whole wheat flour. The particle size distribution of flours was determined in triplicate, using a Buhler Plan sifter.

Moisture, total ash, acid insoluble ash, Hagberg's falling number, diastatic activity, damaged starch were determined according to AACC procedures<sup>6</sup>. Crude protein (N x 5.7) was estimated by micro-Kjeldahl method. Dough properties of whole wheat flour were studied using the farinograph, extensograph, amylograph and mixograph according to standard AACC procedures<sup>6</sup>. Breads were prepared according to remix procedure of Irvine and McMullan<sup>7</sup> with a reduced fermentation of 120 min for the dough instead of

165 min. Evaluation of breads was carried out after 24 h by a panel of six judges. Duncan's new multiple range test was used for finding out the results of test of significance<sup>8</sup>.

## Results and Discussion

**Effect of milling:** The particle size distribution of flours (Table 1) indicates significant variation in the severity of grinding in different mills. The quantity of flour passing through 12xx sieve was highest in RM and HM flours followed by DM and SM flours.

**Chemical characteristics:** The total ash, acid insoluble ash and protein contents of flours milled in different mills were 1.48-1.54, 0.02-0.04 and 11.8-11.9 per cent, respectively. Falling number, diastatic activity and damaged starch content varied among samples significantly with variation in particle

TABLE 1. PARTICLE SIZE DISTRIBUTION OF WHOLE WHEAT FLOUR OBTAINED FROM DIFFERENT MILLS

Sieve	Opening (μ)	Overtailings (%)				SEM
		Hammer mill.	Disc mill	Stone mill	Roller mill	
32	670	0.1 <sup>a</sup>	0.6 <sup>b</sup>	0.1 <sup>a</sup>	—	+0.00
45	480	0.7 <sup>a</sup>	2.4 <sup>b</sup>	0.8 <sup>a</sup>	0.1 <sup>c</sup>	+0.04
7xx	193	0.5 <sup>a</sup>	1.7 <sup>b</sup>	0.2 <sup>c</sup>	0.2 <sup>c</sup>	+0.04
10xx	129	31.1 <sup>a</sup>	34.0 <sup>b</sup>	36.4 <sup>c</sup>	5.7 <sup>d</sup>	+0.06
12xx	112	62.1 <sup>a</sup>	49.1 <sup>b</sup>	38.8 <sup>c</sup>	62.5 <sup>d</sup>	+0.07
15xx	85	3.0 <sup>a</sup>	5.6 <sup>b</sup>	13.2 <sup>c</sup>	24.2 <sup>d</sup>	+0.07
25P	62	0.5 <sup>a</sup>	3.7 <sup>b</sup>	6.9 <sup>c</sup>	3.4 <sup>d</sup>	+0.03
Pan	—	2.0 <sup>a</sup>	2.9 <sup>b</sup>	3.6 <sup>bc</sup>	3.9 <sup>c</sup>	+0.25

Means in the same row followed by different superscripts differ significantly ( $P < 0.05$ ).

size (Table 2) due to the difference in severity of grinding. The damaged starch content and diastatic activity were highest and lowest in SM and HM flours respectively. Similar values of damaged starch content and diastatic activity for whole wheat flours from different mills were reported by Leelavathi *et al*<sup>9</sup> who studied the chapati making quality of differently milled whole wheat flours. The flours also showed significant differences in dough raising capacity, which indicates gas retention properties. These values in SM and DM flours reflect the poor gas retention properties of the doughs from these flours.

**Rheological characteristics:** Farinograph water absorption of whole wheat flour was highest in SM flour (Table 3). The water absorption was found to increase with the increase in damaged starch content of the flours. Sharma and Bains<sup>10</sup> have also reported highly significant relation between damaged starch content and the water absorption of whole wheat meals. The effect of milling on farinograph dough development time and mixograph peak time may be attributed to the variation in particle size distribution of flours. The flours from HM and RM showed higher strength and farinograph valorimeter value as compared to DM and SM flours. The amylograph gelatinization temperatures of whole wheat flours were in the range of 58.5-61.5°C (Table 3). The peak viscosity, which showed inverse relation to  $\alpha$ -amylase activity, was maximum in HM flour. These values are generally in agreement with the falling number and diastatic activity values of flours (Table 2).

The dough properties measured in extensograph exhibited highest resistance to extension and extensibility with HM flour (Table 3). Both DM and SM flours showed similar values for resistance to extension and extensibility. The HM flour had highest dough strength with an area value of 77.2 cm<sup>2</sup>.

**Bread making quality:** The specific loaf volume of HM flour was highest followed by that of RM flour. Bread from both flours had normal crust shape, brown crust colour, medium fine and uniform crumb grain and soft crumb texture

TABLE 2 EFFECT OF MILLING METHOD ON THE CHEMICAL CHARACTERISTICS\* OF WHOLE WHEAT FLOUR

Type of mill	Falling No.	Diastatic activity**	Damaged starch (%)	Dough raising capacity (%)
Hammer mill	561.2 <sup>a</sup>	187.0 <sup>a</sup>	10.7 <sup>a</sup>	79.6 <sup>a</sup>
Disc mill	486.0 <sup>b</sup>	272.8 <sup>b</sup>	14.3 <sup>b</sup>	59.6 <sup>b</sup>
Stone mill	479.8 <sup>c</sup>	380.0 <sup>c</sup>	21.2 <sup>c</sup>	53.4 <sup>c</sup>
Roller mill	530.0 <sup>d</sup>	192.8 <sup>d</sup>	11.7 <sup>d</sup>	68.0 <sup>d</sup>
SEM	+0.34	+0.49	+0.07	+0.08

Means in the same column followed by different superscripts differ significantly (P < 0.05).

\*Values expressed on 14% moisture basis

\*\*mg of maltose/10g flour

TABLE 3. EFFECT OF MILLING METHOD ON THE RHEOLOGICAL AND BREAD MAKING CHARACTERISTICS OF WHOLE WHEAT FLOUR

Characteristics	Hammer mill	Disc mill	Stone mill	Roller mill
<b>Farinograph</b>				
Water absorption (%)	64.9	70.5	72.6	65.9
Dough development time (mm)	3.5	4.0	4.5	5.0
Dough stability (min)	4.0	2.0	3.0	5.0
Mixing tolerance index at 20 min (BU)	80	120	120	100
Valorimeter value	58	52	52	58
<b>Mixograph</b>				
Peak time (min)	2.0	2.0	3.0	3.0
Peak height (cm)	5.4	5.5	5.5	5.4
Weakening angle (°)	10	10	10	8
Area (cm <sup>2</sup> )	63.2	48.4	46.5	60.8
<b>Extensograph</b>				
Resistance to extension, R (BU)	440	320	330	420
Extensibility, E (mm)	133	113	110	128
Ratio figure (R/E)	3.3	2.8	3.0	3.3
Area (cm <sup>2</sup> )	77.2	50.5	46.9	68.7
<b>Amylograph</b>				
Gelatinization temp (°C)	61.5	60.0	61.5	58.5
Peak viscosity (AU)	570	500	500	520
<b>Bread making</b>				
Specific loaf vol (m/g)	2.31	2.15	1.97	2.17
Crumb grain score**	7.0	6.0	6.0	7.0

\*\*Maximum score - 8

with the grain score of 7.0. However, bread from DM and SM flours had slightly flat crust shape, 6.0 score with slightly coarse crumb and slightly hard texture. All the flours produced breads with typical wholesome wheaty taste.

The results indicated that milling method has a profound influence on particle size distribution, damaged starch content,  $\alpha$ -amylase activity, water absorption and mixing strength of the dough. These, in turn, influence the bread quality. The whole wheat flour obtained in hammer mill is best suited for use in bread making followed by that obtained in roller mill. The flours from disc and stone mills are not upto the mark.

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## Production of Medium Fat Soyflour by Dry-Extrusion-Expelling of Raw Soybean and Its Use in Bread Fortification

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Dry heat treatment of soybean through dry-extrusion-expelling was used to produce medium fat soyflour. The flour of the cake obtained after expelling the extrudate of coarsely ground soybean contained 3.3–5.3% moisture and 7–9% oil, while trypsin inhibitor was inactivated to an extent of 35.5–46.8%. Soyflour thus obtained was used at 12% flour substitution level for making bread, which was acceptable.

A medium fat soyflour can be useful in providing good quality protein to those population whose fat requirements are partially met from other sources. It is necessary to partially extract the oil from soybeans to lower the oil content of cake for obtaining soyflour with 6–9 per cent oil. It has been reported<sup>1</sup> that mechanical oil expelling of soybean requires pre-treatment such as steaming/flaking, and 2, 3, 4 passes. Extrusion has been widely used for the production of full fat soyflour with and without pre-conditioning of soybeans<sup>5,6</sup>. However, for medium fat soyflour production, the extrusion-expelling of pre-conditioned soygrits is advocated<sup>7</sup>. In this study, the extrusion-expelling was tried without pre-conditioning of soybeans and the flour quality was evaluated for its potential as protein-rich additive for nutritional improvement of bakery products<sup>8</sup>.

### Materials and Methods

**Soybeans:** 'Sherman' variety of soybean was coarsely ground in a Bauer Mill at the natural moisture level of 8.43 per cent dry basis. The ground soybeans had a bulk density of 700 kg/m<sup>3</sup>. The data on particle size distribution indicated that 5.5, 23.5, 17.2, 43.3, 5.9, 2.3 and 2.3% of the ground soybeans were retained on US sieve Nos. 7, 8, 10, 20, 40, 50 and pan, respectively. In an earlier study<sup>7</sup>,

over 66 per cent of the ground material was passed through US sieve No 20 and retained on No. 60. Thus, the ground soybean was coarser than the size reported by Nelson *et al.*<sup>7</sup>

**Equipment:** A single screw extruder (Insta-Pro. Model, 600 Jr, Insta-Pro International Des Moines: IA, USA) with L/D ratio of 7.34 was used. Its design is such that it can be used for dry or wet extrusion cooking. The extruder is powered by 50 HP motor and provided with two (main and side) raw material feeders. The hander (Japan) expeller powered with 15 HP motor and operated at around 70 r.p.m. was used.

**Extrusion-expelling:** The coarsely ground soybeans were extruded at various feed rates by using side feeder after calibration. The sequence of steam locks was 10-8-8-10; die diameter was 12.7 mm; and the extruder screw speed was 500 r.p.m. The barrel temperature at three locations, feeding rate and extrudate quality were allowed to stabilize for each run before recording data or taking samples. The extrudate was collected in a container and immediately fed to the expeller (Fig.1) which was operated at 8 threads pressure from full choke. Cake was collected for 2 min duration for each run. The temperatures of expeller barrel, oil and cake were measured for each run.



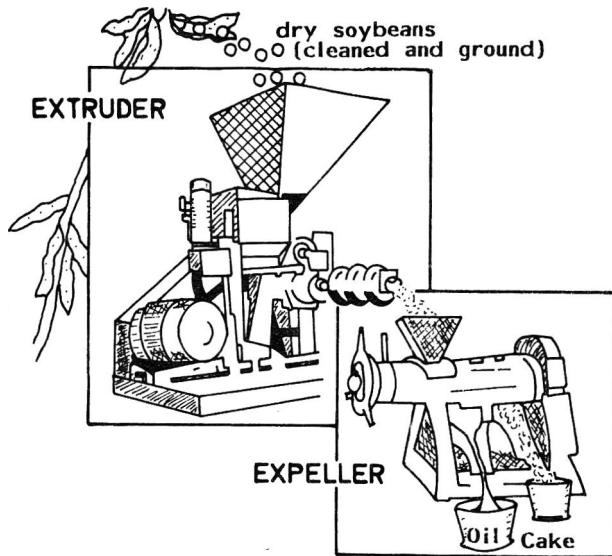


Fig.1. Schematic diagram for dry extrusion and expelling of soybean.

**Product quality evaluation:** The cake was allowed to cool and analysed for oil and moisture contents by AOAC methods<sup>9</sup> and trypsin inhibitor (T.I.) activity by the method of Hammerstrand *et al.*<sup>10</sup>. The colour of medium fat soyflour was measured with Hunter colour meter (model D-25 A 9) and expressed in terms of whiteness and a/b ratio.

The K-State process<sup>11</sup> was followed in bread making with 12 per cent wheat flour supplementation. Other ingredients used were 480 ml water, 14 g active dry yeast, 14 g salt, 42 g sugar and 21 g shortening in both the cases. In addition, 3.5 g sodium stearoyl-2-lactylate (SSL) was added in case of soy-fortified bread. The soyflour obtained after extrusion-expelling at the feeder dial setting of 35 was used in baking test. Also, the medium fat soyflour produced by method of extrusion expelling of conditioned soybeans<sup>7</sup> was used at 12 per cent level for comparison of product quality. The bread acceptability was evaluated by using the Bread Score Report (American Institute of Baking, Manhattan, KS, USA), for judging the bread quality on various accounts. A trained panel of nine members evaluated the soy supplemented bread in comparison with standard white bread, giving maximum allowed score to it. The data were statistically analysed and the acceptability was judged by the significance tested within the products evaluated.

## Results and Discussion

**Extrusion:** Feed rate of coarsely ground whole soybean varied with the feeder dial setting and feeder screw speed, as:

$$Y = 10.000 \times X - 53.780 \quad (r=0.999) \quad \dots (1)$$

where,

$$Y = \text{feed rate, kg/hr}$$

$$X = \text{dial setting}$$

and

$$Y = 4.570 \times X - 33.290 \quad (r=0.998) \quad \dots (2)$$

Where,

$$Y = \text{feed rate, kg/hr}$$

$$X = \text{screw speed, rpm}$$

The extruder barrel temperature was observed at three locations where first is near feeder side and third near extruder die. The respective temperature ranges for the first, second and third locations were 88-103, 105-110 and 125-130°C. The extrusion temperature obtained during this set of studies was within the suggested<sup>7</sup> temperature limits of 121-148°C. Extrudate temperature ranged between 107 and 120°C. The extrudate was in the form of semi-fluid material with oil oozing out from the extrudate due to high temperature short time treatment and tissue disruption during extrusion.

The trypsin inhibitor (T.I.) inactivation for the extrusion duration of 25-30 sec linearly varied with the increase in feed rate to the extruder and was negatively correlated as:

$$Y = 61.475 - 0.101 \times X \quad (r = -0.975) \quad \dots (3)$$

Where

$$Y = \text{trypsin inhibitor inactivation after extrusion, per cent}$$

$$X = \text{feed rate to the extruder, kg/hr}$$

This is because of less heat treatment to the material during extrusion at higher feed rates. This aspect is further clarified when the T.I. inactivation is just half the original for the double feed rate than original. On the other hand, the whiteness of the extrudate after milling to flour appears to depend more on both the heat treatment and oil oozing than on heat treatment (feed rate) thereby giving a fairly positive correlation as

$$Y = 50.351 + 0.021 \times X \quad (r=0.869) \quad \dots (4)$$

Where,

$$Y = \text{whiteness (100 for standard white)}$$

$$X = \text{feed rate to the extruder, kg/hr}$$

In this study, the maximum T.I. inactivation of 43.45 per cent was achieved as against 91 per cent reported in a study conducted using pre-conditioned finer soy grits in the moisture content range of 10-14 per cent.

**Expelling:** Since the extrudate was fed manually to the expeller, some variation is found in throughput of the expeller (Table 1). The oil was expelled in one pass only, with expeller

TABLE I. EXPELLING PROCESS CONDITIONS

Dial setting (extruder)	Feed rate to extruder (kg/hr)	Expeller throughput (kg/hr)	Expeller barrel temp (°C)	Oil temp (°C)	Cake temp (°C)
25	196.57	180.90	77	92	94
30	246.56	167.40	77	77	82
35	296.55	195.75	82	96	97
40	346.54	216.00	82	98	92
45	396.53	216.00	82	91	94

barrel temperature ranging from 77 to 82°C. However, in an earlier study<sup>1</sup> where the steamed soyflakes were expelled in three passes to get 6-12 per cent residual oil under different conditions, expeller barrel temperatures were 110, 125 and 135°C for first, second and third passes, respectively. The combination of extrusion-expelling therefore, is advantageous for lower process temperatures and reduced number of passes to get about the same residual oil content (Table 2). The oil and cake temperatures varied from 77-98°C and 82-97°C respectively and were dependent on throughput of expeller (Table 1). The extrudate with oil oozing out, when expelled and analysed for T.I. inactivation (Table 2) indicated the following relationship:

$$Y = 64.578 - 0.103 \times X \quad (r=-0.961) \quad \dots (5)$$

Where

Y = trypsin inhibitor inactivation after expelling, per cent

X = feed rate to extruder, kg/hr

However, when correlated with expeller throughput it gave a relationship with slightly inferior correlation ( $r=0.812$ ):

$$Y = -575.21 + 3.464 \times X \quad (r=0.812) \quad \dots (6)$$

Where

Y = trypsin inhibitor inactivation, per cent.

X = expeller throughput, kg/hr

The better correlation of per cent T.I. inactivation in cake with feed rate to extruder reflects on the quantum of heat treatment received during extrusion over expelling. This is further clarified by only three per cent T.I. inactivation during expelling (intercepts of eqn. 3 and 5 with same slope).

In this study, it has been established that major portion of heat treatment received was during extrusion only. The residual oil content in the cake positively correlated well ( $r=0.952$ ) with feed rate to extruder than with throughput of expeller ( $r=0.900$ ):

$$Y = 4.901 + 0.02 \times X \quad (r=0.952) \quad \dots (7)$$

Where,

Y = residual oil content in cake, per cent

X = feed rate to extruder, kg/hr

and

$$Y = 0.481 + 0.040 \times X \quad (r=0.900) \quad \dots (8)$$

Where,

Y = residual oil content in cake, per cent

X = expeller throughput, kg/hr.

This reflects on the role of feed rate to extruder in disruption of bean tissue cells containing the bodies known as spherosomes depositing soybean oil<sup>12</sup>. The contribution of extrusion conditions to processing can be rated as more significant than expelling conditions. However, the overall cake quality in terms of colour of soyflour may be predominantly affected by oil expelling and to some extent with the degree of heat treatment during extrusion. The expeller throughput, which was influenced by manual feeding, could not yield any direct and acceptable correlation ( $r = -0.079$ ) with whiteness of soyflour. Also, the extrusion was mainly responsible for improved oil recovery and thereby colour of soyflour. The soyflour had a light creamy yellow colour with a/b ratio in the range of 0.073 to 1.0 (Table 2).

*Nutritional quality aspects:* The residual oil content of medium fat soyflour ranged from 7 to 9 per cent and is comparable with earlier studies<sup>17</sup>. Trypsin inhibitor inactivation ranged from 25 to 47 per cent and was more effectively inactivated at lower feed rates. Though the level of inactivation was appreciably less than 91 per cent reported<sup>7</sup>, it should be enough if the medium fat soyflour is to form an ingredient of a food product where it is expected to receive additional moist heat. Soyflour has been reported to balance amino acids in cereal-based diets and bakery products<sup>5,13,14</sup> and give good nutritional responses. It has good amounts of nutritional components<sup>7</sup>. Other advantages of presence of soyhulls in the product have been reported as good source of dietary fibre<sup>15</sup>, excellent source of iron<sup>16</sup> and blood cholesterol lowering food ingredient<sup>17</sup>. Overall, the product type exhibits good nutritional profile.

*Baking test:* The possibility of incorporation of medium fat soyflour in bread making at the 12 per cent supplementation level, as recommended by earlier workers<sup>11,18,19</sup> was tried. Properties of bread studied confirmed the earlier reports<sup>15,19</sup> on higher water absorption (199.7 and 199.8 g bread weight as well as 34.96 and 35.00 per cent moisture content) in case of medium fat (35 EE) and medium fat<sup>7</sup> breads and lower bread volume (2.41 and 2.65 cc/g for these breads) compared to standard white bread which showed 193.83 g bread weight, 2.95 cc/g specific volume and 33% moisture content. The quality of bread produced using the medium fat soyflour (35 EE) was compared with the bread with INTSOY medium fat soyflour and standard white bread. The properties of these breads prepared under similar conditions indicate the reduction in loaf volume and increase in loaf weight due to higher moisture retention by soy supplemented breads. The specific volume of bread with medium fat soyflour (35 EE) was lower as compared to other breads. The data on acceptability score, and its analysis of variance<sup>20</sup> (Table 3) revealed that the difference between the quality of bread supplemented with medium fat soyflour

TABLE 2. EXPELLED PRESSCAKE QUALITY

Dial setting (extruder)	Moisture (% d.b)	Oil (%)	% T.I. inactivation	a/b ratio	Soyflour whiteness
Raw	8.43	20.73	0.00	0.086	69.80
25	3.30	7.06	46.83	0.080	62.90
30	4.77	7.62	37.76	1.000	65.60
35	4.58	8.52	31.27	0.082	64.20
40	4.92	9.28	—	0.073	63.60
45	5.25	9.11	25.53	0.093	63.40

TABLE 3. QUALITY CHARACTERISTICS AND ACCEPTABILITY SCORE OF MEDIUM FAT SOYFLOUR SUPPLEMENTED BREAD (SOYFOUR : 12% ON FLOUR WEIGHT BASIS).

Quality character	Bread		
	Soyflour (35 EE)	Soyflour INTSOY*	Standard White
External characteristics (Vol. symmetry, crust colour, baking, crust break and shred)	26.50 <sup>a</sup> ± 3.6	29.4 <sup>b</sup> ± 1.7	35.00 <sup>c</sup>
Internal characteristics (Texture, grain, crumb colour slicing)	25.2 <sup>a</sup> ± 3.2	25.9 <sup>a</sup> ± 2.8	30.00 <sup>c</sup>
Eating qualities (Aroma, taste, mouthfeel)	30.20 <sup>a</sup> ± 3.5	29.9 <sup>a</sup> ± 3.5	35.00 <sup>c</sup>
Overall quality	81.9 <sup>a</sup> ± 7.9	85.2 <sup>a</sup> ± 6.7	100.00 <sup>c</sup>

\*By method of Nelson *et al.*, 1987. Maximum allowable points were assigned for standard white bread. Means ± S.D. for 9 panelists. Means not having a common superscript in a row are significantly different ( $P < 0.05$ ) by analysis of variance.

(35 EE) produced by direct extrusion, and extrusion of pre-conditioned soybeans<sup>7</sup> was non-significant ( $P < 0.05$ ) with respect to internal characteristics, eating quality and overall bread quality. This indicates that the process time and pre-conditioning cost can be lowered by adopting the approach suggested in this paper. The product needs improvements in external characteristics, specially the loaf volume. Also, the medium fat soyflour can be tried in a variety of traditional food products to suit the local requirements. Studies on use in traditional food products would be a desirable step for utilization of nutritionally rich-by-product of extrusion-expelling of soybeans for attending to malnutrition problems and gainfully utilizing the advantages of such energy efficient systems for developing world.

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## Storage Stability of Full-fat Soy Flour and Soy-Wheat Flour Blend

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**Raw full fat soy flour (RSF), processed full fat soy flour (FFSF) with and without antioxidant and soy-wheat flour blend (SWF), stored at different temperatures and relative humidities (RH), showed that the temperature and RH had pronounced effect on storage stability of the flours which were found to be more stable at lower temperatures and RH ranges than at higher ones. Thermally processed and prepared FFSF had good keeping quality and could be stored safely for more than 4 months either alone or in combination with wheat flour. Addition of antioxidant had little effect in checking peroxide formation of the flour during the later stages of the storage.**

Because of its increasing popularity, soybean is assuming a significance in human diet. However, careful processing is required to ensure good flavour, acceptability, desirable functional properties and nutrient stability. Full-fat soy flour can be easily incorporated into wheat flour which is extensively used in different Indian food items. The problems in effective utilization of full-fat soy flour are its storage stability due to the presence of highly unsaturated fats<sup>1</sup> and loss in functional properties of such high protein flours<sup>2</sup>. There is little information available on the storage stability of soy flours. Being hygroscopic, soy flour may absorb moisture from the atmosphere resulting in increased free fat acidity (FFA)<sup>3</sup>. High moisture along with high temperature adversely affects the nitrogen solubility index (NSI) of the soy flour during long term storage<sup>4</sup>. In view of these, it was considered necessary to monitor the storage behaviour of processed full-fat soy flour under different conditions of temperature and RH. Since the FFSF has been proposed to be used primarily for the fortification of wheat flour, studies were also conducted on the shelf-life of the soy-wheat flour blend.

### Materials and Methods

Cleaned and sound soybeans (cv. 'Bragg') were dehulled, the splits were water blanched at 100°C for 5 min, dried at 60°C to 7-8 per cent (db) moisture content in a tray dryer and milled to FFSF in a hammer mill. Details of the process technology have been described earlier<sup>5</sup>. The FFSF was then treated in four different ways, i.e., i) 1 kg of FFSF was mixed with 3 kg wheat flour obtained from the local market to give a soy-wheat-flour blend (SWF) of 1:3, ii) 3.5 kg of FFSF was thoroughly mixed with 0.02 per cent (v/w) butylated hydroxy anisol (BHA) and butylated hydroxy toluene (BHT) (1:1) dissolved in a small quantity of absolute

ethanol, iii) 3.5 kg of FFSF was used as such, and iv) raw full-fat soy flour (RSF) made from whole soybeans was used as control.

The storage temperature and RH were so chosen as to represent the relative variations prevalent during the major seasons of the region. The relative humidities were obtained by using saturated salt solutions in desiccators placed in incubators or refrigerators maintained at the desired temperatures. Samples (450g) (7 Nos.) from each were filled into polyethylene bags (50-60 gauge) and stored at 5,15,25 and 40°C at varying RH for 120 days. Samples were drawn initially and at 10,20,30,45,60,90 and 120 days of storage periods and analysed for moisture<sup>6</sup>, free fatty acids (FFA)<sup>7</sup>, peroxide value (PV)<sup>8</sup>, nitrogen solubility index (NSI)<sup>9</sup> and insect infestation<sup>10</sup>.

### Results and Discussion

The initial moisture contents of RSF and FFSF samples were found to be 7.1 and 6.3 per cent (db) respectively. The moisture contents of samples increased at all the temperatures and RH levels of storage studied (Table 1). However, the increase was less pronounced in the FFSF compared to the RSF samples, irrespective of whether the former was blended with wheat flour or not. This reduced moisture rise in FFSF may be due to the denaturation of proteins (as indicated by NSI values) during the thermal processing of soy splits while preparing the product<sup>11</sup>. No appreciable difference was noted in the moisture absorption rate of FFSF samples stored with or without antioxidants. The temperature and RH combinations, however, had some effects. The minimum absorption of moisture during storage was noted at 40°C and 40 per cent RH, followed by those at 15°C and 43 per cent RH. It seems that RH had greater effect than temperature on the absorption of moisture by the stored samples. Moisture

TABLE 1. MOISTURE CONTENT OF FLOUR SAMPLES STORED AT DIFFERENT TEMPERATURE (°C) AND RELATIVE HUMIDITY (% RH) LEVELS

Storage temp. (°C)	RH (%)	Moisture (%) at indicated days of storage							
		0	10	20	30	45	60	90	120
<b>Raw full fat soy flour</b>									
5	59	7.1	7.5	7.8	8.3	8.8	9.4	10.3	10.8
15	45	7.1	7.1	7.2	7.2	7.8	8.4	9.0	9.6
15	92	7.1	7.5	7.9	8.5	9.1	9.8	10.6	11.5
25	43	7.1	7.1	7.1	7.3	7.5	8.0	8.5	9.1
25	90	7.1	7.4	7.6	7.9	8.4	8.8	9.0	10.5
40	40	7.1	7.1	7.1	7.2	7.3	7.5	8.1	8.8
40	91	7.1	7.3	7.4	7.7	8.0	8.6	9.1	10.0
<b>Full fat soy flour</b>									
5	59	6.6	6.7	6.8	7.1	7.4	7.9	8.2	8.9
15	45	6.6	6.7	6.8	7.0	7.4	7.7	7.9	8.2
15	92	6.6	6.8	6.9	7.2	7.6	8.3	8.8	9.4
25	43	6.6	6.8	6.9	7.2	7.3	7.5	7.6	8.1
25	90	6.6	6.7	6.8	7.0	7.3	7.5	8.1	8.7
40	40	6.6	6.7	6.7	6.9	7.0	7.3	7.5	7.7
40	91	6.6	6.7	6.8	7.2	7.4	7.7	8.2	8.5
<b>Soy-wheat flour blend</b>									
5	59	6.5	6.6	6.6	7.0	7.4	7.9	8.4	9.0
15	45	6.5	6.6	6.6	6.8	7.2	7.6	7.8	8.3
15	92	6.5	6.7	6.8	7.0	7.5	8.2	8.9	9.7
25	43	6.5	6.5	6.6	6.8	7.1	7.3	7.7	8.2
25	90	6.5	6.6	6.7	6.9	7.4	7.7	8.3	8.2
40	40	6.5	6.5	6.5	6.7	6.8	7.1	7.5	7.9
40	91	6.5	6.5	6.6	6.9	7.3	7.5	8.2	8.7
<b>Full fat soy flour + antioxidant</b>									
5	59	6.6	6.7	6.8	7.0	7.5	7.9	8.3	8.8
15	45	6.6	6.7	6.8	6.9	7.1	7.4	7.7	8.0
15	92	6.6	6.7	6.8	7.1	7.5	8.2	8.6	9.2
25	43	6.6	6.7	6.7	6.8	7.1	7.3	7.6	7.9
25	90	6.6	6.7	6.8	7.1	7.3	7.7	8.1	8.5
40	40	6.6	6.6	6.7	6.8	7.0	7.2	7.5	7.6
40	91	6.6	6.7	6.8	6.9	7.3	7.4	7.8	8.1

absorption increased with the increase in RH, but decreased with the increase in temperature of storage. At a given temperature, however, the samples at higher RH absorbed moisture faster than those at lower ones.

The effects of temperature and RH levels on FFA development in the stored samples are presented in Fig 1. The initial FFA value in the RSF was 0.58 per cent, in contrast to 0.41 and 0.14 per cent for FFSF and SWF (1:3), respectively. The lower value of FFA in the FFSF was due to thermal inactivation of lipolytic enzyme and in the blend due to a relatively smaller proportion of the FFSF. With progress of storage, FFA increased in all RSF samples, particularly at higher levels of temperature and RH. On the other hand, FFSF samples with or without wheat flour showed no appreciable development of FFA at any temperature and RH level. The higher values of FFA in RSF

samples were due to higher lipolytic activity. Temperature of storage had a somewhat greater effect on the development of FFA. At lower temperature and RH levels, the rise in FFA content was at a slower rate compared to that at higher temperature and RH levels. Samples stored at all temperatures showed faster rate of rise in FFA at higher RH levels than at lower ones.

The effect of temperature and RH on the changes in PV during storage is illustrated in Fig 2. There was a sharp increase in PV of RSF samples stored at all temperatures and RH levels. In FFSF samples, on the other hand, the rise in PV during storage was smaller. This was due to the inactivation of the enzyme lipoxigenase. Addition of antioxidants had only limited effect on checking peroxide development in FFSF. This was evident during the later period of storage at high temperature and RH levels. The PV of RSF

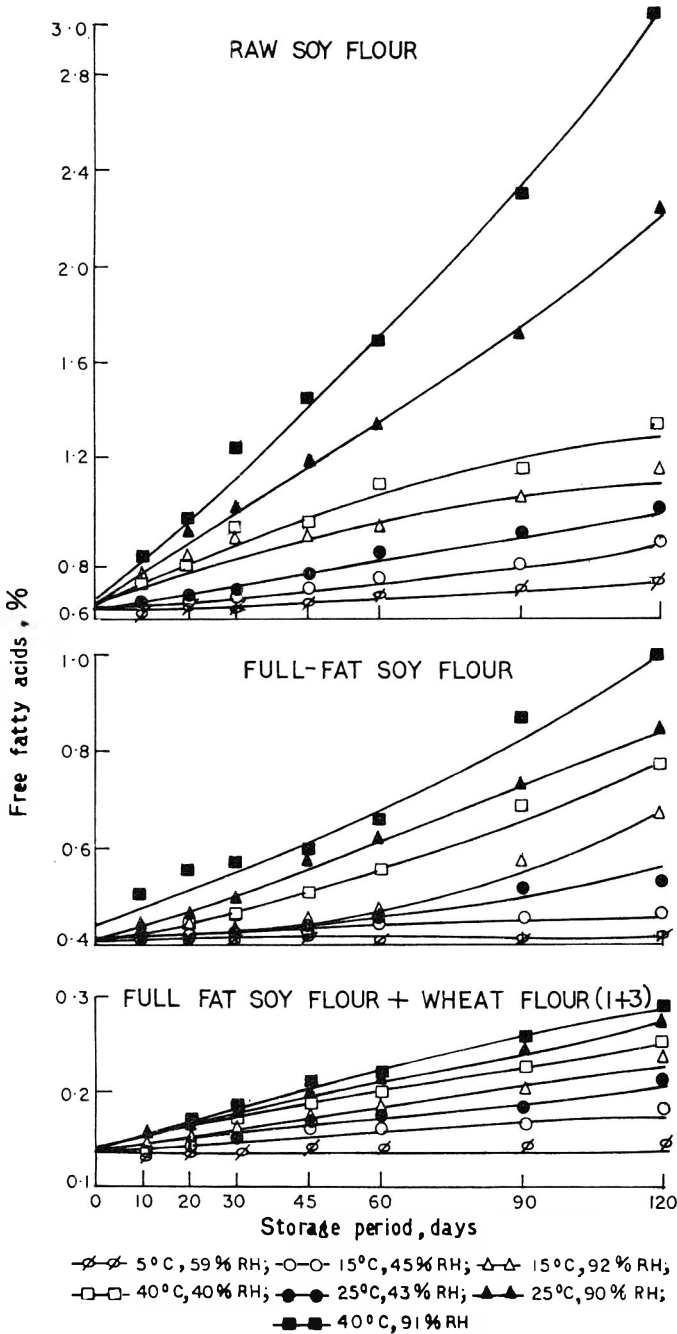


Fig.1: Changes in free fatty acids in flour samples stored at different temperatures (°C) and relative humidity (RH) levels.

and FFSF with antioxidant samples stored at 40°C and 91 per cent RH for 120 days was recorded as 4.25 and 2.98 meq per kg of oil, respectively. Pratt<sup>12</sup> observed no appreciable effect of addition of antioxidant on peroxide development in soy flour. Addition of wheat flour to FFSF brought down the initial PV of the sample substantially, and hence, the rise in PV of WSF samples during storage was at a lower rate as compared to FFSF samples.

Effects of storage conditions on NSI are presented in Fig 3. The initial NSI of RSF samples was 80.15 per cent, whereas

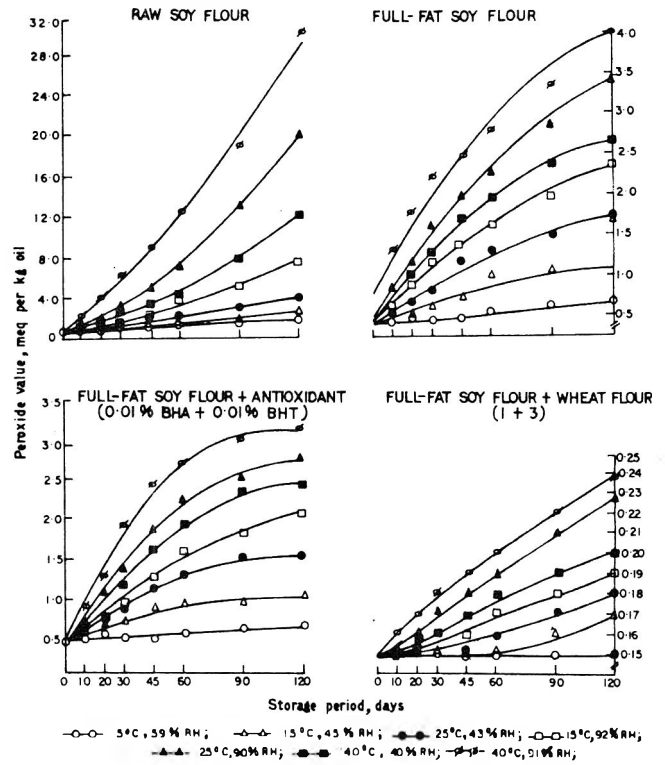


Fig.2. Development of peroxides in flour samples stored at different temperatures (°C) and relative humidity (RH) levels.

the same for FFSF and SWF samples were 35.15 and 23.63 per cent, respectively. The NSI decreased during storage, the decrease being more pronounced in RSF samples than in FFSF samples with or without antioxidants. However, no appreciable change occurred in any sample under refrigerated storage condition. It is evident that as the storage temperature and RH increased, the NSI decreased faster and with greater magnitude. The maximum decrease was recorded in the samples stored at the highest temperature (40°C) and RH (91 per cent) in all the cases. At any temperature, lesser reduction in NSI was observed at lower RH than at higher RH levels. Decline in NSI during storage has also been reported in whole soybeans<sup>4</sup> and in soy flour<sup>13</sup>.

RSF was more susceptible to infestation than FFSF and WSF. No infestation was recorded upto four months in any of the FFSF and WSF samples. However, the RSF became infested after two or three months depending upon whether the RH was higher or lower.

In conclusion, it may be stated that the FFSF and WSF samples were more stable than RSF samples probably because of the denaturation of enzymes during thermal processing of soybeans. Both temperature and RH had pronounced effect on storage stability of the flours, the influence being more prominent at lower temperature and RH ranges than at higher levels.

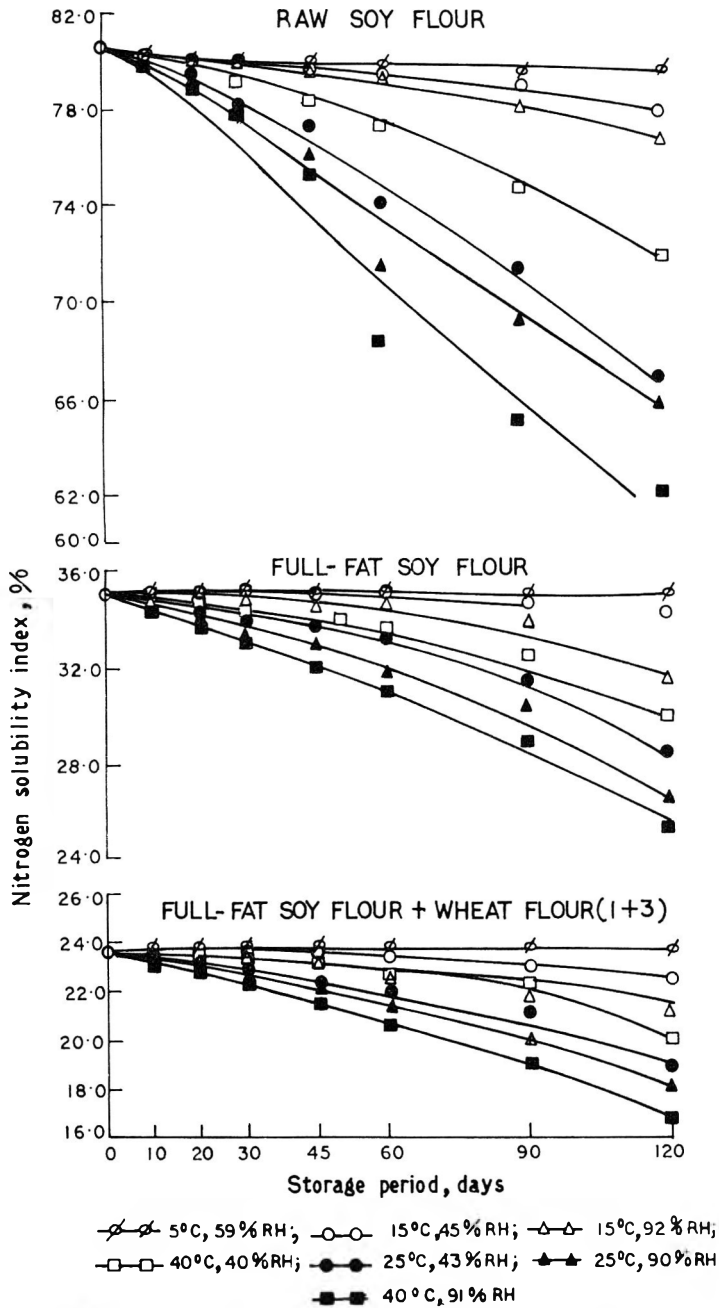


Fig.3. Changes in nitrogen solubility index of flour samples stored at different temperatures ( $^{\circ}\text{C}$ ) and relative humidity (RH) levels.

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## Seed Mycoflora of Some Spices

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Survey of fungal contaminants of bishopweed, black pepper, coriander and cumin from district Sagar of Madhya Pradesh, India showed occurrence of *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *Penicillium*, spp., *Rhizopus arrhizus*, *R. Stolonifer* and *Syncephalastrum racemosum*. However, their frequency varied widely. Samples of test spices yielded slightly lower fungal species with the use of spice extract agar medium as compared to malt-salt agar.

Depending on chemical nature, different organic substrates harbour different microorganisms. These may spoil the quality of the substrate by discolouration, formation of foul odour, change of chemical make up or production of toxic substances. Spices are consumed daily in India and hence, microbiological examination of spices is important from the points of view of their quality which can be affected by contaminating microorganisms during storage. Contamination of various toxigenic moulds in spices is also known<sup>1,2</sup>, but no data are available for spices from Central India. The present paper reports the major fungal contaminants of four test spices.

### Materials and Methods

**Mycoflora:** In all, 100 samples of 4 spices viz; bishopweed (*Trachyspermum ammi*), black pepper (*Piper nigrum*), coriander (*Coriander sativum*), cumin (*Cuminum cyminum*) were collected in sterile polythene bags from different markets of district Sagar (M.P.) and stored at  $2 \pm 1^\circ\text{C}$ , if not processed immediately. The seeds (about 200 Nos.) were plated on sterilized malt-salt agar medium (20 g malt extract, 20 g NaCl, 20 g agar-agar, 1000 ml distilled water) and spice extract agar medium (spice extract 200 ml, agar-agar 20 g, distilled water 800 ml) following "Direct plate method" as suggested by ISTA<sup>3</sup>. For the preparation of spice extract, 20 g of each spice was powdered and boiled separately with 200 ml distilled water for 20 min. The extract was filtered and diluted with water to 200 ml. The media used were sterilized by autoclaving at  $121^\circ\text{C}$  for 20 min. The plates were incubated at  $28 \pm 1^\circ\text{C}$  and observed daily after 3 days. The fungal growth appearing over the surface of the seeds or on media adjacent to plated seeds was picked up and transferred to the fresh culture medium for purification. The fungi were identified using standard methodologies<sup>4,5</sup>. While few cultures were

identified at CAB International Mycological Institute, Kew, England, U.K.

The per cent frequency and relative density of each fungal species were calculated as follows:

$$\% \text{ Frequency} = \frac{\text{No. of samples from which an organism was isolated}}{\text{Total no. of samples tested}} \times 100$$

$$\text{Relative density (\%)} = \frac{\text{Total no. of colonies of an organism}}{\text{Total no. of colonies of all organisms}} \times 100$$

**Mycotoxin production:** Potentiality of dominant *Aspergillus* species to elaborate mycotoxin on spices have been tested by following the method of Flannigan and Hui<sup>10</sup>.

### Results and Discussion

During the present survey, a total of 26, 21, 20 and 25 fungal species were recorded from the samples of bishopweed, black pepper, coriander and cumin, respectively, when these samples were plated on malt-salt agar medium containing 2 per cent NaCl. However, a little less number of fungal species appeared when the same samples were plated on spice extract agar medium. The details of per cent frequency and relative density of each fungal species occurring in test spices are given in Tables 1 and 2.

The data indicate the presence of *A. flavus*, *A. niger*, *A. ochraceus*, *Curvularia lunata*, *Emericella regulosa*, *Penicillium* spp., *Rhizopus arrhizus*, *R. stolonifer* and *Syncephalastrum racemosum* in all the four spices. Other fungal forms were recorded from fewer number of test spices and their distribution was less frequent with poor density. Plating of washing of test spice samples on malt-salt agar medium indicated no significant variation in the recovery of total number of fungal contaminants as compared to direct plating of test spice samples on malt-salt agar medium. Surface sterilized seeds have yielded only 2-3 per cent fungal



TABLE 1. FUNGAL CONTAMINANTS OF BISHOPWEED AND BLACK PEPPER

	Malt-salt agar				Spice extract agar			
	Bishopweed		Black pepper		Bishopweed		Black pepper	
	Frequency (%)	Relative density (%)	Frequency (%)	Relative density (%)	Frequency (%)	Relative density (%)	Frequency (%)	Relative density (%)
<i>Acrophialophora fusispora</i>	8	0.4	—	—	—	—	—	—
<i>Aspergillus flavus</i>	100	10.5	84	87.6	84	13.3	76	37.8
<i>A. fumigatus</i>	80	9.2	76	16.6	60	7.8	52	16.7
<i>A. nidulans</i>	4	0.1	—	—	—	—	—	—
<i>A. niger</i>	88	20.4	36	5.9	80	19.1	32	6.1
<i>A. ochraceus</i>	32	0.6	40	2.9	—	—	28	5.9
<i>A. terreus</i>	40	1.2	8	0.6	12	2.2	8	0.6
<i>Aspergillus</i> sp.	8	1.5	—	—	4	0.7	—	—
<i>Chaetomium globosum</i>	12	1.1	—	—	12	3.9	8	0.6
<i>Chaetomium</i> sp.	4	0.7	—	—	—	—	—	—
<i>Cladosporium herbarum</i>	24	1.4	—	—	12	1.2	—	—
<i>C. Sphaerospermum</i>	32	3.0	—	—	16	4.5	—	—
<i>Curvularia lunata</i>	16	0.4	12	0.9	8	0.9	4	0.3
<i>C. pallesense</i>	8	0.3	—	—	16	3.9	—	—
<i>Emericella nidulans</i> var. <i>echinulata</i>	—	—	8	0.5	—	—	4	0.3
<i>E. rugulosa</i>	40	1.7	12	0.2	4	0.3	—	—
<i>Fusarium</i> spp.	24	2.9	12	0.2	12	3.3	20	1.7
<i>Helminthosporium haviense</i>	—	—	16	0.6	—	—	8	0.5
<i>Helminthosporium</i> sp.	28	2.0	—	—	12	1.5	—	—
<i>Penicillium</i> spp.	60	4.0	48	2.6	72	14.1	40	12.7
<i>Rhizopus arrhizus</i>	84	28.9	64	23.5	60	17.2	40	12.7
<i>R. stolonifer</i>	20	1.9	60	3.9	8	2.0	36	4.7
<i>Syncephalastrum racemosum</i>	20	0.7	16	0.7	4	0.3	—	—
<i>Thielavia</i> sp.	4	0.3	—	—	—	—	—	—
<i>Thielaviopsis</i> sp.	32	1.9	44	1.3	4	0.9	—	—
<i>Trichothecium roseum</i>	8	0.3	—	—	4	0.6	—	—
Unidentified spp.	40	3.3	28	1.2	12	1.4	52	5.7
	(2)		(5)		(2)		(3)	

Number given in parentheses indicates the number of unidentified species. — = Not detected.

species of the total contaminating fungi in most of the test spice samples (unpublished data). Surface sterilized seeds have yielded species of *Aspergillus* (i.e., *A. fumigatus*, *A. terreus*) and some dematiaceous (*Helminthosporium*) and coelomycetous (*Phoma*) fungi. This indicates that majority of fungi occur as superficial contaminants in spices due to the contamination during their harvesting, drying in farms or marketing. For the enumeration of contaminating fungi from spices, in general, malt-salt agar medium was found suitable in comparison to spice extract agar medium. On the other hand, spice extract agar was found to provide an artificially selective growth condition comparable to those found in whole spices for fungal growth. Thus, fungi appearing on spice extract agar indicate their potentiality to utilize various ingredients of spices as their sole source of nutrients and to deteriorate them during storage. *Aspergillus*

*flavus* and *A. niger* have been found as major contaminants in test spice samples (Tables 1 and 2). These fungi were found to synthesize aflatoxin B<sub>1</sub> during their growth on bishopweed, black pepper, coriander and cumin samples. A number of species belonging to genus *Aspergillus* are known to produce mycotoxins on a variety of substrates during their growth<sup>11-15</sup>. The present paper indicates the importance on surveys for the enumeration of microbial contaminants and evaluation of their ability to produce mycotoxins in various spices.

#### Acknowledgement

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TABLE 2. FUNGAL CONTAMINANTS OF CORIANDER AND CUMIN

	Malt-salt agar				Spice extract agar			
	Coriander		Cumin		Coriander		Cumin	
	Frequency (%)	Relative density (%)	Frequency (%)	Relative density (%)	Frequency (%)	Relative density (%)	Frequency (%)	Relative density (%)
<i>Aspergillus flavus</i>	76	14.9	96	10.2	60	18.2	84	12.6
<i>A. fumigatus</i>	24	1.7	—	—	16	4.6	—	—
<i>A. nidulans</i>	—	—	24	1.2	—	—	—	—
<i>A. niger</i>	80	21.0	100	23.6	56	14.9	96	21.4
<i>A. ochraceus</i>	4	0.5	20	1.7	4	0.3	24	4.0
<i>A. terreus</i>	—	—	16	1.2	—	—	4	0.6
<i>Aspergillus</i> spp.	—	—	8	0.1	—	—	—	—
<i>Cephalosporium acremonium</i>	—	—	4	0.2	—	—	—	—
<i>Chaetomium globosum</i>	—	—	8	0.4	—	—	4	0.1
<i>Chaetomium</i> sp.	—	—	—	—	—	—	20	3.6
<i>Corynascus sepedonium</i>	—	—	—	—	12	3.3	—	—
<i>Curvularia clavata</i>	—	—	28	3.3	—	—	28	4.4
<i>Curvularia lunata</i>	20	2.2	52	7.3	12	1.7	32	7.6
<i>C. pallesense</i>	—	—	12	0.5	—	—	—	—
<i>Curvularia</i> sp.	12	1.0	28	2.5	4	0.3	8	0.6
<i>Emericella nidulans</i> var. <i>echinulata</i>	20	1.1	16	0.8	—	—	20	1.1
<i>E. nidulans</i> var. <i>latus</i>	—	—	32	2.2	—	—	—	—
<i>E. nidulans</i>	—	—	20	0.4	—	—	—	—
<i>E. rugulosa</i>	28	1.2	36	1.5	—	—	4	0.4
<i>Fusarium</i> spp.	12	1.0	—	—	8	0.9	—	—
<i>Helminthosporium haviense</i>	4	0.4	16	1.0	—	—	8	1.8
<i>Helminthosporium</i> sp.	—	—	20	0.6	—	—	8	0.3
<i>Myrothecium roridum</i>	—	—	16	0.7	—	—	8	0.3
<i>Penicillium</i> spp.	4	0.0	88	14.8	—	—	52	9.8
<i>Rhizopus arrhizus</i>	96	41.0	80	17.3	52	20.3	56	10.3
<i>R. stolonifer</i>	12	3.2	56	4.0	60	32.6	52	15.3
<i>Rhizopus</i> sp.	20	5.4	—	—	—	—	—	—
<i>Syncephalastrum racemosum</i>	24	3.6	44	2.4	16	2.4	32	3.7
<i>Verticillium</i> sp.	8	0.2	—	—	—	—	—	—
Unidentified spp.	12	0.9	16	1.0	—	—	8	0.6

(3)

Number given in parentheses indicates number of unidentified species. — = Not detected.

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## Changes in Solubility, $\beta$ -Carotene and Development of Non-enzymatic Browning of Spray-Dried, Foam-Mat-Dried and Freeze-Dried Whole Egg Powders Packed in Different Packaging Materials

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Drying conditions and packaging materials did not significantly influence solubility, destruction of  $\beta$ -carotene and development of non-enzymatic browning of spray-dried, foam-mat-dried and freeze-dried egg powders during storage at 4, 19-27 and 37°C upto 365 days. However, the storage at 42 and 55°C had significant effect on these attributes in all the three types of egg powders packed in cans and flexible pouches.

During storage, the dehydrated raw egg powder undergoes many undesirable changes such as loss of solubility, discolouration, development of off-flavour, destruction of nutrients and alterations in functional properties like sponge cake volume and foaming values. The loss of solubility, one of the most serious problems in the storage of dehydrated egg powder is mainly attributed to denaturation of proteins in egg melange and interactions of proteins and lipids. The loss of solubility can be minimized by removal of glucose present in the egg melange. The solubility index in egg powder has been widely used as main criterion for assessing the damage caused to proteins during drying and storage.

Egg yolk pigments contribute a pleasing colour to many foods, especially baked products, noodles, ice creams, custards, sauces and omelettes. The naturally occurring pigments in hen's egg yolk are mainly alcohol-soluble xanthophylls, lutein and zeaxanthin. Very little  $\beta$ -carotene and cryptoxanthin are present. These carotenoids lose their colour upon oxidation. Moreover, dark brownish to blackish compounds are also formed in stored whole egg powder and such browning is usually accompanied by undesirable changes in flavour and nutritive value. The factors responsible for such browning are temperature, moisture, oxygen, sugar, protein and water activity. Dutton and Edwards<sup>1</sup> have studied the changes in colour of carotenoids and their interactions in non-enzymic browning.

So far, no systematic work has been done on the changes in solubility, destruction of  $\beta$ -carotene content and the formation of brown colour in different types of egg powders packed using different packaging materials and stored at different temperatures. The results of such an investigation are reported in the present paper.

### Materials and Methods

Spray-dried egg powder was procured from M/s Foods and Inns Ltd., Bombay. The eggs of 'White Leg Horn' birds were procured and pre-treated as per the method of Rao *et al.*<sup>2</sup> for preparing foam-mat-dried and freeze-dried egg powders. The egg melange was de-sugared from an initial 400 to 20 mg per cent as per the method described earlier<sup>3</sup>.

Spray dried, foam-mat-dried and freeze-dried egg powders were packed in butter size cans (401×300) and in paper-aluminium foil polyethylene laminate (PFP) pouches of 0.02 mm under air and nitrogen. In addition, high density polyethylene (HDPE) 300 gauge bags were also used. The packages were stored at 4, 19-27, 37, 42 and 55°C for different periods and analyzed periodically.

The moisture content and the solubility were determined as per A.O.A.C.<sup>4</sup> method and modified Haenni method as described by ISI<sup>5</sup> respectively.  $\beta$ -carotene was estimated by extracting 5 g egg powder with 25 ml acetone in pestle and mortar. The extraction was repeated with small portion of acetone and final volume was made up to 100 ml. The extract was filtered through Whatman No. 1 filter paper. The absorbance of the extract was measured at 435 nm against the standard and the values are expressed as  $\mu$ g  $\beta$ -carotene in 100 g sample. The non-enzymatic browning (NEB) was determined in alcohol extract as per the method of Kannur *et al.*<sup>6</sup> with modification. Egg powder (5 g) was extracted with small quantity of absolute alcohol in pestle and mortar as the extraction was repeated two to three times and volume was made up to 100 ml and the extract was filtered through Whatman No. 1 filter paper. The per cent transmittance of the combined extract was read at 420 nm in Spectronic-20 colorimeter.

## Results and Discussion

The moisture contents in spray-dried, freeze-dried and foam-mat-dried whole egg powders were 2.15, 1.87 and 2.17 per cent, respectively. Though the egg powders were prepared by different drying methods initially, they had the same solubility value of 95.3 per cent (Table 1). Higher temperatures of 55°C and 42°C were chosen as these were prevailing during summer season in Defence Stores Depots. At 55°C storage, the decrease in solubility was higher in spray-dried egg powder than foam-mat-dried or freeze-dried egg powders at the end of storage period of 90 days. These changes at high temperatures are probably due to lipid peroxidation, lipid and protein interaction and non-enzymatic browning that set in during foam-mat-drying. The spray-drying is likely to aggravate the rate of deteriorative reactions. Oxygen has been reported<sup>7</sup> to be detrimental to the solubility and other functional characteristics of egg powders during storage and this fact was confirmed in the present study also. The decrease in the solubility value was higher and significant changes were observed in all the packaging materials used at 42°C. All the samples showed values lower than 80.0 per cent as specified by ISI.

There are no significant changes in solubility values of egg powders packed in cans and in flexible pouches under nitrogen at 4, 19-27 and 37°C upto a period of 365 days (Table 1). Iyengar *et al.*<sup>8</sup> have observed a loss of 5-6 per cent solubility in spray-dried egg powder packed in cans under nitrogen. Similar results but with lower loss in solubility were observed during storage at ambient temperature and these are in conformity with earlier observation<sup>7,9</sup>. The solubility value remained unchanged at 4°C during the entire period of study and all the samples showed about 80 per cent solubility as prescribed by ISI.

Table 2 shows the changes in  $\beta$ -carotene content of three types of egg powders packed in different packaging materials with air and under nitrogen and stored at 55°C and 47°C for a period of 90 days and 365 days respectively. The spray-dried egg powder initially contained 68  $\mu$ g  $\beta$ -carotene/100g egg powder which decreased to 20-40  $\mu$ g in different packs at 55°C (Table 2). However, air packed and HDPE packed samples showed higher loss of  $\beta$ -carotene. These reductions in carotenoid pigments are indicative of oxidative changes involving atmospheric oxygen either directly or indirectly or through chemical intermediates. Such losses were also reported by Baloch *et al.*<sup>10</sup> in dehydrated carrots and in case of foam-mat-dried and freeze, dried egg powders<sup>11,12</sup>. The loss of carotenoid pigment in egg powders does not seem to depend upon the moisture contents since moisture content in these egg powders were below 2 per cent. Similar observations were made by Dutton and Edwards<sup>13</sup>.

The stability of carotenoid seems to be better at storage of 42°C when compared to 55°C and HDPE packed samples showed more loss. Oxidative changes in  $\beta$ -carotene were more in case of air packed than nitrogen packed samples.

TABLE 1. CHANGES IN THE SOLUBILITY (%) OF EGG POWDERS PACKED IN DIFFERENT PACKAGING MATERIALS AND STORED AT DIFFERENT TEMPERATURES

Egg powder type	Storage period (days)	Cans		PPF laminate		HDPE
		N <sub>2</sub> pack	Air pack	N <sub>2</sub> pack	Air pack	
<b>55°C</b>						
Spray-dried	30	93.8	90.9	87.7	85.6	83.3
	60	85.6	61.4	83.3	61.4	71.1
	90	69.6	66.9	66.9	55.5	55.4
Foam-mat-dried	30	78.7	75.9	83.3	83.3	81.0
	60	75.9	70.1	73.1	70.1	70.1
	90	66.9	61.4	61.4	61.4	66.9
Freeze-dried	30	90.0	85.6	85.6	85.6	83.3
	60	85.6	81.0	78.6	73.1	73.1
	90	78.5	73.1	78.6	70.1	70.1
<b>42°C</b>						
Spray-dried	120	83.3	83.3	83.3	83.3	83.3
	240	75.1	73.1	75.1	70.1	70.1
	365	61.9	61.4	61.4	61.4	57.6
Foam-mat-dried	120	81.3	81.3	81.0	81.0	78.6
	240	73.1	70.1	70.0	75.9	70.0
	365	61.4	61.4	61.4	61.4	55.5
Freeze-dried	120	85.6	83.3	83.3	83.3	81.0
	240	75.9	73.1	75.9	75.9	70.0
	365	66.9	66.9	66.9	66.9	61.4
<b>37°C</b>						
Spray-dried	180	87.2	87.7	90.0	87.7	87.7
	365	83.3	78.5	81.0	78.5	81.0
Foam-mat-dried	180	87.7	87.7	87.7	87.7	87.7
	365	75.5	73.1	75.9	75.9	75.9
Freeze-dried	180	87.7	87.7	87.7	85.6	87.7
	365	83.3	78.5	81.0	78.5	81.0
<b>19-27°C</b>						
Spray-dried	180	93.5	93.5	93.5	91.7	93.5
	365	90.0	87.7	90.0	87.7	87.7
Foam-mat-dried	180	90.0	90.0	90.0	87.7	87.7
	365	85.6	83.3	83.3	81.0	81.0
Freeze-dried	180	91.7	91.7	91.7	91.7	90.0
	365	87.7	85.6	87.7	87.7	87.7
<b>4°C</b>						
Spray-dried	180	93.5	93.5	93.5	93.5	93.5
	365	91.7	90.0	93.5	91.7	91.7
Foam-mat-dried	180	93.5	93.5	93.5	93.5	93.5
	365	90.0	90.0	91.7	90.0	90.0
Freeze-dried	180	93.5	93.5	93.5	93.5	93.5
	365	93.5	93.5	93.5	93.5	93.5

Initial solubility was 95.3 per cent in all the samples obtained by different drying methods.

TABLE 2. CHANGES IN  $\beta$ -CAROTENE CONTENT ( $\mu$ G%) OF EGG POWDERS PACKED IN DIFFERENT PACKAGING MATERIALS AND STORED AT DIFFERENT TEMPERATURE

Egg powder type	Storage period (days)	Cans		PPF laminate		HDPE
		N <sub>2</sub> pack	Air pack	N <sub>2</sub> pack	Air pack	
55°C						
Spray-dried	30	56	44	52	42	40
	60	46	40	42	36	28
	90	40	30	40	28	20
Foam-mat-dried	30	60	54	60	50	48
	60	50	40	52	40	34
	90	48	40	48	36	28
Freeze-dried	30	60	50	60	50	48
	60	50	44	50	40	34
	90	50	42	50	40	30
42°C						
Spray-dried	60	60	58	60	58	54
	180	54	52	56	54	50
	365	56	48	52	50	46
Foam-mat-dried	60	66	62	66	62	60
	180	60	60	62	58	54
	365	56	52	60	50	48
Freeze-dried	60	66	62	68	60	60
	180	62	58	60	56	56
	365	58	52	60	50	52
37°C						
Spray-dried	60	64	60	60	60	58
	180	60	58	60	58	58
	365	56	56	58	56	54
Foam-mat-dried	60	72	68	72	68	66
	180	70	66	70	66	64
	365	68	62	66	62	60
Freeze-dried	60	72	68	72	68	66
	180	72	68	70	68	62
	365	68	62	68	64	60
19-27°C						
Spray-dried	90	64	62	64	62	60
	180	62	60	62	62	60
	365	60	58	60	58	58
Foam-mat-dried	90	72	70	72	70	70
	180	72	70	72	70	68
	365	70	70	70	68	66
Freeze-dried	90	74	72	74	72	70
	180	72	70	74	70	68
	365	72	70	72	70	66
4°C						
Spray-dried	90	68	68	68	68	66
	180	66	66	66	60	60
	365	64	62	64	64	62
Foam-mat-dried	90	76	76	76	76	74
	180	76	74	76	74	74
	365	74	72	74	74	72
Freeze-dried	90	76	76	76	76	76
	180	76	76	76	74	74
	365	74	74	74	72	72

The initial values were 68, 76 and 76  $\mu$ g/100 g spray-dried, foam-mat-dried and freeze-dried samples, respectively.

TABLE 3. CHANGES IN BROWNING (% TRANSMISSION) OF EGG POWDERS PACKED IN DIFFERENT PACKAGING MATERIALS AND STORED AT DIFFERENT TEMPERATURE

Egg powder type	Storage period (days)	Cans		PPF laminate		HDPE
		N <sub>2</sub> pack	Air pack	N <sub>2</sub> pack	Air pack	
55°C						
Spray-dried	30	76	76	77	77	74
	60	76	78	77	77	75
	90	76	76	76	78	76
Foam-mat-dried	30	74	72	72	70	71
	60	74	74	72	72	74
	90	74	74	72	72	75
Freeze-dried	30	73	73	74	75	74
	60	73	74	74	77	76
	90	76	77	77	77	75
42°C						
Spray-dried	60	74	75	74	76	74
	180	74	76	74	78	74
	365	76	76	77	78	73
	60	68	74	72	74	72
Foam-mat-dried	180	73	76	72	76	72
	365	76	76	76	76	76
	60	74	75	78	78	72
Freeze-dried	180	75	75	76	78	76
	365	74	76	77	77	75
	37°C					
Spray-dried	60	74	76	74	75	78
	180	76	78	74	76	78
	365	78	78	78	78	78
	60	68	68	68	68	68
Foam-mat-dried	180	74	75	76	74	74
	365	76	76	76	76	76
	60	74	76	77	77	78
Freeze-dried	180	76	78	77	77	78
	365	76	78	78	78	79
	19-27°C					
Spray-dried	90	75	75	74	75	74
	180	75	77	74	74	75
	365	75	77	74	74	76
	90	68	68	68	68	68
Foam-mat-dried	180	68	68	68	72	68
	365	70	70	70	73	69
	90	72	74	74	74	72
Freeze-dried	180	74	74	74	74	74
	365	75	72	75	73	74
	4°C					
Spray-dried	90	74	74	75	74	74
	180	76	77	74	74	74
	365	76	77	76	77	76
	90	68	68	68	68	68
Foam-mat-dried	180	68	68	68	68	68
	365	68	70	68	70	71
	90	74	74	75	74	74
Freeze-dried	180	74	74	76	75	75
	365	74	74	74	76	76

The initial values were 75, 68 and 71 per cent transmittance for spray-dried, foam-mat dried and freeze dried samples, respectively.

Lipid oxidation at this temperature would not be as high as that of  $\beta$ -carotene, though Bonner<sup>14</sup> suggested that lipid oxidation corresponded well with that of carotenoids. Since no appreciable amount of lipid oxidation was noticed in these samples, it can be assumed that lipids are oxidised preferentially and the oxidised lipid namely phospholipids act as antioxidant for  $\beta$ -carotene oxidation.

Table 3 indicates the changes in browning of egg powders packed in different packaging materials and stored at 4, 19-27 and 37°C. The rate of change at these temperatures was much lower than at higher temperatures. The loss in browning at these temperatures appears to be independent of the packaging materials and the atmosphere.

It is seen that absorbance values at 55°C have increased from 74-78, 68-75 and from 71-77 in spray-dried, foam-mat-dried and freeze-dried egg powders respectively within a period of 90 and 365 days (Table 3). A similar increase in absorbance values was observed both at 37°C and 42°C in all the egg powders packed both under air and nitrogen. Egg powders packed and stored at ambient temperature showed browning to a lesser extent in all the packs.

The data indicate that the changes in  $\beta$ -carotene and non-enzymatic browning are not significant at lower temperature. Even at high temperature, the loss of carotenoid is not well reflected in the increased non-enzymatic browning and the reasons for the same are not fully understood.

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## Evaluation of Wooden Boxes Fabricated from Lesser Valued Farm Tree Species for Packaging and Transportation of Plum

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'Santa Rosa' plums packed in the packages fabricated from lesser valued farm tree species were transported by truck to the market covering a distance of about 400 km. All the packing cases remained intact except cracking of one side strip in each single box fabricated from the woods of *Albizia Chinensis*, *Bombax ceiba* and *Ficus roxburghii*. The quality of fruits was found to be acceptable with respect to loss in net and gross weights, fruit damage, fruit firmness and total soluble solids.

India has got varied agroclimatic conditions suitable for growing specific kinds of fruits. The fruits grown in one area are transported to all over the country generally in wooden containers. It has been reported that improvement in packaging has contributed greatly to more efficient transportation and marketing of fresh fruits<sup>1</sup>. In Himachal Pradesh, the demand for packing material is simultaneously increasing every year with the increase in production of fruits. To meet this requirement, a tremendous pressure is being exerted on our forest resources thereby leading to disturbance in the ecosystem. In the last decade, efforts were made to use corrugated fibre board (CFB) cartons<sup>2</sup>. However, the use of these cartons has not given enough confidence to growers because of their susceptibility to moisture absorption, poor storage conditions at transit points, the hilly terrain and poor roads through which they are to be carried on human/mule backs. The present studies on packing and transporting of plum fruits in wooden boxes, prepared from the lesser valued farm tree species were, therefore, conducted so as to reduce the pressure on commercial forest tree species of long rotation. The alternate tree species selected had moderate to fast growing rate.

'Santa Rosa' plum fruits, from a local orchard near University campus were harvested at proper maturity and brought to packing shed where they were stored, graded and packed in wooden boxes fabricated from 10 different species of less valued trees viz. *Pinus roxburghii* (conventional species), *Ficus palmata*, *Ficus roxburghii*, *Albizia chinensis*, *Bombax ceiba*, *Toona ciliata*, *Celtis australis*, *Lannea coromandalica*, *Prunus puddum* and *Bauhinia variegata*. The inner dimension of each box was 35.0×20.0×12.5 cm. The packed boxes were loaded on mule back and transported to road head, about 1 km away. The boxes were then loaded on the truck and transported to Delhi covering a distance of about 400 km. Observations like net weight of 10 marked fruits, gross weight of box alongwith fruits, bruising damage, at the time of packing and immediately upon reaching Delhi were noted. Fruit pressure of representative sample was

measured with 'Effegi' fruit pressure tester. Total soluble solids were ascertained using hand refractometer calibrated to 20°C. The experiment was carried out in complete randomised design<sup>3</sup>. There were three replications for a treatment and each replication had one box thereby leading to a total of 30 packing cases.

*Condition of the boxes:* The boxes remained intact during transportation except cracking of one strip in one replication of *Ficus roxburghii*, *Albizia chinensis* and *Bombay ceiba*; but this has not damaged the fruits. Loosening of nails was also observed in *Ficus palmata* and *Pinus roxburghii*. No damage was observed in other boxes which may be due to their stronger and tough character. Safe transportation of apple packed wooden boxes along with CFB carton during transportation has also been reported<sup>4</sup>.

*Net weight and gross weight of fruits:* There were significant losses in net and gross weights of fruits (Table 1). The minimum net weight loss was recorded in *Pinus roxburghii* and *Prunus puddum* boxes. This may be due to lesser porosity of the wood. In contrast, the maximum net weight loss of fruits was recorded in *Albizia chinensis* boxes. Minimum significant loss in gross weight of fruits was recorded in *Toona ciliata*, *Celtis australis* and *Lannea coromandalica* as compared to maximum recorded in *Albizia chinensis* boxes, which may be due to loss of moisture from the green toon leaves used as cushioning material. Similar results have been reported by other workers with the use of different types of wooden and other containers for apple<sup>2</sup>.

*Bruising damage:* The data presented in Table 1 reveal significant damage to fruits in different wooden boxes. The damage to fruits during transportation was minimum in *prunus puddum* and maximum in *Albizia chinensis* and *Ficus roxburghii* boxes. The bruising damage of 36 per cent has been reported in conventional wooden boxes<sup>5</sup>.

*Fruit firmness:* Maximum fruit firmness loss was recorded in *Ficus roxburghii* which was closely followed by *Ficus palmata*, *Bombax ceiba* and *Albizia chinensis* in contrast to minimum loss in *Prunus puddum*. The fruit firmness loss in

TABLE 1. QUALITATIVE EVALUATION OF PLUM FRUITS AFTER TRANSPORTATION

Wood used for box	Net wt loss (%)	Gross wt loss (%)	Bruising damage (%)	Fruit firmness loss (lb)	Total soluble solids (°B)
<i>Pinus roxburghii</i>	1.1 (1.0)	2.1 (1.5)	7.1 (2.7)	3.1	1.0 (1.0)
<i>Ficus palmata</i>	2.0 (1.4)	2.0 (1.4)	7.6 (2.7)	4.1	1.8 (1.3)
<i>Ficus roxburghii</i>	4.4 (2.1)	2.3 (1.5)	8.5 (3.0)	4.9	1.2 (1.1)
<i>Albizia chinensis</i>	5.0 (2.3)	3.5 (1.9)	10.1 (3.2)	4.0	1.5 (1.2)
<i>Bombax ceiba</i>	3.3 (1.8)	2.7 (1.6)	8.4 (2.9)	4.1	1.2 (1.1)
<i>Toona ciliata</i>	2.9 (1.7)	1.8 (1.4)	5.5 (2.3)	3.1	0.9 (1.0)
<i>Celtis australis</i>	1.8 (1.4)	1.8 (1.4)	4.4 (2.4)	3.5	0.9 (1.0)
<i>Lannea coromandalica</i>	2.8 (1.6)	1.8 (1.4)	4.4 (2.2)	3.7	0.9 (1.0)
<i>Prunus puddum</i>	1.3 (1.1)	2.6 (1.6)	4.4 (2.1)	3.0	0.6 (0.8)
<i>Bauhinia variegata</i>	2.4 (1.5)	2.1 (1.5)	7.1 (2.7)	3.9	1.2 (1.1)
C.D. (0.05)	0.28	0.04	0.33	—	0.15

Data in parenthesis are square root transformed values.

wooden containers is possibly due to the hygroscopic nature of wooden boxes and more breakdown of protopectins into soluble pectins or pectic acids.

**Increase in total soluble solids:** There was increase in the total soluble solids (TSS) of fruits during transportation. The maximum increase in TSS of fruits was recorded in *Ficus palmata*, which was at par with *Albizia chinensis* as against the minimum increase in *Prunus puddum*. The increase in TSS of fruits is probably caused by the hydrolysis of insoluble starch into sugars and higher respiration rate of fruits inside the boxes.

From the above findings, it can be concluded that all the experimental wooden boxes except *Albizia chinensis* and *Ficus roxburghii* are suitable for packing the plum fruits.

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## Water Vapour Transmission Rates of Multi-layer Flexible Packaging Materials

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**Water vapour transmission rate values of co-extruded films having different webs and laminates at conditions of  $38 \pm 1^\circ\text{C}$  with  $90 \pm 2\%$  relative humidity and  $27 \pm 1^\circ\text{C}$  and  $65 \pm 2\%$  relative humidity indicated a linear relationship between RH and WVTR for low density polyethylene while hydrophilic polyamide containing composites indicated dependence on RH. The water vapour transmission rate of multi-layer films containing polyamide as the core layer ranged between 4.1 and 5.4  $\text{g/m}^2 \cdot \text{day}$  at  $38 \pm 1^\circ\text{C}$  and  $90 \pm 2\%$  relative humidity, while monofilms of polyolefins had values between 1.9 and 3.0  $\text{g/m}^2 \cdot \text{day}$ .**

Permeation of packaging materials to water vapour is of utmost importance in the selection of suitable packaging materials<sup>1</sup> as all food products are sensitive to moisture interchanges. Data on the water vapour transmission rates (WVTR) are also required<sup>2,3</sup> to predict the shelf-life of the packaged products under different climatic conditions. In recent years, newer types of multi-layer flexible packaging materials have made significant inroads in food packaging field. These include polyester (PET or polyethylene terephthalate), polyamides (PA or nylons), ionomers (Surlyns) and co-polymers of ethylene-acrylic acid (EAA or Primacor). Another recent entrant in this field<sup>4</sup> is the multi-layer co-extruded films. These are made of polyolefinic plastic such as low-density polyethylene (LDPE), linear LDPE (LLDPE), high-density polyethylene (HDPE) and polypropylene (PP). When dissimilar webs are co-extruded, they are bonded by proprietary bonding agents<sup>5</sup> such as Admer, Nucrel and CXA compositions. These form the "tie" layer between the films.

Although WVTR data are available for individual plastic films at the accelerated condition of  $38^\circ\text{C}$  and 90 per cent relative humidity (RH) gradient, information regarding the composite films and values at normal conditions and at different relative humidities is scarce. The present work was undertaken to provide complete WVTR data. The most ubiquitous LDPE and multi-layer structures comprising polyester and polyamide with good gas and aroma barrier properties<sup>6</sup> were selected to study the effect of RH on the transmission behaviour. Another objective of the study was to determine the WVTR values of different co-extruded films and also the variations in values of a composite film having similar plies.

The materials tested were: (i) Low-density polyethylene (75 micron), (ii) plain polyester (12 micron)/LDPE (100 micron), (iii) polyamide (30 micron)/ionomer (60 micron)

and (iv) LLDPE/BA/PA/BA/EAA (100 micron) where BA stands for bonding agent. The different co-extruded structures studied are listed in Table 1. WVTR values were determined at  $38 \pm 1^\circ\text{C}$  with 90 per cent RH or  $27 \pm 1^\circ\text{C}$  with 65 per cent RH according to the Bureau of Indian Standards method<sup>7</sup>. Relative humidity was maintained by using appropriate saturated salt solutions<sup>8</sup>. Standard WVTR aluminium dishes with exposed sample area of 50  $\text{cm}^2$  were used in quadruplicate.

The WVTR values of LDPE, PET/PE, PA/Ionomer and the co-extruded film, LDPE/BA/PA/BA/EAA at  $27^\circ\text{C}$  indicate that the last three packaging materials have higher values especially at above 70 per cent RH (Fig. 1). At the high humidity condition of 90 per cent, LDPE was found to be a good barrier with a WVTR of 1.3 while the PA/Ionomer showed higher dependence on RH, in consonance with the multi-layer moisture adsorption above 70 per cent RH<sup>8</sup>. Similar results have been reported by Davis<sup>10</sup> for hydrophilic cellulose films and de Leiris<sup>11</sup> for PA. This suggests that WVTR is not directly proportional to RH, but exhibits relatively higher permeation rates per unit vapour pressure gradient at higher RH levels than at lower RH levels. In case of PET/PE and PA based co-extruded films, the effect of RH was found to be less pronounced. In the latter film, PA, being the core layer, is protected on either side by LDPE and EAA indicating that it is preferable to have hydrophilic PA as the middle layer.

Table 1 shows WVTR data of a range of co-extruded plastic films at two conditions of RH and temperature. At  $38^\circ\text{C}$  and 90 per cent RH, the values ranged between 4.1 and 5.4  $\text{g/m}^2 \cdot \text{day}$  while the corresponding values at  $27^\circ\text{C}$  with 65 per cent RH varied between 1.2 and 1.5. The variations in the values could be attributed to differences in the thickness of barrier layers and/or variations in their densities. The ratio of WVTR values obtained at  $38^\circ\text{C}$  and 90 per cent RH

TABLE 1. WATER VAPOUR TRANSMISSION RATES OF CO-EXTRUDED FILMS

Film structure	Thickness $\mu\text{m}$	WVTR $\text{g}/\text{m}^2 \cdot \text{day}$ at	
		38°C/90% RH	27°C/65%RH
LLDPE/BA/BA/EAA	90	4.6-5.4	1.4-1.5
-do-	100	4.1-4.3	1.2-1.3
PP/BA/PA/BA/EAA	90	4.1	1.1
HDPE/BA/PA/BA/EAA	125	2.9-3.0	0.9
HDPE/LDPE/EAA	130	2.1-2.3	0.6
HDPE/LDPE/HDPE	100	2.1-2.2	0.7
LLDPE/LDPE/HDPE/HDPE/EAA	130	1.9	0.6
HDPE/LDPE/LDPE	130	1.9	0.6

LDPE: Low density polyethylene, LLDPE: Linear low-density polyethylene, HDPE: High density polyethylene, PP: Polypropylene, EAA: Ethylene-acrylic acid copolymer, PA: Polyamide, BA: Bonding agent.

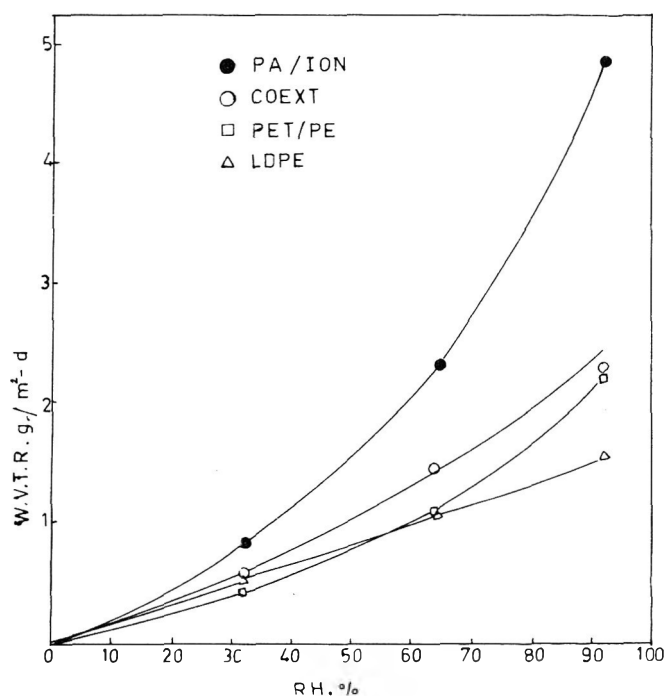


Fig. 1. Water vapour transmission rates of packaging materials at different relative humidities. PA/ION: Polyamide/Ionomer; COEXT: Co-extruded; PET/PE: Polyester/polyethylene LDPE: Low-density polyethylene.

condition to those of 27°C and 65 per cent RH were between 3.0 and 3.5. This suggests that the shelf-life of a food product with respect to moisture interchanges would be nearly three fold at the normal conditions vis-a-vis those at the accelerated conditions.

Further, the results indicate that extrapolation of data obtained, as generally practised at the accelerated condition for the prediction of WVTR at lower temperature/RH conditions, could lead to erroneous estimates in the case of hydrophilic film structures. This, in turn, would lead to selection of costlier barrier films. Thus, it is imperative to obtain WVTR data at use conditions for computing actual shelf-life of packed food products.

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## Development of A Mathematical Model to Predict Kinetics of Osmotic Dehydration

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**A mathematical model based on mass balances to study the kinetics for osmotic dehydration was developed. The equation is useful for any geometrical configuration and was tested using published data on apple, beef and pineapple. In all cases of relatively short time experiments, good predictions were obtained for long drying times until equilibrium was reached.**

During recent years, only a few works have been reported on osmotic dehydration of foodstuffs<sup>1-8</sup>. Some authors<sup>3,5,9</sup> used models developed for a flat plate geometry as defined by Crank<sup>10</sup>. Lenart and Flink<sup>11</sup> formulated an equation based on experimental data to predict solid gain in potato cubes. In these reports, it was demonstrated that water loss, soluble solids gain and weight loss of the foodstuff behaved similarly with respect to time. The curves displayed in the plots reported by these authors show an asymptotic trend when equilibrium has been reached. In order to study in more detail the kinetics of dehydration of a food, it is necessary to put long work hours for experimentation, sampling and analysis, sometimes without being able to reach equilibrium due to external problems, such as spoilage of sample due to long times needed to equilibrate.

The purpose of the present work was to develop and test the applicability of an equation or model that, irrespective of the geometrical shape of the foodstuff, may predict the kinetics of osmotic dehydration, along with the concentrations of the diffusing substances upon equilibrium. For this, short time experimental data published by other authors were used.

*Equation development:* Very few mathematical models have been successfully employed to study the osmotic dehydration process. Among these, the most widely known are: 1) Crank's<sup>10</sup> sorption equation for a semi-infinite medium, which has limitations such as: i) the geometry of the foodstuff must be a flat slab, ii) it provides good fits only during the first stages of dehydration, iii) a large osmotic solution to foodstuff ratio is required, and iv) the equilibrium point has to be experimentally determined. 2) Crank's<sup>10</sup> sorption equation in a solution of limited volume, which also

has some disadvantages: vizi i) limited to flat slab geometry, ii) the equilibrium point has to be experimentally determined, and iii) its use is rather cumbersome. In addition to these two models, other equations have been proposed for specific foodstuffs<sup>7,11</sup>. Their major restriction is narrow applicability, besides the fact that they were derived from Crank's equation for a semi-infinite medium, carrying along its limitations.

Next, the development of a simple model is presented. The equation is useful to study the process of osmotic dehydration, without any of the weaknesses described above for other models.

Performing a mass balance on water movement inside the foodstuff:

$$\begin{array}{l} \text{Fraction of water} \\ \text{lost by the foodstuff} \\ \text{at time "t"} \end{array} = \begin{array}{l} \text{Fraction of water lost} \\ \text{by the foodstuff when} \\ \text{equilibrium is reached} \\ \text{"t" = } \infty \end{array} - \begin{array}{l} \text{Fraction of water that} \\ \text{can diffuse out, but} \\ \text{remains in the foodstuff} \\ \text{at time "t"} \end{array} \quad (1)$$

If we define WFL = fraction of water lost by the foodstuff at time t, WFL $\infty$  = fraction of water lost by the foodstuff at equilibrium, and WFS = fraction of water that can diffuse out, but which remains inside the foodstuff at time t. Equation (1) can be written in terms of these variables as:

$$WEL = WFL\infty - WFS \quad (2)$$

In this equation, WFL $\infty$  has a fixed value for the established conditions of temperature and concentration of the osmotic solution. On the other hand, WFL and WFS are functions of the rate of water loss and time; however, WFL increases as these later variables also increase, whereas WFS decreases. From this, we may suggest that the relationship between WFL and WFS is:

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$$WFS = \frac{WFL}{K} \tag{3}$$

where  $K = f(\text{time, rate of water loss})$

The rate of water loss is a function of temperature and initial concentration of the osmotic solution. Most of the experiments in osmotic dehydration are carried out at constant temperature and at a given initial concentration<sup>1-9,11</sup> and hence we can assume that under these conditions the rate of water loss is not noticeably affected during the dehydration process. Based on this, it is possible to propose a simple function for  $K$ , time and the rate of water loss:

$$K = st \tag{4}$$

where  $s = \text{constant related to the water loss, and } t = \text{time}$ . The relationship between  $K$ ,  $s$  and  $t$  is assumed to be linear, due to the similarity that exists between this situation and a zero order reaction rate.

Substituting equation (4) into equations (2) and (3), and rearranging terms, we obtain:

$$WFL = \frac{st(WFL\infty)}{1 + st} \tag{5}$$

This equation associates the water lost (WFL) with time (t), by means of two constants:  $s$  and  $WFL\infty$ . When  $t \rightarrow \infty$  (at equilibrium), equation (5) asymptotizes at a value corresponding to  $WFL\infty$ . This can be demonstrated by calculation of the limit of equation (5) when  $t \rightarrow \infty$ . In order to predict the fraction of water lost by the foodstuff (WFL) at time  $t$  in equation (5), it is necessary to know the values for  $s$  and  $WFL\infty$ . These values can be estimated using a non-linear regression program, or by linear regression, using experimental data obtained during short time and the linearized form of equation (5):

$$\frac{t}{WFL} = \frac{1}{s(WFL\infty)} + \frac{t}{WFL\infty} \tag{6}$$

Similar equations to (5) and (6) can be written for the loss of weight and gain of soluble solids of the product:

$$WL = \frac{s_1 t (WL\infty)}{1 + s_1 t} \tag{7}$$

$$\frac{t}{WL} = \frac{1}{s_1 (WL\infty)} + \frac{t}{WL\infty} \tag{8}$$

(linear form)

$$SG = \frac{s_2 (SG\infty)}{1 + s_2 t} \tag{9}$$

$$\frac{t}{SG} = \frac{1}{s_2 (SG\infty)} + \frac{t}{SG\infty} \tag{10}$$

(linear form)

where  $WL = \text{weight fraction lost by the food at time } t$ ,  $WL\infty = \text{weight fraction lost by the food at equilibrium}$ ,  $s_1 = \text{constant related to the rate of weight loss}$ ,  $SG = \text{fraction of soluble solids gained by the food at time } t$ ,  $SG\infty = \text{fraction of soluble solids gained by the food at}$

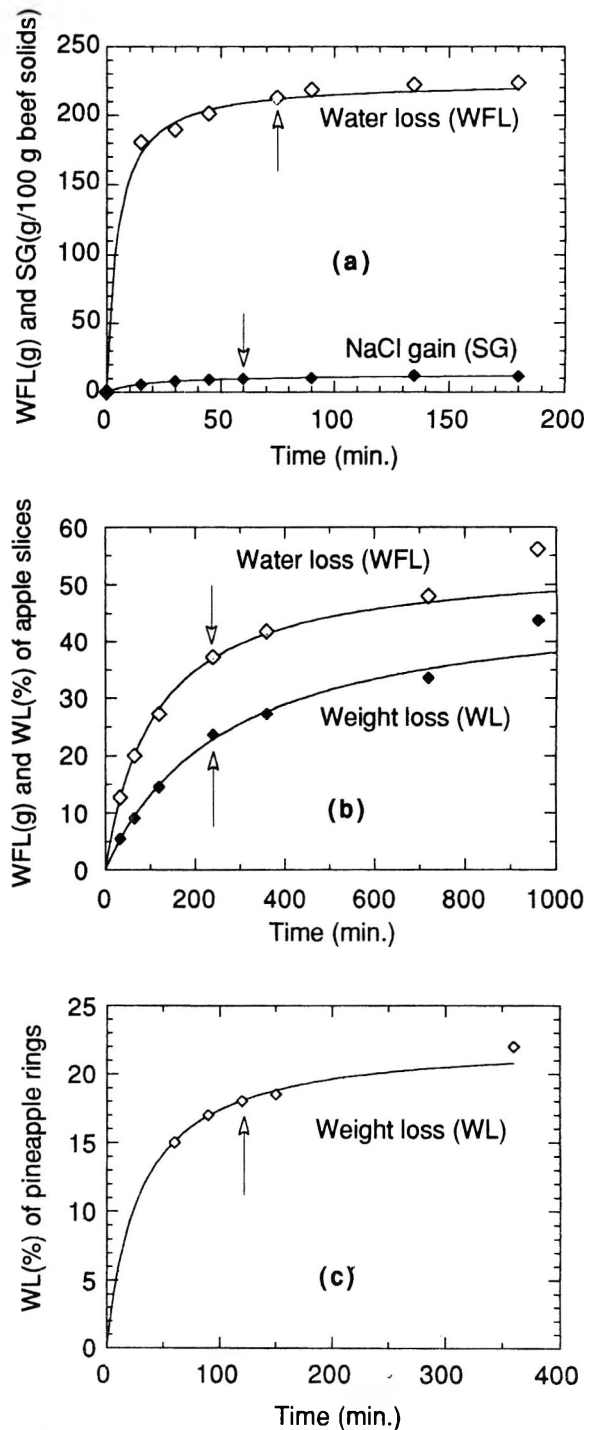


Fig. 1. Graphical representation of the experimental data (symbols) and the models (lines) proposed for water loss (WFL), weight loss (WL) and soluble solids gain (SG) for three different products (apple slices, beef solids and pineapple rings). The arrows represent the time point until which data were used to evaluate model parameters according to equations 5, 7 and 9. Source of (a): Favetto *et al.*<sup>7</sup>, (b): Lerici *et al.*<sup>8</sup>, and (c): Beristain *et al.*<sup>9</sup>.

equilibrium, and  $s_2 = \text{constant related to the rate of incoming of soluble solids to the foodstuff}$ .

Deviation of the model from experimental data was evaluated with the equation for root mean square (RMS)<sup>12</sup>:

TABLE 1. EVALUATION OF THE PROPOSED MODEL FOR THE DATA OF LERICI *et al.*<sup>3</sup>, FAVETTO *et al.*<sup>4</sup> AND BERISTAIN *et al.*<sup>5</sup>. CONSTANTS WERE ESTIMATED USING LINEAR REGRESSION FROM THE LINEARIZED FORMS OF THE MODEL (EQUATIONS 6, 8 AND 10 FROM TEXT)

Product, Variable and source	Conditions	R. M. S. %	Time interval (min.) used for prediction	Experimental time (min.) to reach equilibrium	Experimental time		Constant related to velocity (min <sup>-1</sup> )	
					Exp.	Calc.		
Water loss from apple	Sucrose solution (51 °Brix) at ambient temp.	2.0	240	960*	(WFL∞) <sup>a</sup>	56.1	54.1	(s) 9.02 × 10 <sup>-1</sup>
Water loss from beef	Solution of 40.9% glycerol, 9.4% NaCl and 0.5% potassium sorbate at 85°C	2.7	75	180	(WFL∞) <sup>b</sup>	223.7	224.9	(s) 2.16 × 10 <sup>-1</sup>
NaCl gain in beef	Solution of 40.9 glycerol, 9.4% NaCl and 0.5% potassium sorbate at 85°C	3.0	60	180	(SG∞) <sup>b</sup>	12.1	13.0	(s <sub>2</sub> ) 5.07 × 10 <sup>-2</sup>
Wt loss in pineapple	Sucrose solution of 50 °Brix at 30°C	0.8	120	360	(ML∞) <sup>c</sup>	22.0	22.5	(s <sub>1</sub> ) 3.38 × 10 <sup>-2</sup>
Wt loss in apples	Glucose solution of 50 °Brix at ambient temp.	3.2	360	960	(WL∞) <sup>c</sup>	43.7	48.3	(s <sub>1</sub> ) 3.72 × 10 <sup>-3</sup>

\*Values reported by the authors at the maximum time studied, <sup>a</sup>grams, <sup>b</sup>g/100 g beef solids, <sup>c</sup>% (wet base).

$$\text{RMS}(\%) = 100 \sqrt{\frac{1}{N} \sum_{i=1}^N \left[ \frac{V_e - V_c}{V_e} \right]^2} \quad (11)$$

where  $V_e$  = experimental value,  $V_c$  = calculated value using the proposed equations.

**Evaluation of the model:** In order to test the applicability of equations (5), (7) and (9), data from reports by three different authors on osmotic dehydration were used<sup>3,8,9</sup>. These were selected because they provided a suitable amount of data during a given time interval and they also indicated the time at which equilibrium was reached. Experimental data (indicated by the symbols), and the curve generated by the model (solid lines) are presented in Fig. 1. In all cases, water and weight losses in apples, water loss and salt gain in beef and water losses in pineapple rings were predicted quite acceptably. RMS's are presented in Table 1, where a maximum of 3.2% for weight loss in apples is observed. Short times were used for this computation (ca. 1/3 of the time needed to reach equilibrium). The models were able to predict the values for the dehydration process beyond the range actually used, and even at equilibrium. The values at equilibrium calculated by the model and those obtained experimentally are quite close to each other, whether the process is water loss, solids gain or weight loss. Data from Leric *et al.*<sup>3</sup> as given in Table 1 refer to the maximum time reported at which samples were taken for analysis (up to 960 min). The differences observed between these values and those obtained from equations (5) and (7) may be attributed

to physical and/or biological instability, which makes determination of the true equilibrium hard to assess. In cases like this, the model proposed in this work could be used to obtain an estimate for the equilibrium point. Correlation coefficients for all cases studied were close to 0.99. A quick look at the data shows that the faster processes have higher values for  $s$ ,  $s_1$  or  $s_2$ . This is attributed to higher diffusion of material per unit time under the conditions given. This model could be applied to food products that show similar dehydration behaviour as those reported in this communication.

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## Prevalence of Salmonella in Meats and Sea Foods of Bombay City

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**Fifteen of the 166(9%) meat samples tested were positive for *Salmonella* in contrast to its absence in 96 fish samples, collected in Bombay. The serotype *S. saintpaul* predominated in the slaughterhouse meat as against the dominance of *S. mbandaka*, *S. adelaide*, *S. liverpool*, *S. enatum*, *S. derby* and *S. butantan* in market meat samples. *S. mbandaka* was frequently isolated from the retail market beef samples.**

The most important form of food poisoning in most countries of the world is salmonellosis. In spite of the hygiene regulation in slaughterhouses, factories and kitchens and tight control of foodstuffs and animal feeds the number of cases of salmonellosis are high. Foodstuffs primarily of animal origin have been incriminated to be the reservoirs for infection in human beings<sup>1</sup>. The results of a survey on occurrence and distribution of *Salmonella* serotypes in meats and seafoods sold in Bombay are reported in this communication.

A total of 262 samples comprising 96 meat samples from large service municipal meat markets, 96 sea food samples from fish markets and 70 meat samples from Deonar abattoir, Bombay were collected in self sealing sterile polythene bags for transporting to the laboratory in cold box between April and September 1987. They were processed immediately for isolation and identification of *Salmonella* as per the method of International Standards Organisation<sup>2</sup>. Twenty five g of the sample in triplicate was pre-enriched in 225 ml of buffered peptone water, followed by enrichment in tetrathionate broth. The enriched broth (0.1 ml) was plated onto Brilliant Green Agar and Bismuth Sulfite Agar or Hektoen Enteric Agar. Presumptive colonies of *Salmonella* were further examined to confirm the genus *Salmonella* as per identification scheme of Edward and Ewing<sup>3</sup>. The isolates were serotyped at National Salmonella Centre (Veterinary), Indian Veterinary Research Institute, Izatnagar.

The results of the study showed that seven isolates (1 from beef, 3 from mutton, 1 from pork and 2 from buffalo beef) belonging to one serotype, *S. saintpaul* was isolated from a total of 70 meat samples analysed from the slaughter house. Slaughtering and dressing operation involved in meat production revealed a large number of points of contact for transfer of bacteria on to the carcass. However, the amount of transfer of *Salmonella* depends upon the number and location of the organisms on the animal and degree of transfer risk within the network<sup>4</sup>. Smeltzer *et al*<sup>5</sup> showed a close correlation between microbial population on the hide and those subsequently found on the carcass, and Peel and Simmons<sup>4</sup> reported the knives used in a meat works to be major source of contamination of Salmonellae. Probably

TABLE 1. SALMONELLA SEROTYPES ISOLATED FROM SAMPLES FROM DIFFERENT PARTS OF BOMBAY

Place	No. of samples	Beef	Mutton	Pork	Chicken
North Bombay	32	1 <i>S. mbandaka</i>	—	1 <i>S. anatum</i>	—
Central Bombay	24	2 <i>S. mbandaka</i>	1 <i>S. liverpool</i>	1 <i>S. derby</i>	—
South Bombay	40	1 <i>S. adelaide</i>	—	—	1 <i>S. butantan</i>

knives and other unhygienic practices may have contributed *S. saintpaul* from a common source to the carcasses in the slaughter house.

*S. mbandaka*, *S. adelaide*, *S. liverpool*, *S. anatum*, *S. derby* and *S. butantan* were recorded in market meat samples (Table I). Occurrence of different *Salmonella* serovars in market samples can be attributed to the different sources of contamination such as carrier animals, cross contamination during transportation and in lairage, faulty evisceration and cross contamination during slaughter<sup>5,6</sup>. Also, lack of adequate hygienic handling of meat during storage, processing and retailing could result in a larger percentage of retail meat samples to be contaminated with *Salmonella*.

The isolation of *S. mbandaka* from beef is of Public Health significance in view of its prevalence in man<sup>7</sup>. Frequent isolation of *Salmonella* from the markets of central Bombay may be due to lower socio-economic status of butchers and workers handling meats and to a poor hygienic status of the retail markets. None of the 96 seafood samples examined were found to contain *Salmonella*, though prevalence of *Salmonella* serotypes in different types of sea foods have been reported by Kulshrestha *et al*<sup>8</sup>. The sea foods viz. crab, shellfish, Bombay duck and prawns examined in the present study are caught in the catchment areas of South coast of Maharashtra which is free of city sewage pollution and industrial effluents.

Therefore, the situation may change depending upon the contamination entering the catchment area as well as the amount of handling and hygienic practices adopted.

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## Chemical Characteristics of Maize Grains and Their Relationship to Roti Quality

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**Protein, starch and sugar contents were found to decrease upon alkali treatment of maize grains in contrast to increase in amylose and ash contents. Untreated rotis of all varieties were found significantly more acceptable than treated rotis. Significant correlation of protein, starch and total sugars with roti qualities was identified.**

More than 90% per cent of maize produced in India is used as human food, roti being the most common product. Studies on different grains have shown that the quality of products prepared is influenced by grain components<sup>1,3</sup>. In Central America and Mexico, alkali cooking of corn is traditionally used for tortillas, an unleavened flat bread and snacks preparation. However, the lime treatment of maize grain is not popular in our country and information on the acceptability of lime treated rotis is limited. Studies on the

effect of alkali treatment of tortillas have shown changes in chemical characteristics of the product<sup>4,5</sup>. Therefore, the relationship between chemical characteristics and the overall quality of roti prepared from maize before and after alkali treatment are studied.

Six varieties of maize consisting of two local (Yellow and white) two composite ('Hemant' and 'Laxami') and two hybrids ('High starch' and 'Ganga 2 safed') grown at R.A.U., Pusa, Samastipur (Bihar) during kharif season of 1986 were



TABLE 1. CHEMICAL COMPOSITION OF SIX VARIETIES OF MAIZE BEFORE (BT) AND AFTER (AT) ALKALI TREATMENT

Maize variety	Protein		Amylose		Starch		Sugar		Ash	
	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT
Hemant	7.1	7.3	19.4	21.3	75.0	71.2	3.1	2.6	1.0	1.3
Laxami	8.1	7.3	18.5	20.4	78.0	75.0	3.6	2.4	1.0	1.5
H-Starch	8.4	8.1	21.3	22.3	82.0	78.0	4.0	3.6	1.1	1.5
Ganga 2 Safed	8.1	7.7	18.5	21.3	71.3	63.7	4.8	3.6	1.0	1.5
Local yellow	10.0	9.2	17.4	19.4	67.5	63.7	4.3	3.8	1.2	1.7
Local white	9.6	8.8	16.5	20.4	67.5	59.0	4.0	3.1	1.2	1.7
Mean $\pm$ S.D.	8.5 $\pm$ 0.97	8.1 $\pm$ 0.74	18.6 $\pm$ 1.51	20.8 $\pm$ 0.91	73.5 $\pm$ 5.3	68.4 $\pm$ 6.7	3.9 $\pm$ 0.52	3.2 $\pm$ 0.52	1.1 $\pm$ 0.10	1.5 $\pm$ 0.13

TABLE 2. DOUGH AND MEAN ROTI QUALITY SCORE OF SIX VARIETIES OF MAIZE FLOUR BEFORE (BT) AND AFTER (AT) ALKALI TREATMENT

Variety	Dough Quality							
	Water required for dough (ml/100 g flour)		Kneading quality Score		Rolling quality (mm)		Mean roti quality Score	
	BT	AT	BT	AT	BT	AT	BT	AT
Hemant	86	104	2.5	1.0	17.5	16.0	3.8	3.2
Laxami	84	110	2.5	1.5	18.5	17.5	3.8	2.9
H. Starch	86	112	3.0	1.5	18.5	17.0	3.8	2.9
Ganga 2 Safed	84	104	3.0	1.5	15.5	16.0	4.2	3.1
Local yellow	90	112	2.5	1.0	17.0	15.0	3.6	2.7
Local white	84	114	3.0	1.0	17.5	16.0	3.7	3.1
Mean $\pm$ S.D.	85.66 $\pm$ 2.13	109 $\pm$ 3.94	2.75 $\pm$ 0.25	1.25 $\pm$ 0.25	17.4 $\pm$ 1.01	16.25 $\pm$ 0.80	3.88 $\pm$ 0.23	3.06 $\pm$ 0.16

TABLE 3. RELATIONSHIP BETWEEN THE CHEMICAL CHARACTERISTICS AND ROTI QUALITY OF UNTREATED (BT) AND TREATED MAIZE (AT)

Characteristics	Colour		Texture		Taste		Flavour		Acceptability	
	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT
Protein	-0.4792	-0.3336	0.2278	-0.2454	-0.2971	-0.7770	-0.8384*	0.7734	-0.6910	-0.4005
Starch	0.5806	0.2267	1.735**	-0.1754	0.2765	0.3347	0.5334	0.3768	0.6555	0.7814
Amylose	0.7533	0.7354	0.2151	0.4230	0.1237	0.6930	0.6238	0.3467	0.6525	0.8112
Total Sugar	0.2214	0.2643	0.8225*	-0.4002	0.4252	-0.6353	-0.2925	-0.7771	0.2157	-0.1910
Ash	0.2525	-0.6	0.1370	-0.3261	-0.3257	-0.7656	-0.5580	-0.4553	0.6151	-0.5593

\*Significant at 5%

\*\*Significant at 1%

divided into two lots each. One lot of maize was cooked for 75 min in 1 per cent calcium oxide and steeped for 15 hr at room temperature<sup>6</sup>. It was washed thoroughly to remove excess alkali and sun-dried. The other lot was used without alkali treatment. All grain samples were ground in a domestic electric flour mill, the flour was sieved and stored in an air tight container. Both untreated and treated flour samples were analysed for total protein and ash<sup>7</sup>, total amylose<sup>8</sup>, total starch<sup>9</sup> and total sugar<sup>10</sup>.

To 50 g of flour, hot water (about 90°C) was added in small increments, mixed well and kneaded by hand until appropriate consistency was obtained. The volume of water required for 100 g flour was noted. After kneading well, the cohesiveness of dough was subjectively evaluated and expressed as kneading quality using a score of 1 to 3, where 3 is good

and one is poor. The rolling ability of dough was determined by the method of Subramanian *et al*<sup>11</sup>. The rolled dough was baked on a tawa (pan) and time taken for baking was recorded. Rotis were evaluated for sensory qualities by a panel of 8 members selected by triangle test. The panel members scored the rotis for colour, appearance, texture, taste, flavour and acceptability.

The chemical composition of the maize flour used in the study is given in Table 1. Alkali treatment decreased the protein, starch and sugar and increased ash and amylose contents in all varieties. Bressani *et al*<sup>12</sup> observed that corn lost 10 per cent nitrogen and 33.44 per cent of ether extractable components in addition to reduction in soluble sugars and an increase in ash content after alkali treatment. The decrease in starch content on lime treatment is due to

gelatinization. Although maize grains do not contain gluten, when maize flour is mixed with water and kneaded, it produces a sticky dough. A good quality dough should be sticky and easily rollable into a roti without any breakage. Dough quality characteristics are presented in Table 2. Water required to make dough for all varieties varied from 84-90 ml/100 g and it increased in all the varieties after alkali treatment. Dough made from untreated maize flour showed good cohesiveness as the kneading quality score was between 2.5 and 3, but cohesiveness and rolling quality decreased after alkali treatment.

The untreated rotis got better scores for all sensory attributes and were significantly more acceptable than treated rotis (Table 2). Maximum score obtained by treated roti is less than minimum score of untreated rotis. The correlation between chemical characteristics of untreated and treated grains and taste panel scores of rotis (Table 3) was significantly negative between protein and flavour. Texture showed significant positive correlation with starch and total sugars.

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## Studies on Processing Properties of Milk Obtained from Crossbred Cows

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The data indicated that the processing qualities of the milk are influenced by the genetic group of cows in terms of rennet and heat clotting times as well as curd tension. Microbial rennet is advantageous for quick coagulation while the milk from HF crossbred cows is more suited for formulating infant foods.

Milk production in India has increased considerably in the recent years mainly due to the crossbreeding of cattle. Of the total milk produced in India, 55.5 per cent is used for manufacturing different milk products. In many product manufacturing technologies, rennet coagulation, heat stability and curd tension of milk play a significant role. However, the reports on the processing characteristics of milk from different crossbred cows are scanty. The work was, therefore, undertaken.

Crossbred cows from the following genetic groups were selected for obtaining the milk samples:

- 1)  $\frac{1}{2}$  (Holstein Fressian) HF +  $\frac{1}{2}$  Gir (HFG); 2)  $\frac{1}{2}$  J (Jersey) +  $\frac{1}{4}$  HF +  $\frac{1}{4}$  Gir (JHG); 3)  $\frac{1}{2}$  (BS) Brown swiss +  $\frac{1}{4}$  HF +  $\frac{1}{4}$  Gir (BHG) and 4) All genetic groups (mixed group). In each group, five cows running between the first and seventh lactation were selected. The cows were maintained under almost uniform management and feeding conditions. Composite milk samples were obtained from each of the

TABLE 1. PROCESSING PROPERTIES OF MILK FROM COWS OF DIFFERENT GENETIC GROUPS

Genetic group	Rennet clotting time (sec.)		Heat clotting time (min.)	Curd tension (g)
	Calf	Microbial		
HFG	280.0	109.0	20.3	19.9
JHG	172.0	54.0	22.1	22.4
BHG	212.0	66.2	20.4	20.6
Mixed group	192.2	70.0	23.1	21.9
S.E.	+23.1	+8.0	+0.7	+0.6
C.D. at 5%	69.4	23.9	N.S.	1.7

N.S. = Non-significant. Each value is the mean of 5 samples.

genetic groups in the morning. Rennet clotting time was determined by using the method described by Salam and Shibiny<sup>2</sup>. The calf and microbial rennets were used for this purpose. Heat stability was measured by using the procedure described by Puri *et al.*<sup>3</sup>, while the curd tension was measured by the method of Chandrasekhara *et al.*<sup>4</sup> The trial was laid out in the Completely Randomised Design. The calf rennet clotting time (CRCT) ranged from 172 to 280 sec, while the microbial rennet clotting time (MRCT) ranged from 54 to 109 sec (Table 1.) The differences in the clotting time were found to be significant ( $P < 0.05$ ). The highest clotting time irrespective of the kind of rennet used was required for the milk from HFG cows while the lowest for the milk from JHG cows. Further, the microbial rennet coagulated the milk much faster than the calf rennet, irrespective of any genetic group. These differences in clotting time for the milk of different genetic groups may be due to the differences in the

casein as well as mineral make up of these milks. Heat clotting time (HCT) ranged from 20.3 to 23.1 min. The differences were, however, non-significant. It indicates that the milk from the different genetic groups behaved similarly for heat stability characteristics.

Curd tension (CT) is one of the major criteria for determining the suitability of different milks for infant feeding. Generally, it is considered that the milk with low curd tension is desirable for infants and people with weak digestive power. The average curd tension was 19.9, 22.4, 20.6 and 21.9 g in the HFG, JHG, BHG and mixed group, respectively (Table 1). The differences were significant. The milk from HFG group had the most soft curd forming properties as indicated by the lowest curd tension value. The results agree with those reported by Jairam *et al.*<sup>5</sup>. The results of this investigation suggest that a) the processing properties of the milk are significantly influenced by the genetic group of the cows, b) the use of microbial rennet is much advantageous when the quick coagulation is desired, and c) the use of milk from HF crossbred cows is advantageous in formulating infant foods.

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## Studies on Pickled Chicken Eggs

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Sensory evaluation of egg pickles prepared with citric, acetic, lactic and tartaric acids indicated overall acceptability of the product made with lactic and tartaric acids when used alongwith 10% common salt, spices, condiments and oil. The storage period did not affect the sensory parameters.

Eggs can be pickled in vinegar and/or oil with added spices, salt and condiments. Pickled eggs are ready-to-eat products and can be prepared from hard cooked eggs of hen, duck or quail. Various researchers have evaluated hard cooked<sup>1,2</sup> and brine pickled chicken eggs<sup>3,5</sup>. Srivastava and Panda<sup>6</sup> conducted a study on pickling of quail eggs to preserve hard

cooked eggs at room temperature so that they could be transported to different parts of the country without refrigeration while Tipshetti and Panda<sup>7</sup> standardised conditions for preparation of pickles with quail eggs. They advocated the use of a pickling solution containing acetic acid and salt. Acton<sup>8</sup> conducted a survey on the sensory panel evaluation of pickled eggs and reported that many a consumer did not like the characteristic pungent flavour of acetic acid used as a major ingredient in pickles. Preliminary studies were conducted to evaluate citric, lactic, tartaric acids and common salt at different concentrations for pickling of chicken eggs and reported that eggs pickled in 3 per cent acids and 10 per cent salt solutions were the most acceptable. The present investigation was undertaken to study the quality and acceptability of oil-cum-spice based pickle prepared from eggs equilibrated in solutions containing a combination of 3 per cent acid (citric/lactic/tartaric/acetic acids) and 10 per cent common salt.

Eight dozen fresh chicken eggs were procured from the poultry farm of the University and held in refrigerator for 48 hr prior to hard cooking in simmering water for 15 min and then immediately cooled in running water for 2 min<sup>2</sup>. Hard cooked cooled eggs were peeled and two dozen each were immersed in solutions containing a combination of 3 per cent of either acid (citric, lactic, tartaric and acetic acids) and 10 per cent common salt for seven days to achieve pH of 3.5 at equilibrium. The equilibrated eggs were used in preparation of oil-cum-spice based pickle. The spices consisted of green curry stuff (50 g each of garlic and ginger, and 100 g of onions) and seasonings (cinnamon 5 g, clove 10 g, coriander powder 50 g, chilli powder 50 g, black pepper 10 g, turmeric powder 30 g and cumin seed 10 g). Mustard oil (100 ml) was heated to smoke point and the juice extracted from green curry stuff as well as the dried ground spices were added. The mixture was fried till light brown in colour, cooled and added to the jars containing equilibrated eggs. Additional 100 ml of heated and cooled mustard oil was poured on the eggs to provide 1 cm layer on the top. The jars containing pickles were screw capped and stored at ambient temperature (20-25°C). The total plate counts of the pickle were conducted by pour plate method<sup>9</sup>. A ten member semi-trained panel evaluated the pickled eggs at 15-days intervals for appearance, texture, flavour and overall acceptability using 4-point Hedonic rating with a score of 1 being the lowest possible value and a score of 4 being the highest. The data were statistically analysed<sup>10</sup>.

The analyses of the data revealed that the sensory parameters were significantly affected by acids while the storage had a non-significant effect (Table 1). The storage period as well as kind of acid did not have a significant ( $P > 0.05$ ) effect on appearance and flavour. The storage periods did not have a significant ( $P > 0.05$ ) effect on body and texture and overall acceptability. However, the body and

TABLE 1. OVERALL MEANS (AVERAGED OVER STORAGE PERIODS) OF SENSORY PARAMETERS AND PLATE COUNTS UNDER DIFFERENT ACIDS

Acids	Storage (days)					Mean
	0	15	30	45	60	
<b>Texture</b>						
Lactic	2.9	2.9	2.9	3.0	3.3	3.3 <sup>a</sup>
Tartaric	3.1	3.1	3.0	2.9	3.0	3.0 <sup>b</sup>
Citric	2.8	2.8	2.4	2.5	2.6	2.6 <sup>c</sup>
Acetic	2.9	2.8	3.0	2.9	2.8	2.9 <sup>b</sup>
Mean	2.9	2.9	2.8	2.8	2.9	
<b>Overall Acceptability</b>						
Lactic	2.8	2.8	2.9	3.3	3.4	3.1 <sup>a</sup>
Tartaric	2.8	2.8	2.7	3.0	3.1	2.9 <sup>a</sup>
Citric	3.2	3.2	2.7	2.6	2.6	2.5 <sup>b</sup>
Acetic	2.3	2.3	2.9	2.5	2.3	2.5 <sup>b</sup>
Mean	2.5	2.5	2.8	2.9	2.9	
<b>Total Plate Count/g</b>						
Citric	4.0	2.3	2.3	2.4	2.3	2.7
Lactic	4.4	2.9	2.7	2.9	2.7	3.1
Tartaric	3.0	2.3	2.3	2.3	2.1	2.4
Acetic	3.5	2.6	2.5	2.6	2.4	2.7
Pickle spices	5.6	4.2	3.9	4.1	4.1	4.4
Mean	4.1	2.9	2.7	2.8	2.7	

\*Means with the same superscript in either row or column are not significantly different ( $P > 0.05$ ).

the texture scores were significantly higher for lactic acid. The overall acceptability scores were significantly higher for lactic acid and tartaric acid samples as compared to that for citric acid and acetic acid which had almost the same mean score (2.5).

McCready<sup>2</sup> reported greatest flavour intensity in eggs aged at ambient temperature. On the contrary, Reddy<sup>5</sup> observed better overall acceptability scores for refrigerated pickled eggs than those stored at ambient temperature. The main parameter affecting the acceptance of pickle is the pickling acid ingredient which is in agreement with the results of the present investigation.

The total plate count values for eggs pickled in various acids revealed that the number decreased after 15 days, as compared to zero day, but did not change much thereafter. The number was in the range of  $10^3$  which is well within the acceptable limits<sup>5</sup>. The decrease in number of organisms may be due to unfavourable conditions caused by spices and oil. Reddy<sup>5</sup> observed a marked decrease in bacterial count of pickled quail eggs during the first week of preservation at room temperature followed by a more or less constant microbial load. Arafa<sup>11</sup> reported no change in microbial population of pickling solutions in sealed jars.

The data indicated that an acceptable shelf stable oil-cum-spice based egg pickle can be prepared using eggs equilibrated

in solution containing 3 per cent lactic acid or tartaric acid in combination with 10 per cent salt and spices/condiments suiting Indian culinary and taste.

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## Effect of Polyphosphate Dip Treatment on Frozen Storage of Indian Squid *Loligo duvauceli* Orbigny

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Squid fillets (Mantles) given a dip treatment in aqueous trisodium polyphosphate solution (5% w/v) prior to freezing showed reduced protein denaturation as evidenced by improved texture, decreased thaw drip and weight loss, retention of salt soluble and alpha-amino nitrogens as compared with control squids during frozen storage. The overall acceptance is better in polyphosphate treated samples than in control.

Squid is exported in the form of frozen mantles and tubes from India. Freezing and frozen storage prolong the shelf life of seafoods by retarding enzymatic and microbial degradation. But protein denaturation occurs during prolonged storage resulting in moisture loss and texture changes<sup>1</sup>. Polyphosphates are found to be effective in preventing oxidation, moisture loss and textural changes in seafoods<sup>2,3</sup>. In the present study, the effect of polyphosphate dip treatment on frozen storage characteristics of Indian squid are investigated.

Fresh samples of *Loligo duvauceli* Orbigny were procured from Tuticorin Fishing Harbour and were stored in ice overnight until use. One group of dressed mantles was given a 5 min dip treatment in 5 per cent (w/v) aqueous solution

of food grade trisodium polyphosphate. The other group served as control. The mantles were packed individually in 150 gauge polyethylene pouches and quick frozen at -40°C in a contact plate freezer and stored at -20°C. Samples were drawn in triplicate for analysis at monthly intervals. The thaw drip, weight loss on thawing and weight loss on cooking were estimated by using standard methods, in addition to salt soluble nitrogen<sup>4</sup> and alpha amino nitrogen<sup>5</sup>. The cooked samples were prepared by boiling the thawed mantles in 3 per cent brine for 10 min<sup>6</sup> and presented to a taste panel consisting of 6 trained personnel for evaluation of colour, appearance, texture and flavour on 5 point scale for calculation of overall acceptability mean score. Point 5 was excellent i.e., Off-white, firm, with sheen, rubbery texture

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and fresh squid (sea water flavour) in contrast to point 1 as spoiled (pinkish brown colour, offensive odour, very mushy and curdy texture, very bitter and cheesy flavour).

The weight loss due to thawing increased gradually during frozen storage (Table 1). However, this was much less in samples treated with polyphosphate even after 9 months storage. This may be attributed to the water holding and thaw drip reducing capacities of polyphosphate<sup>2,7</sup>. A similar effect on thaw drip was also reported in frozen prawns<sup>3,8</sup>. Weight loss on cooking increased from 16.3 from the first month to 32.2 per cent at the end of 9 months storage in polyphosphate treated samples, the corresponding losses in control being 22.5 and 43.1 per cent respectively. Thus, polyphosphate is effective in reducing cooking loss in squid.

The data on variations in the alpha amino and salt soluble nitrogens in the polyphosphate treated and control samples at the end of 9 months storage indicate that the protein denaturation was less in the polyphosphate treated than in the control samples. The salt soluble nitrogen represents the protein fraction that has not undergone denaturation. The lesser decrease in polyphosphate treated samples indicated the protective effect of polyphosphate on denaturation of proteins<sup>8</sup>.

The sensory evaluation (Table 2) showed that the polyphosphate treated samples (both cooked and uncooked) scored above 3 throughout the study, while the control samples showed less than 3 on 6th month itself. The control samples became fairly soft at the end of 8th month and very

TABLE 1. PHYSICAL CHANGES IN FROZEN SQUID MANTLES DURING STORAGE

Storage period (months)	Thaw drip (ml)	Thawing wt loss (%)	Cooking wt loss (%)	$\alpha$ -amino N (mg %)	Salt soluble N (g %)
<b>Control</b>					
1	6.0	8.7	22.5	212.9	2.1
2	7.8	10.3	24.4	188.7	1.9
3	8.4	13.9	26.8	152.0	1.8
4	9.5	14.3	28.8	132.5	1.7
5	12.0	16.4	39.0	106.4	1.5
6	14.3	19.3	33.7	100.3	1.3
7	15.1	23.5	38.8	94.5	1.2
8	17.2	25.7	40.3	89.0	1.0
9	18.0	28.7	43.1	83.1	0.9
<b>Polyphosphate treated</b>					
1	1.6	6.9	16.3	232.4	2.2
2	2.0	7.5	18.6	200.6	2.1
3	2.5	9.5	20.5	174.8	2.0
4	2.8	10.4	23.8	156.8	1.9
5	3.1	12.7	24.4	123.2	1.7
6	3.7	14.0	26.4	115.4	1.6
7	4.2	15.7	28.2	106.4	1.5
8	4.9	17.3	30.0	100.4	1.4
9	5.6	20.3	32.2	93.8	1.3

TABLE 2. SENSORY EVALUATION OF POLYPHOSPHATE TREATED COOKED AND UNCOOKED FROZEN STORED *LOLIGO DUVAUCELI*.

Storage period (month)	Uncooked sample		Cooked sample	
	Control	Polyphosphate treated	Control	Polyphosphate treated
0	5.0	5.0	5.0	5.0
1	4.8	5.0	4.9	5.0
2	4.4	5.0	4.7	4.9
3	3.9	5.0	4.3	4.9
4	3.5	4.9	4.0	4.9
5	3.3	4.6	3.5	4.7
6	3.0	4.5	3.0	4.5
7	2.6	4.2	2.3	4.2
8	2.1	3.7	2.0	3.8
9	1.2	3.6	1.3	3.6

soft and sticky by the 9th month. However, the cooked sample of control had a hard and rubbery texture indicating denaturation. The results show that the polyphosphate treatment at 5 per cent (w/v) level on squid fillets have a noticeable effect in controlling denaturation as evidenced by less thaw drip, less weight loss on cooking, higher salt soluble nitrogen content and soft texture of the cooked sample.

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## Storage Stability of Refined Sunflower Oil in Tins and HDPE Bottles

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**Refined sunflower oil remains stable for two years at room temperature when stored in high density polyethylene (HDPE) bottles and sealed tins without development of perceptible off flavour or odours. Peroxide, TBA, total carbonyls and anisidine values increased during storage and the changes correlated linearly with the storage period. Increases in these values were more in leaking containers.**

In India, sunflower oil (0.2 million MT/annum) has emerged as the third largest edible oil after groundnut (1.5 million MT) and mustard oil (1.2 million MT). It is mainly marketed as double refined prime grade cooking oil in plastic bottles and is a rich source of essential fatty acids. At present, refined sunflower oil gets sold within about 3-4 months because of shortage and no off-flavour or rancidity is observed during this period. But for Defence Forces, a minimum shelf life of one year under ambient conditions is considered essential, before any oil is considered suitable for inclusion in ration scale.

In sealed tins and mild steel containers, raw sunflower and groundnut oils remained stable oxidatively and hydrolytically for 1080 and 660 days respectively<sup>1</sup>. The raw sunflower oil obtained from crops grown under rainfed conditions was found to have better keeping quality than the one obtained from crops under irrigated conditions<sup>2</sup>. Huang *et al.*<sup>3</sup> have reported data on comparative storage stabilities of refined sunflower oil and corn oil. Under normal usage conditions, rate of peroxide formation was faster in sunflower oil than in corn oil. Similarly, the rate of storage degradation was found to be slower in brown than in colourless bottles<sup>4</sup>. In view of scanty information on the storage stability of refined sunflower oil, the present study was undertaken to determine its storage life in 15 kg tins and 5 kg HDPE bottles.

Two popular commercial brands (A and B) of refined sunflower oil were procured in 5 kg HDPE bottles and 15 kg square sealed tins and stored at room temperature (15-35°C) and in cold room (5-10°C). To study the effect of citric acid and tertiary butylated hydroquinone (TBHQ) on oil stability, requisite quantity of citric acid (1.5 g) and TBHQ (3.0 g) were dissolved in propyleneglycol (20 ml) and incorporated in vegetable oil stored in tins. Control samples were treated with only propyleneglycol. All the tins were hermetically sealed after treatments. Initially and after every three months, stored samples were analysed for peroxide value (PV)<sup>5</sup>, thiobarbituric acid value (TBA)<sup>5</sup>, free fatty acids (FFA)<sup>6</sup>, anisidine value (AV)<sup>7</sup>, and total carbonyls (Tc)<sup>8</sup>. For

sensory acceptance tests, potato chips were fried in stored oil samples and served to a panel of 25 persons for grading for aroma, taste and overall acceptability on a 9-point Hedonic scale having 9 for excellent in all respects and 1 for complete unacceptability. Oil samples as such were also served to panel members for detection of any stored odour.

Both brands of refined sunflower oil remained stable during storage in sealed tins and plastic bottles. There was no perceptible rancidity or off flavour during the entire two years of storage. The acceptability of chips fried in stored sunflower oil also did not decrease significantly. The aroma, taste and overall acceptability scores of chips fried in two brands of refined sunflower oil at the beginning of storage were  $7.1 \pm 0.6$ ,  $7.1 \pm 0.6$ ,  $7.4 \pm 0.5$  and  $7.5 \pm 0.5$ ,  $7.4 \pm 0.5$ ,  $7.6 \pm 0.5$  respectively. After two years storage, the corresponding values were  $6.9 \pm 0.5$ ,  $7.0 \pm 0.4$ ,  $7.1 \pm 0.4$  and  $7.1 \pm 0.5$ ,  $7.0 \pm 0.5$ ,  $7.3 \pm 0.4$  respectively. In a few containers in which the oil had leaked during storage, the oil developed stored odour suggesting detrimental effect of air exchange on the keeping quality of oil.

Off-flavour resulting from peroxidation of unsaturated fatty acids is the major cause of spoilage in stored oils. In the present investigation, the rate of peroxidation was followed by measuring PV, TBA, AV, FFA, refractive index and Tc (Table 1). The initial PV in brands A and B were 4.5 and 6.7 respectively. After 12 months storage, the PV increased to 12.5 and 14.6 at room temperature stored samples and to 10.9 and 12.8 in cold room stored samples when packed in HDPE bottles. The changes in TBA, AV and Tc also followed the same pattern. The increases in PV, TBA, AV and Tc were linearly correlated with the storage period and coefficient of correlation ranged between 0.87 and 0.99 at 95 and 99% confidence. The changes in PV, TBA, AV and Tc though significant, were not large enough to cause perceptible change in sensory quality of refined sunflower oil upto two years storage at room temperature. The increases in FFA and refractive index during storage were only marginal and did not correlate with the storage period. The iodine value of brands A and B were 117.5 and 121.8 respectively. In both



TABLE 1. CHANGES IN PEROXIDE VALUE, FREE FATTY ACIDS, THIOBARBITURIC ACID, ANISIDINE VALUE AND TOTAL CARBONYLS OF SUNFLOWER OIL DURING STORAGE

Parameters	Brand	Con- tainer	Storage period (months)									
			0		6		9		12		24	
				Room temp		Cold storage		Room temp		Cold storage		Room temp
Peroxide value (meq O <sub>2</sub> /kg fat)	A	HDPE	4.5	10.6	8.6	11.2 (35.3)	10.4	12.5	10.9	—	—	
	B	HDPE	6.7	12.9	10.5	12.9	10.8	14.6	12.8	22.6	14.6	
	A	Tin	4.3	4.7	5.1	4.8	4.7	4.9	4.8	8.5	8.0	
Free fatty acids (%oleic acid)	A	HDPE	0.19	0.27	0.22	0.28 (0.28)	0.23	0.28	0.23	—	—	
	B	HDPE	0.20	0.20	0.20	0.22	0.20	0.23	0.20	0.28	0.21	
	A	Tin	0.20	0.26	0.23	0.29	0.25	0.29	0.26	0.39	0.28	
Thiobarbituric acid (mg malonaldehyde/kg fat)	A	HDPE	0.24	0.41	0.33	0.46 (0.81)	0.34	0.51	0.36	—	—	
	B	HDPE	0.10	0.23	0.19	0.25	0.22	0.28	0.25	0.78	0.39	
	A	Tin	0.23	0.25	0.25	0.27	0.26	0.27	0.27	0.36	0.34	
Anisidine value	A	HDPE	17.52	25.57	23.94	25.90 (26.50)	24.17	28.92	25.31	—	—	
	B	HDPE	18.23	23.56	21.62	26.02	23.35	28.37	24.56	32.45	29.40	
	A	Tin	17.36	22.62	22.09	24.67	24.09	26.69	24.69	29.73	28.02	
Total carbonyls (mg n-hexanal/100g fat)	A	HDPE	7.95	15.58	15.48	16.81 (19.70)	16.11	20.21	16.57	—	—	
	B	HDPE	7.96	11.92	11.31	11.96	11.51	14.17	13.14	15.75	14.92	
	A	Tin	8.69	13.12	13.01	13.29	13.02	14.18	13.86	15.44	14.75	

Values in parenthesis are of leaking bottles.

brands, there was a decrease of 3 to 4 units in iodine value after 12 months of storage in HDPE bottles at room temperature.

Relatively, increases in PV, TBA, AV and Tc were significantly higher in oil samples stored in HDPE bottles as compared to sealed tins. After 12 months storage, the PV, TBA, AV and Tc of brand A sunflower oil packed in tins (Table 1) were 4.9, 0.27, 26.7 and 14.2 respectively as compared to 12.5, 0.51, 28.9 and 20.2 in HDPE bottles at room temperature. Addition of TBHQ and citric acid in refined sunflower oil did not exert significant effect on the rate of peroxidation during storage. Previously also, incorporation of anti-oxidants in refined sunflower oil has not been found beneficial in enhancing storage stability and flavour scores<sup>9</sup>. Synthetic antioxidants are generally more effective in oils containing low levels of natural tocopherols. Limited published report indicates that sunflower oil contains 500-700 g tocopherol per gram<sup>10</sup>. This level seems to be sufficient to impart adequate storage stability to refined sunflower oil in sealed tins and bottles. The rate of peroxidation in leaking containers was considerably higher. The PV and TBA values of oil stored in leaking HDPE bottles were 35.3 and 0.81 as compared to 11.2 and 0.46 respectively in a non-leaking container after 9 months storage at room temperature. Yousuf Ali Khan *et al.*<sup>1</sup> have observed that in raw sunflower oil

stored odours became perceptible only when its PV increased above 25 meq O<sub>2</sub>/kg. In the present study also, perceptible off flavours were detected only in leaking bottles where PV had reached above 35. In all other samples, PV had remained below 22.8 upto two years storage (Table 1) and no off flavour was detected in any of these samples.

From the foregoing discussion, it is evident that refined sunflower oil remains in acceptable condition for two years without any significant change in flavour when stored in sealed tins and HDPE bottles. Increases in PV, TBA, AV and Tc correlate linearly with the storage period and can be used as objective parameters for storage deterioration.

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## Influence of Water Activity on Autoxidation of Methyl Linoleate During Storage

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**Peroxide and total carbonyl values showed no noticeable difference at 0.02 and 0.91 water activities. Anisidine value and polymer content were appreciably higher at  $a_w=0.91$ . The results showed that the development of hydroperoxides and their subsequent secondary reactions were influenced to a considerable extent at high water activity.**

Water activity is known to have an influence on autoxidation of lipids and consequent formation of secondary reaction products<sup>1,2</sup>. The effect of low and high water activities on autoxidation of methyl linoleate has been examined in the present studies due to its importance as a model for monitoring the autoxidation behaviour of oils and fats during storage.

Methyl linoleate was prepared and 10 ml sample was autoxidized at  $a_w=0.02$  and  $a_w=0.19$  at  $40 \pm 1^\circ\text{C}$  as reported earlier<sup>1</sup>. All other chemicals and solvents used were of AR grade and purified whenever necessary<sup>1</sup>. Samples were withdrawn at regular intervals for analysis. AOCS method<sup>3</sup> was followed for the determination of peroxide value using  $0.5 \pm 0.01$  g of the sample. The total carbonyl value was determined by dissolving the autoxidized samples ( $1 \pm 0.01$  g) in carbonyl free hexane (25 ml) and then converting into DNPH derivatives on the 2,4-dinitrophenyl hydrazine-phosphoric acid-celite column<sup>4</sup>. Anisidine value was determined by reacting the autoxidized linoleate (2-10mg) in chloroform with p-anisidine in ethanol as outlined by Jirousova<sup>5</sup>. Autoxidized/fresh methyl linoleate sample (1 g) was weighed into a 10 ml Erlenmeyer flask. Hexane (5 ml) was added and shaken thoroughly to extract unoxidized and non-polymerized methyl linoleate. The supernatant hexane layer was carefully decanted. The residue was extracted (six times) with hexane (5 ml), pooled hexane extracts were

desolventized under reduced pressure and the residue was weighed to calculate per cent polymer content. Use of n-propanol, or acetone (generally used for determination of polymers in oils and fats<sup>6</sup>) for determination of polymerized linoleate content was not possible due to the complete solubility of the sample in the above solvents.

Thin layer chromatography of autoxidized methyl linoleate was performed using silica gel G coated plates (300  $\mu$  thickness activated at  $120^\circ\text{C}$  for 150 min). The sample in petroleum ether and diethyl ether (3:1) was spotted and developed in petroleum ether (40-60°C) and diethyl ether (60:40 v/v) for 40 min. The spots were visualised by either spraying with 50 per cent sulphuric acid followed by heating at  $160^\circ\text{C}$  for 4 hr or spraying a hydroperoxide specific spray reagent<sup>7</sup>.

TABLE 1. PEROXIDE VALUES OF METHYL LINOLEATE DURING STORAGE AT A LOW AND HIGH WATER ACTIVITY AT  $40 \pm 1^\circ\text{C}$

Storage period (days)	Peroxide values (meq O <sub>2</sub> /kg linoleate) at indicated water activities.	
	$a_w=0.02$	$a_w=0.91$
0	2	2
7	13	14
14	23	24
28	46	57
56	2243	1936

The peroxide values in the stored methyl linoleate at 0.02 and 0.91  $a_w$  were similar (Table 1). However, it is interesting to note that the physical characteristics of the linoleate oxidized at  $a_w=0.02$  were different from those at  $a_w=0.91$ . Methyl linoleate oxidized for 56 days at 40°C and  $a_w=0.02$  was a free flowing colourless liquid in contrast to dark brown coloured viscous liquid in case of sample stored at  $a_w=0.91$ . However, its peroxide value (PV) of 1936 meq  $O_2/kg$  was not very much lower than the PV at  $a_w=0.02$  (PV=2243 meq  $O_2/kg$ ). Also, the sample at  $a_w=0.91$  was insoluble in hexane, indicating polymerization of linoleate. The hexane insoluble matter was high at  $a_w=0.91$  (52.5 per cent) and was negligible in fresh linoleate (0.06 per cent) as well as in the sample autoxidized at an  $a_w=0.02$  (0.12 per cent). Similar observation on the polymerization of walnut oil/cellulose system, stored at a high  $a_w=0.86$  (86 per cent RH) has been reported<sup>8</sup>. The influence of high water activity on polymerization of linoleate has not been reported so far although polymerization has been generally recognised during prolonged and high temperature autoxidation of lipids (e.g., deep-fat-frying of foods).

Total carbonyl values of methyl linoleate autoxidized for 28 days have increased to 357 M/M and 344 M/M at  $a_w$  values of 0.02 and 0.91 respectively from an initial value of 185 M/M. The anisidine value, a measure of the monocarbonyl class, viz., 2-alkenals, showed slightly higher value at an  $a_w$  of 0.91 (184) than at an  $a_w$  of 0.02 (135), thereby indicating that the composition of the total carbonyl compounds formed during autoxidation was dependent on  $a_w$  of the system, corroborating the earlier findings<sup>1</sup>. The odour intensity (subjective) of methyl linoleate stored for 56 days at an  $a_w$  of 0.02 was much different from that stored for the same period at an  $a_w$  of 0.91. However, different odour scores of walnut oil/cellulose system stored at different  $a_w$ -values have also been reported<sup>9</sup>.

Thin layer chromatography of the linoleate autoxidized for 56 days at  $a_w$  values of 0.02 and 0.91, and fresh linoleate, indicated presence of at least 3-4 types of peroxides having

different  $R_f$  values. The hydroperoxides found at  $a_w=0.02$  had  $R_f$  values of 0.85, 0.75, 0.68 and 0.35, as against that of 0.93, 0.85 and 0.68 in case of  $a_w$  of 0.91. The fresh linoleate had a small amount of hydroperoxide of  $R_f=0.85$ , which is possibly, for the monohydroperoxide. The formation of different types of hydroperoxides in an aqueous dispersion of methyl linoleate after autoxidation as well as further autoxidation of secondary reaction products in the aqueous environment have also been reported by other workers<sup>10,11</sup>.

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## Antibacterial Activity of Eugenol in Comparison with Other Antibiotics

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The antibacterial activity of eugenol was tested and compared with that of various antibiotics of known concentration against several Gram positive and Gram negative microorganisms. The results showed that eugenol possessed property of inhibiting the growth of microorganisms *in vitro* at a concentration of 5 g per assay. It was found to be sensitive against organisms like *Escherichia coli*, *Enterobacter sakazaki* and *Klebsiella pneumoniae* which were resistant to antibiotics like Ampicillin, Erythromycin and Sulphamethizole.

Since ancient times, spices and condiments have been considered indispensable in the culinary arts, as they are used to flavour foods. They are also recognised for their physiological and medicinal properties<sup>1</sup>, Eugenol, an active principle of clove, is used widely as food flavouring agent<sup>2</sup>. It is considered as an antiseptic and often employed as a preservative<sup>3-5</sup>. It possesses some local anaesthetic action, being a favourite remedy for toothache<sup>6,7</sup>. Information is scanty about the functional aspect of eugenol. The present investigation attempts to assess and compare the antibacterial property of eugenol in relation to known antibiotics.

Eugenol oil was obtained from Spice Board, Government of India, Cochin. The nutrient agar was prepared as described by Baker and Breach<sup>8</sup>. Blood agar was prepared by adding 5 per cent sheep blood in the nutrient agar (a modified procedure of Bauer *et al*<sup>9</sup>). Microbial sensitivity test discs of known concentrations, supplied by Span diagnostics, UDHNA (Surat), India were used. Eugenol discs were prepared by impregnating filter paper discs with eugenol. This concentration was arrived at by conducting minimum inhibitory concentration (MIC) test. Gram positive and Gram negative organisms isolated in pure cultures were identified upto species level by using Microbial Identification test kits from API system Sa-La Blameless Grottes 38390 Montalieu Vercieu, France. Antibacterial action of eugenol against Gram positive and Gram negative organisms was assessed. These were considered adequate to represent wide range of pathogenic organisms varying in their susceptibility to antibacterial agents, clinical pathogenicity, staining and cultural characteristics to draw useful conclusions about antibacterial quality of eugenol. Broth cultures of organisms

derived from stock cultures maintained in the Department of Microbiology, LAISC, Hyderabad were used.

The test proper was carried out by inoculating 5 ml of nutrient broth with individual organism. The tubes were incubated for 18 h at 37°C and 1 ml of culture was spread over the agar plates with the help of spreader for uniform distribution. The antibiotic discs were put on to the surface of the plate along with eugenol disc. The antibiotic discs commercially available were used against all the organisms to maintain the uniformity of the dose. The plates were incubated for a period of 18 hr at 37°C and were observed for the growth and the zones of inhibition around the discs to read the antibacterial activity of antibiotics along with eugenol. The zones around the discs were measured in millimeters and compared with standard inhibitory zones prescribed for each antibiotic. The inhibitory zones exhibited by eugenol were compared with the zones of the various antibiotics.

The results showed that eugenol possessed antibacterial activity which was demonstrated by large and measurable zones of inhibition against most of the organisms tested (Tables 1 and 2). Among the Gram negative organisms, *Enterobacter cloacae* was found to be highly sensitive to eugenol. Similar effect was found against *Enterobacter sakazaki* which was resistant to ampicillin, erythromycin and sulphamethizole. Organisms like *Klebsiella pneumoniae* and *Klebsiella oxytoca* showed sensitivity comparable with other antibiotics like ampicillin, cephaloridine, cotrimoxazole and gentamycin. *Escherichia coli* which was resistant to ampicillin, bacitracin, erythromycin and gentamycin also showed sensitivity towards eugenol. However, the effect of

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TABLE 1. ANTIMICROBIAL ACTIVITY OF EUGENOL AND OTHER ANTIMICROBIAL AGENTS AGAINST GRAM NEGATIVE ORGANISMS

Antimicrobial agents	Concn ( $\mu\text{g}$ )*	Min. sensitive zone dia. (mm)	<i>Escherichia coli</i>	<i>Enterobacter</i>		<i>Klebsiella</i>		<i>Proteus mirabilis</i>	<i>Citrobacter freundii</i>
				<i>cloacae</i>	<i>sakazaki</i>	<i>pneumoniae</i>	<i>oxytoca</i>		
Ampicillin	10	14	R	12	R	15	R	15	R
Bacitracin	10	13	R	R	R	R	14	R	R
Cephalexin	30	18	17	22	17	18	15	18	R
Cephaloridine	30	16	12	18	17	20	18	28	R
Chloramphenicol	30	18	—	—	25	—	24	—	24
Chlortetracycline	30	19	18	15	18	20	19	13	17
Co. trimoxazole	25	16	R	30	30	30	28	30	29
Erythromycin	15	18	R	40	R	40	R	—	R
Framycetin	50	21	15	18	17	16	16	19	17
Gentamycin	10	13	R	18	20	15	19	20	19
Kanamycin	30	18	19	17	22	20	20	16	20
Nitrofurantoin	300	17	17	17	18	20	15	14	18
Penicillin	10	22	R	R	—	R	—	R	—
Polymyxin-B	300	12	R	R	—	R	—	R	—
Streptomycin	10	15	16	31	24	20	18	15	14
Sulphamethazole	300	17	R	R	R	10	19	29	R
Tetracycline	300	19	13	15	15	28	18	25	12
Eugenol	5		12	25	16	15	15	14	12

R = Resistant                      — = test not conducted

\*concentrations of bacitracin, penicillin and polymyxin-B reported are in units.

TABLE 2. ANTIMICROBIAL ACTIVITY OF EUGENOL AND OTHER ANTIMICROBIAL AGENTS AGAINST GRAM POSITIVE ORGANISMS

Antimicrobial agents	Concn ( $\mu\text{g}$ )*	Min. sensitive zone (mm)	<i>Bacillus</i> spp	<i>Micrococcus</i> spp	<i>Staphylococcus</i>		<i>Streptococcus</i> spp
					<i>aureus</i>	<i>epidermis</i>	
Ampicillin	10	29	12	39	20	16	30
Bacitracin	10	13	12	21	30	20	10
Cephalexin	30	18	17	39	40	21	36
Cephaloridine	30	16	31	48	42	35	R
Chloramphenicol	30	18	30	29	—	20	—
Chlortetracycline	30	19	29	36	36	26	18
Co. trimoxazole	25	16	32	32	30	24	R
Erythromycin	15	18	18	17	R	12	R
Framycetin	50	21	17	17	12	15	16
Gentamycin	10	13	20	22	20	18	10
Kanamycin	30	18	22	24	20	18	R
Nitrofurantoin	300	17	16	29	18	20	20
Penicillin	10	29	—	—	14	—	15
Polymyxin-B	300	12	—	—	7	—	R
Streptomycin	10	15	24	14	19	10	R
Sulphamethazole	300	17	32	30	38	32	18
Tetracycline	300	19	22	31	25	20	9
Eugenol	5		15	18	25	15	5

R = Resistant                      — = test not conducted

\*concentrations of bacitracin, penicillin and polymyxin-B reported are in units.

eugenol against *Proteus mirabilis* and *Citrobacter freundii* was not significant.

Among the Gram positive organisms tested, maximum antibacterial effect was shown towards *Staphylococcus aureus* which was comparable to the effect of antibiotics like cephalaxin, cephaloridine, co-trimoxazole, chlortetracycline, gentamycin and sulphamethazole. A similar effect was

observed against *Micrococcus* spp and *Bacillus* spp. However, the effect against *Streptococcus* spp was not significant.

Eugenol could inhibit the growth of *Escherichia coli*, *Enterobacter sakazaki* and *Klebsiella pneumoniae* which are not inhibited by antibiotics like ampicillin, erythromycin, and sulphamethazole. Thus, the broad spectrum antibacterial effect of eugenol was comparable to the antibiotic effect of

tetracycline as evidenced by similar zones of inhibition. A kinetic study to understand the dose and response effect of eugenol needs to be carried out, which may provide the information on the minimal effective dose of eugenol to inhibit the growth of microorganisms.

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## Studies on Colour Retention in Pepper Subjected to Different Treatments

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Fresh pepper (*Piper nigrum* L.) was subjected to treatments such as microwave exposure, microwave exposed boiling water blanching and direct boiling water blanching to study the extent of green colour retention. The variation in the chlorophyll content at different stages was insignificant among the samples subjected to the same treatment. The best colour retention was observed in the microwave exposed boiling water blanched samples.

Pepper (*Piper nigrum* L.) one of the most important spices, is used mainly as black dried berries, skin removed white pepper or as dehydrated green pepper<sup>1,2</sup>. Green coloured dried pepper has become attractive due to good export market<sup>1</sup>. The reports on chemistry of green pigment of pepper are scanty<sup>3</sup>. It is only recently that the enzyme involved in the biochemical changes of the blackening of pepper during drying was isolated and studied<sup>4</sup>. According to this, study the blackening of the pepper is due to the enzymatic oxidation of (3,4-dihydroxy phenyl)- ethanol glycoside by *o*-diphenol oxidase enzyme, present in the fresh pepper. The chemistry of the compounds involved in blackening is also studied to some extent<sup>5</sup>. It is worth mentioning that chlorophyll degradation during biological activities<sup>6,7</sup> in some plants and heating or blanching<sup>8</sup> of chlorophyll containing materials during processing are reported. Similar studies have not been carried out in pepper. Chlorophyll content and colour of the pepper, dried after subjecting the fresh green pepper to different techniques for arresting enzymes are reported here.

Fresh mature green pepper was collected from the local plantation. The berries were separated and cleaned. About 75 g of the berries were subjected to the following treatments at different times: (a) *Direct microwave exposure*: Fresh pepper berries were uniformly spread in a petri dish and exposed to 2450 MHz frequency microwaves using a domestic model microwave oven. (b) *Microwave exposed water blanching*: Fresh pepper was added to boiling water in a beaker and exposed to microwaves in the oven and (c) *Water blanching*: Fresh pepper was added to boiling water heated using an electric heater. All the above samples were dried overnight in a cross flow dryer at  $55 \pm 3^\circ\text{C}$ . Two g each from the dried samples were extracted with ether and the chlorophyll content was estimated by AOAC method<sup>9</sup>.

At different duration of boiling water blanching, the chlorophyll content remained fairly constant in the dried product at  $0.03 \pm 0.002$  per cent level (Table 1) though the colour of dried berries blanched in boiling water at different duration varied from black in the shortest time blanched berries to mixed black and green (blackish green) in the

TABLE 1. CHLOROPHYLL CONTENT AND COLOUR OF PEPPER SUBJECTED TO DIFFERENT TREATMENTS

Time* (min)	Chlorophyll (% wt)**			Product colour		
	Boiling water	Microwave in boiling water	Microwave	Boiling water	Microwave in boiling water	Microwave
2	0.029	0.043	0.033	A	C	A
5	0.030	0.043	0.032	A	C	A
8	0.032	0.045	0.029	B	D	A
12	0.033	0.042	0.032	B	D	A
15	0.031	0.045	0.035	B	D	C

\*Time sequence for microwave treatment was 10,20,30,60 and 120 sec.

\*\*Average of three values

In case of microwave treatment, the chlorophyll content was 0.030% and the product was of faded green colour at 300 sec.

A-Black; B-Mixed black and green; C-Faded green and D-Green.

prolonged blanched materials. The fresh pepper extracted with ether after mixing with anhydrous sodium sulphate was also found to have the same level of chlorophyll as the above samples. This shows that chlorophyll degradation is not taking place during processing while the colour variation of dried pepper is probably due to the masking effect of the compounds of oxidation.

Faded green coloured product was obtained in 2 min microwave exposed boiling water blanched sample as against black colour in the 2 min boiling water blanched samples. This showed the possible involvement of partial inactivation of enzymes and possible removal of polyphenols with water. Presence of sufficiently large amount of water appears to be desirable for avoiding the blackening of pepper when exposed to microwaves. The green colour intensity of the dried product steadily increased in samples with increase of time of microwave exposure. The colour of the dried berries obtained from 8,12 and 15 min exposed fresh pepper (in boiling water) were green, probably due to inactivation of polyphenol oxidase. In our earlier studies<sup>10</sup> it was shown that oil palm fruit lipase can be inactivated in 30 sec and that of rice bran<sup>11</sup> in 2-3 min by microwaves. Higher level of chlorophyll in the microwave exposed boiling water blanched samples could, however, not be explained.

The berries exposed to direct microwave energy for 10 to 30 sec were more or less uniformly black in colour after drying, indicating that the changes are like those taking place as in the pepper dried by conventional method. Berries exposed to microwaves for 3 to 5 min showed only faded green colour. Further exposure above 6 min resulted in charred

berries. In case of the exposure of fresh pepper to microwaves directly, the water ejected out from inside the berries and was visible as droplets. Chlorophyll content remained fairly constant in samples directly exposed to microwave for different time durations, probably due to different degrees of polyphenol oxidation. Even at 5 min of direct exposure, the chlorophyll was not degraded by the microwave energy in pepper.

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## Chlorophyll Losses During Preparation, Canning and Storage of Brassica Greens (SAG)

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Total chlorophyll content decreased to 80% of its initial value after cooking and canning and to 40-50%, during 6 months storage in Brassica greens. 'RLM-240' and 'Brown sarson' retained maximum chlorophyll and Chinese cabbage contained minimum chlorophyll in fresh, processed as well as stored form among the five varieties studied. Higher chlorophyll was retained under refrigerated storage.

In green leaves, the chlorophyll is broken down during senescence and the green colour tends to disappear. Colour changes from bright green to olive brown in processed and stored green vegetables<sup>1,2</sup>. Attempts were made to preserve the green colour by pH control<sup>3</sup> and minimizing heat exposure duration in high temperature short time processing or their combination<sup>4</sup>. Recently, it was observed that pyropheophytin a and b were the major chlorophyll degradation products found in all commercially canned vegetables<sup>5</sup>. Clydesdale and Francis<sup>6</sup> recommended the use of high quality raw materials, handling of the product with utmost care, employing optimum storage conditions and use of quality maximized process for the best retention of chlorophyll in the processed food. Since information on this aspect for canned sag was lacking, this investigation was undertaken to study the rate of chlorophyll degradation in

canned sag during storage at room and low temperatures.

Five Brassica (*Brassica species*) varieties, namely 'Torla-ITSA', 'Brown sarson', 'Chinese cabbage', 'RLM-240' and 'Gobhi sarson', were cooked and processed into ready-to-eat canned sag and stored for six months at room temperature (10-39°C) or low temperature (0-4°C). Chlorophyll was estimated according to Anderson and Boardman<sup>7</sup>. Tintometer colour readings were recorded against Red (R), Yellow (Y), Blue (B) and Neutral (N) filters by taking the sample into the colour matching cell of universal lovibond tintometer (Tintometer Ltd. Salisbury, England). Data on chlorophyll degradation were examined by the analysis of variance<sup>8</sup>.

Raw leaves and stems (composite sample) of five Brassica varieties varied widely among the varieties (Table 1). Major portion (80 per cent) of chlorophyll was lost during cooking.

TABLE I. CHLOROPHYLL CONTENT OF RAW, COOKED, AND CANNED BRASSICA GREENS

Variety	Stages	Chlorophyll (mg/100 g db*)			Tintometer readings			
		a	b	Total	Red	Yellow	Blue	Neutral
Torla-ITSA	Raw	217.0	183.0	400.0	2.3	13.1	8.2	0.1
	Cooked	18.9	14.1	33.0	4.2	15.0	5.4	0.4
	Canned	18.4	14.1	32.5	4.2	15.0	5.4	0.4
Brown sarson	Raw	326.0	262.0	588.0	2.3	12.8	9.4	0.1
	Cooked	19.9	16.0	35.9	4.2	14.2	5.5	0.4
	Canned	19.6	15.8	35.4	4.2	14.2	5.5	0.4
Chinese cabbage	Raw	206.0	186.0	392.0	2.1	13.1	8.5	0.2
	Cooked	15.3	13.9	29.2	4.0	15.5	5.5	0.4
	Canned	15.8	13.3	29.1	4.0	15.5	5.5	0.4
RLM-240	Raw	288.0	229.0	517.0	2.0	12.7	9.6	0.2
	Cooked	25.9	17.5	43.4	4.2	14.0	5.4	0.6
	Canned	24.4	17.6	42.0	4.2	14.0	5.4	0.6
Gobhi sarson	Raw	308.0	248.0	556.0	2.1	13.0	8.7	0.1
	Cooked	21.5	16.4	37.9	4.2	15.4	5.1	0.1
	Canned	20.8	15.3	36.1	4.2	15.4	5.1	0.1

\*On dry weight basis



TABLE 2. EFFECT OF VARIETY AND STORAGE ON THE CHLOROPHYLL CONTENT OF CANNED SAG

Variety	Temp (°C)	Stages period (months)	Chlorophyll (mg/100 g db*)			Tintometer readings			
			a	b	Total	Red	Yellow	Blue	Neutral
Torja-ITSA	0-4	3	16.3	12.5	28.8	3.0	16.5	4.0	0.1
		6	10.0	8.4	18.4	3.1	16.9	4.1	0.2
	10-39	3	15.0	12.5	27.5	3.1	16.2	4.1	0.2
		6	9.2	8.0	17.2	3.8	17.0	4.0	0.2
Brown sarson	0-4	3	17.4	13.6	31.0	3.0	15.8	4.2	0.1
		6	12.1	9.6	21.7	3.0	16.4	4.0	0.2
	10-39	3	16.6	12.8	29.4	3.4	15.8	4.4	0.2
		6	10.4	8.8	19.2	3.2	16.6	4.2	0.2
Chinese cabbage	0-4	3	13.2	10.7	23.9	3.1	16.0	5.2	0.4
		6	8.3	7.4	15.7	3.2	17.2	4.9	0.4
	10-39	3	12.6	9.6	22.2	3.4	16.4	4.2	0.4
		6	6.9	6.9	13.8	3.0	17.0	4.6	0.4
RLM-240	0-4	3	19.9	14.7	34.6	3.2	14.2	5.1	0.4
		6	14.3	9.2	23.5	3.1	15.2	4.2	0.2
	10-39	3	18.7	14.5	33.2	3.3	14.5	4.6	0.4
		6	13.3	8.7	22.0	3.2	15.4	4.0	0.2
Gobhi sarson	0-4	3	18.6	12.2	30.8	4.0	16.4	4.1	0.1
		6	8.7	8.6	17.3	4.2	17.1	4.0	0.1
	10-39	3	17.6	11.2	28.8	4.1	16.6	4.2	0.1
		6	8.1	8.5	16.6	4.0	17.4	4.0	0.1

\*On dry weight basis

Negligible losses (about 2 per cent) of chlorophyll were found immediately after canning. After six months of storage of canned Brassica at room temperature (10-39°C) and low temperature, (0-4°C) percentage losses of chlorophyll were 40-50 per cent depending upon the variety (Table 2). During storage of canned Brassica, retention of chlorophyll was slightly better at 0-4°C than at 10-39°C (Table 2). Statistically high significant differences were observed on the effect of variety of Brassica and storage conditions on chlorophyll retention. On an average, 'RLM-240' and 'Brown sarson' retained maximum chlorophyll after processing and storage. Both the varieties contained good amounts of chlorophyll in the fresh form also (Table 1). 'Chinese cabbage' was found to have minimum chlorophyll content in fresh, processed as well as stored form among all the Brassica varieties. The red and blue colours measured through tintometer decreased during storage with corresponding increase in yellow units (Table 2). These changes were more pronounced in Brassica stored at room temperature for 24 weeks as compared to low temperature storage.

Therefore, it is concluded that for better retention of chlorophyll, the product should be stored at low temperature

(0-4°C). The raw material should be with high chlorophyll content and should be exposed to heat for minimum time as possible. Hence there is a need to grow new varieties with high chlorophyll content.

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## A New Spectrophotometric Method for the Determination of Fenvalerate (Synthetic Pyrethroid)

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A new spectrophotometric method, developed for the determination of fenvalerate (Cyano (3-phenoxyphenyl) methyl 4-chloro - $\alpha$  - (1-methyl ethyl) benzene acetate (51630-58-1), involves the hydrolysis of fenvalerate under alkaline conditions and the coupling of liberated aldehyde with 2,4-dinitrophenylhydrazine to yield a chromophore ( $\lambda_{\max} = 465$  nm). Beer's law is obeyed in the range 0.1 – 3.0 p.p.m. The method is sensitive (Sandell sensitivity = 0.0048) and it could be successfully used for the determination of fenvalerate in water sources and formulations.

Fenvalerate, a broad spectrum pyrethroid insecticide, is exceptionally active as a contact and stomach poison against lepidopterous larvae. Besides high insecticidal activity, it has moderately long persistence in plants<sup>1</sup>. It is also toxic to birds and mammals ( $LD_{50} = 450$  mg/kg)<sup>2</sup>. This insecticide is intensively used for a variety of crops and its residue enters into inland water sources from the fields where it has been applied and contaminates the adjacent streams, ponds, lakes, wells, etc. The insecticide is highly stable under acidic conditions, though unstable at pH 8.0<sup>1</sup>. Several chromatographic methods are available for determination of fenvalerate<sup>3-5</sup>. The development of a new simple and accurate spectrophotometric method has been reported in the present communication.

The formulations used in the investigation were 20 per cent E.C. fenvalerate samples supplied by two different manufacturers. Stock solutions of fenvalerate, prepared in carbonyl-free methanol, were progressively diluted with methanol to obtain solutions of desired concentrations. Aliquots containing 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 p.p.m. of standard fenvalerate solutions were taken in clean, dry 10 ml graduated test tubes, 1 ml of 2 per cent potassium hydroxide solution was added to each test tube, heated to 40 – 45°C for 30 min and neutralised with 0.1 N hydrochloric acid. A 0.5 ml of 0.1 per cent methanolic 2,4-dinitrophenylhydrazine was added followed by one drop of concentrated hydrochloric acid and heated to 50-55°C for 30 min. Red coloured solutions, with an absorption maximum at 465 nm (Fig. 1), were obtained by the addition of 2.5 ml of 4 per cent potassium hydroxide. 2,4-dinitrophenylhydrazine and fenvalerate solutions in alcohol showed maximum absorption at 360 and 300 nm respectively (Fig.1). The

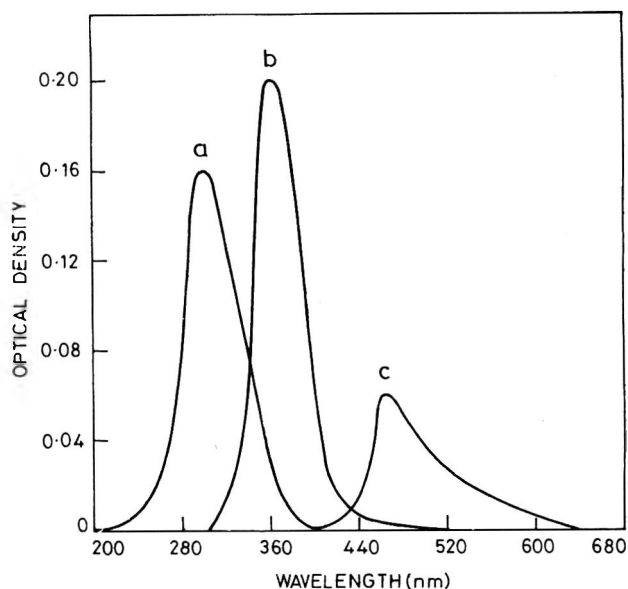


Fig.1. (a) Absorption spectrum of fenvalerate  
(b) Absorption spectrum of 2,4-dinitrophenylhydrazine  
(c) Absorption spectrum of coloured compound

absorption maximum value for fenvalerate in carbon tetrachloride reported by the manufacturers is 278 nm. The colour of the phenylhydrazone derivative is stable for 48 hr and is maximum in the pH range 11.5–12. Excess reagent had no effect on the absorbance of the coloured compound. It was found to obey Beer's law for the fenvalerate concentration of 0.1–3 p.p.m.

Distilled water samples (1 l) were taken and spiked at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 p.p.m. of fenvalerate, the pH of these samples was adjusted to 3-4 with 50 per cent sulphuric acid solution and anhydrous sodium

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sulphate (10 g) was added to each sample. The pyrethroid in the samples was extracted thrice using approximately 50 ml chloroform for each extraction by shaking for 5-10 min. The combined extracts were dried over 10 g anhydrous sodium sulphate and chloroform was evaporated to dryness by exposing to air. Residue left was dissolved in carbonyl - free methanol and the amount of fenvalerate was determined using the above indicated procedure. The data are reported in Table 1 along with those for ground water (Borewell and dugwell). Two commercial formulations of fenvalerate were analysed using the above indicated procedure and by a standard curve, the percentage of active ingredient was determined.

TABLE 1. RECOVERY OF FENVALERATE FROM SPIKED WATER AND ANALYSIS OF 20% FENVALERATE FORMULATIONS

Fenvalerate added (p.p.m)	Spiked water samples		Fenvalerate taken (p.p.m)	Fenvalerate formulations (20%)	
	Recovery (%)			Fenvalerate determined (%)	
	Distilled water	Ground water		Sample A	Sample B
0.5	96.0	96.0	0.5	19.2	19.2
1.0	96.0	96.0	1.0	19.2	19.6
1.5	97.3	97.3	1.5	19.5	19.5
2.0	98.0	99.0	2.0	19.6	19.6
2.5	98.4	99.2	2.5	19.7	19.7
3.0	98.6	98.0	3.0	19.7	19.7
Mean $\pm$ S.D.	97.4 $\pm$ 0.39	97.6 $\pm$ 0.35		19.5 $\pm$ 0.1	19.55 $\pm$ 0.06

The data presented in Table 1 show that the recovery of the pyrethroid from the spiked water samples is 97.4 per cent. The results indicate that the percentages of the active ingredient present in the formulations confirm the efficiency of the method.

The spectrophotometric method developed is also sufficiently sensitive. Further, the colour development is quick and is also stable for a long period. The coloured compound has maximum absorption at 465 nm at which the reagent has no absorption. The method thus, is of value in the determination of fenvalerate in the insecticide formulations and also in water samples.

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## ANNOUNCEMENT

An "International Symposium on Pulses Research" will be organised by the Indian Society of Pulses Research and Development (ISPRD) at Kanpur from 4-8 December, 1993. It is co-sponsored by the ICAR, New Delhi. The programme will cover topics on grain legumes of cool and warm seasons and will include enhancement of genetic resources, breeding for resistance (biotic and abiotic) and productivity, genetics, cytogenetics, physiology, biotechnology, disease and pest management, production technology (legumes based cropping systems, fertility management including biological nitrogen fixation, weed management and sustainable agriculture), grain quality, post-harvest technology and developmental strategies.

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## Digestibility of Protein and Starch in Malted Weaning Foods

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**Roller-drying reduced *in vitro* protein digestibility of weaning foods based on malted/roasted maize/rice and malted cowpea. Cooking of weaning foods improved protein digestibility as compared to uncooked blends. *In vitro* starch digestibility was similar in various blends of cooked weaning foods. Increasing the malted material in weaning foods improved the digestibility of protein, but not the digestibility of starch.**

Cereals and millets form staple foods for the majority of population in India, Africa and other developing countries. Along with these basic staples, legumes such as chickpea (*Cicer arietinum*), and cowpea (*Vigna sinensis*) are generally consumed after cooking.

Cereals and legumes are subjected to different kinds of processing such as germination, roasting, roller-drying during the course of preparation of weaning foods. Such processing procedures have beneficial effect on the digestibility of protein and starch. Germination of sorghum significantly improved the *in vitro* digestibility of starch and protein<sup>1</sup>. Roasting at 160°C for 10 min enhanced significantly *in vitro* digestibility of starch and protein of soya bean<sup>2</sup>. Cooking resulted in an improvement in *in vitro* digestibility of starch in roller-dried wheat flour<sup>3</sup>. Since data on the *in vitro* digestibility of proteins and starch of weaning foods based on maize, rice and cowpea subjected to treatments such as malting, heat processing are limited, the present studies were undertaken.

Maize, rice and cowpea, from the local market, were cleaned and stored in tins at room temperature. Both maize and cowpea were found to possess over 95 per cent germination. The maize grains were washed, soaked in water for 18 hr and allowed to germinate for 48 hr. Soaking and germination times for cowpea were 16 and 24 hr respectively. The germinated grains were dried in the sun for 10 hr. Maize was derooted, kilned in an electric roaster at 70°C for 15 min, sprinkled with 5 per cent water, allowed to stand for 10 min and milled slightly to loosen the husk. Dried cowpea was split in a disc abrasion mill to loosen the husk, which was removed by air aspiration. The split grains were kilned and milled to obtain the germinated cowpea flour. Rice was washed, soaked for 2 hr and dried in the sun for 8 hr roasted for 25 min at 80°C in an electric roaster and pulverized.

Three weaning food formulations based on malted maize and cowpea were prepared. In the first formulation, germinated cowpea flour (90) and skim milk powder (10) were mixed with water (20 per cent) to form a dough, placed in a tray and steamed in an autoclave for 25 min, followed by drying in a tray drier at 65°C for 6 hr. In the second formulation, malted maize (60) and germinated cowpea (40 per cent) were dry mixed for 30 min. In the third formulation, roasted rice (55), germinated cowpea (35) and malted maize (10 per cent) were dry mixed.

Two roller-dried formulations were prepared. One consisted of malted maize (48), germinated cowpea (32), skim milk powder (8), sugar (8) and groundnut oil (4 per cent). The other consisted of roasted rice (44), germinated cowpea (28), malted maize (8), skim milk powder (8), sugar (8) and groundnut oil (4 per cent). In both the cases, the ingredients were mixed with double the quantity of water, the slurry was blended for 15 min, passed through a homogeniser and poured slowly on to two stainless steel revolving drums, rotating in opposite direction at 4 r.p.m. and under steam pressure of 2-2.5 kg/sq.cm. The slurry was cooked and dried simultaneously, producing thin, paper-like flakes, which were reduced to flour in a hammer mill.

*In vitro* protein digestibility was determined according to Walter *et al*<sup>4</sup> with following modifications: Slurried samples containing about 100 mg protein were shaken with 12.5 mg of pepsin in 50 ml 0.1 N HCl at 37°C for 3 h. After neutralisation with 0.5 N NaOH, 6 mg pancreatin dissolved in 25 ml of phosphate buffer (pH 8.0) was added and the digestion continued for 24 h at 37°C. The volume was made to 100 ml and an aliquot (50 ml) was treated with 10 per cent TCA overnight to precipitate the proteins. The suspensions were centrifuged (6,000 × g for 40 min) and the residue

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TABLE 1. *IN VITRO* DIGESTIBILITY OF PROTEIN AND STARCH IN MALTED AND ROLLED-DRIED WEANING FOODS

Food formulation	% protein digested		mg of glucose equivalents released from 2 g material			
	Uncooked	Cooked	30 min	60 min	90 min	120 min
Malted maize + malted cowpea	75.5	80.5	700	790	840	980
Roller-dried malted maize + malted cowpea	72.3	76.5	802	852	961	1080
Roasted rice + malted cowpea	74.7	76.7	717	794	909	925
Roller-dried roasted rice + malted cowpea	60.6	67.6	780	825	941	1020
Steamed cowpea + skim milk powder	79.6	83.5	746	858	926	1050
Skim milk powder	87.1	—	—	—	—	—

(undigested protein) was assayed for protein by micro-kjeldahl method. To study the effect of cooking, the materials were steamed with 10 ml water for 5 min prior to treatment with pepsin. The protein digested by pepsin and pancreatin was calculated using the formula:

$$\text{Protein digestibility} = \frac{\text{Protein content of sample} - \text{Undigested protein}}{\text{Protein content of sample}} \times 100 \text{ per cent}$$

*In vitro* starch digestibility was determined as follows: Two per cent slurry of the food material was cooked on a boiling water bath for 15 min. Thirty ml of 0.2 M glycine-HCl buffer (pH 2.0) containing 10 mg of pepsin was added to 50 ml slurry and incubated at 37°C for 2 h. After incubation, the slurry was neutralized with 0.2 N NaOH and volume was made to 100 ml. Five ml of 0.05 M phosphate buffer containing 15 mg of pancreatin and 15 mg of amyloglucosidase were added to 10 ml aliquot and incubated for 2 h at 37°C. At 30 min intervals, the reaction was stopped by keeping the digest on a boiling water bath for 5 min. Aliquots (0.5 ml) of the samples were mixed with dinitrosalicylic acid reagent (2 ml) for determination of reducing sugar<sup>5</sup>. Glucose was used as a standard and the degree of hydrolysis was expressed as mg of glucose liberated from the food products, after correction for blank values.

Cooking increased the digestibility in all the blends (Table 1). Malted maize-cowpea blend showed slightly higher protein digestibility as compared to the roasted rice-cowpea mixture. However, the protein digestibility of roller-dried product based on maize and cowpea was markedly greater than that of the similar product based on rice and cowpea. Steam-cooked cowpea-skim milk powder blend showed the highest digestibility among the various blends. The protein

digestibilities of the different blends were lower as compared to skim milk powder. The differences in protein digestibility between the rice-based and maize-based blends may be due to the lower content of the malted components in the blends containing rice as compared to the maize blends. The improvement upon cooking may be due to the inactivation of proteinase inhibitors and opening up of protein structure through denaturation.

*In vitro* starch digestibility is similar in malted and roller-dried blends of maize, cowpea and rice during the course of digestion of starch, as observed from the rate of release of glucose (Table 1). The rate of digestibility in all the blends was rapid upto 30 min and thereafter slowed down considerably. The data obtained in these studies indicate that while there is some improvement in the overall digestibility of proteins in weaning foods with the increase in the incorporation of malted material, no marked differences are observed in the digestibility of starch due to such incorporation.

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## Effect of Some Mycotoxins on Reproduction in Pregnant Albino Rats

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**Effect of oral administration of aflatoxin B<sub>1</sub>, patulin and kojic acid on reproductive performance of pregnant rats showed significant anti-implantational activity. Patulin and kojic acid exhibited significant abortifacient activity. A significant loss of viability among the litters was also noticed in mycotoxin fed rats except for those fed with patulin.**

Several workers have studied the effect of aflatoxins on growth and reproduction of laboratory animals. Progressive degeneration of germinal cells of testis<sup>1</sup>, intra-uterine resorption in pregnant rats<sup>2,3</sup> and cannibalistic behaviour in mother albino rats have been reported after feeding of aflatoxin<sup>4</sup>. Patulin has also been found to possess toxic property<sup>5</sup>. Domestic and pet animals are exposed to these mycotoxicological effects upon consumption of contaminated feed. The present communication describes the effects of oral administration of some mycotoxins such as aflatoxin B<sub>1</sub>, patulin and kojic acid to pregnant rats from day 1 to 5 post coitum on implantation, intra-uterine resorption and litter.

Mycotoxins (aflatoxin B<sub>1</sub>, patulin and kojic acid) were procured from Sigma Chemical Company, St. Louis, USA. Adult male and female albino rats (140 to 160 g of Sprague Dawley strain) of proven fertility were caged together in the ratio of 1:2 in the evening. Next morning females were separated and vaginal smears examined under the microscope for presence of spermatozoa. Sperm positive smear indicated that the rat had mated and the day of mating was counted as the first day of pregnancy.

Four groups of mated albino rats, each consisting of seven females were taken. The first group acted as control and were

fed orally with propylene glycol (0.1 ml) from day 1 to 5 of pregnancy. Second, third and fourth groups acted as experimental groups and were fed orally with different mycotoxins (aflatoxin B<sub>1</sub>, patulin and kojic acid) dissolved separately in glycol at a dose level of 50 µg/day/rat for 1 to 5 days of pregnancy. These rats were then laparotomized on day 8 of pregnancy to examine the corpora lutea and implantation sites. Litter size was recorded at term. Care was also taken to detect teratogenic defects or death of young ones if any as well as behaviour of mothers. Student's test was used for statistical evaluation.

Post-coital oral administration of all the three mycotoxins showed significant reduction in implantation sites as well as loss of viability among the litter, 2 to 3 days after littering (Table 1). Significant loss in litter size was observed in females treated with patulin and kojic acid. Though no teratogenic effects could be observed in the young ones born from the mycotoxin fed groups, the mortality of litter was significant (except in patulin treated group). The cannibalistic behaviour in the mothers after two days of delivery was also noticed in these groups. Out of eight pregnant females treated with kojic acid one died before delivery and two showed acute nasal and mouth infection.

TABLE 1. POST-COITAL TREATMENT OF SOME MYCOTOXINS (50 µg/rat/day) ON MATED FEMALE RATS

Treatment	Corpora lutea sites	Implantation sites	Total	Size dead	Litter viable
Glycol	10.14 ± 0.51	7.28 ± 0.36	6.57 ± 0.20	0	6.57 ± 0.20
Aflatoxin B <sub>1</sub>	11.43 ± 0.48 <sup>NS</sup>	5.42 ± 0.42 <sup>c</sup>	4.85 ± 1.00 <sup>NS</sup>	1.42 ± 0.53 <sup>b</sup>	3.43 ± 1.19 <sup>c</sup>
Patulin	9.42 ± 0.29 <sup>NS</sup>	5.57 ± 0.36 <sup>c</sup>	3.37 ± 0.75 <sup>c</sup>	0.57 ± 0.57 <sup>NS</sup>	3.00 ± 0.78 <sup>d</sup>
Kojic acid	9.42 ± 0.71 <sup>NS</sup>	3.85 ± 1.18 <sup>b</sup>	2.71 ± 0.84 <sup>d</sup>	2.00 ± 0.84 <sup>d</sup>	0.71 ± 0.18 <sup>d</sup>

NS - Not significant; a (P < .05); b (P < .02); c (P < .01) & (P < .001)

7 animals were used in each experiment

Mean ± SEM;

Pregnant females fed with all the different mycotoxins showed significant decrease in the number of implantations. It indicates inhibition of implantation<sup>6</sup> (anti-implantation effect). The secretory activity of corpus luteum controls implantation<sup>7</sup> but there is no decline in the number of corpora lutea. This indicates that the mycotoxins have acted directly at the uterus/endometrium. This may be due to either changes in maternal estrogen/progesterone ratio<sup>8</sup> or may be due to the toxicity of mycotoxins<sup>9</sup>. Decline in the litter size (indicating foetal resorption or abortifacient effect), death of litter as well as acute infection on nose and mouth in mother rats are due to the toxic effect of mycotoxins. However, toxicity of aflatoxin B<sub>1</sub> is mainly reflected by increase in the number of dead litters. The pregnant females treated with these three mycotoxins started eating their litter after two days of delivery. The cannibalistic behaviour may be due to disturbances in the physical state of the animal, perhaps causing irritability which leads to the cannibalistic tendency<sup>4</sup>. Since the mothers in the control group included in the present study did not show cannibalism, the possibility of any nutritional deficiency is ruled out.

It is, therefore, concluded that aflatoxins B<sub>1</sub>, patulin and kojic acid bring about anti-implantation, abortifacient effect. Death of the litter in mated females is mainly due to their toxicity.

Financial assistance provided by UGC, New Delhi for this project is gratefully acknowledged.

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## Critical Control Points in the Slaughter and Dressing of Farmed Crocodiles

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Microbial contamination exceeding 300 colony forming units (CFU) per cm<sup>2</sup> has been observed on the skin surface of crocodiles. Mechanical removal by scrubbing and rinsing had little effect. Handling during skinning resulted in contamination ranging from 2 – 50 CFU/cm<sup>2</sup> on the meat surface. Dipping the meat in a 30 p.p.m. chlorine solution for 10 min reduced surface contamination in one abattoir by approximately 90%, whereas no effect was recorded at another abattoir, most likely because of handling subsequent to dipping.

The Nile crocodile, *Crocodylus niloticus*, has for many years been exploited for its skin which is highly valued by the leather industry. This has generated a strong interest for the rearing of crocodiles in captivity, a venture initially based

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on collection of eggs from the wild, but during the last decade proper crocodile farms were established with breeding stock<sup>1</sup>. The development seems to have been particularly successful in Southern Africa - in Zimbabwe alone more than 60 crocodile farms are now in commercial operation<sup>2</sup>. The reptile meat is reported to be pleasant to eat and considered as a potential food source<sup>3</sup>. Crocodile tail meat, originally a by-product from skin production, has become increasingly popular as an exotic dish on the Zimbabwean market especially among tourists, and the export of frozen crocodile meat to overseas markets is increasing. However, the rearing of crocodiles in ponds with high stocking densities combined with the regular occurrence of *Salmonella sp.* and other enteropathogens<sup>4,7</sup> necessitate very strict hygienic procedures during slaughter, skinning, dressing and packing of crocodile meat<sup>8</sup>. This study was undertaken to identify critical points of microbial contamination, and to evaluate control measures at two crocodile abattoirs in Zimbabwe.

Fifteen crocodiles from two farms were sampled during normal slaughter operations at various points from capture to packaging of the tail meat for freezing. Both farms were operating abattoirs approved by the veterinary authorities. The animals for slaughter were either captured, stunned and pithed (Farm 1) or shot in the pens and collected (Farm 2), vigorously scrubbed and rinsed with running water, and the throat and cloaca plugged with cotton wool or paper. Before skinning, the carcass was either dipped in a 0.5 per cent iodophore (Mikrokleen<sup>®</sup>) solution (Farm 1), or left for 10 min in a bath containing 100 p.p.m. chlorine (Alginate<sup>®</sup>) (Farm 2). Skinning was performed on stainless steel tables that were regularly cleaned and disinfected.

After removal of the skin, the tail was severed immediately behind the cloacal area and held in a 30 p.p.m. chlorine bath for approximately 10 min. The tail was then drained for 5 min, packed in a plastic bag and transferred to a blast freezer. On Farm 1, the tails were removed from the bath by hand before being placed on hooks to drain, whereas at Farm 2

the tails were placed on stainless steel hooks before being submerged in the chlorine bath, and not touched thereafter.

Bacteriological samples were obtained as surface impressions of a 20 cm<sup>2</sup> area approximately 5 cm posterior to the cloaca by standard agar sausage sampling techniques<sup>9</sup>. The samples were incubated aerobically for 2-3 days at 30°C for determining CFU/cm<sup>2</sup>. Similarly, impression samples were obtained from knives, table surfaces and workers' hands.

The disinfection was satisfactory at Farm 2 (Table 1), whereas a considerable build-up of bacterial table contamination was noted at Farm 1. The hand and knife hygiene was unsatisfactory at both farms.

Data in Table 2 show that skin surface contamination was heavy, and it probably depended on water temperature and stocking density. The surface contamination of alligators (*Alligator mississippiensis*) caught in the wild was reported to be around 10<sup>4</sup> CFU/cm<sup>2</sup>.

Scrubbing and rinsing appeared to have little effect on skin contamination whereas the procedure of dipping the carcass in a disinfectant-containing tank before skinning resulted in a considerable reduction of surface contamination. Although most skin samples taken before disinfection showed confluent growth, it was estimated that this was roughly equivalent to 300 CFU/cm<sup>2</sup>. By using this estimate, it can be stated that the procedure reduced skin surface contamination on Farm 1 by about 90 per cent and on Farm 2 by about 99 per cent.

The dipping of the tail meat in chlorine was an effective hygienic measure on Farm 2 which recorded a 90 per cent reduction. On Farm 1, this procedure appeared to have no effect whatsoever, probably due to the handling and recontamination of tails after dipping. Further, it was observed that dipping of just 8-10 crocodile tails resulted in an almost complete depletion of free chlorine to a level of less than 1

TABLE 1. GENERAL SLAUGHTER HYGIENE AS MONITORED BY SURFACE IMPRESSION SAMPLES FROM CRITICAL POINTS IN THE SLAUGHTER PROCESS. COUNTS EXPRESSED AS CFU/cm<sup>2</sup>.

Item	Start		After 10		After 15 crocs
	Farm 1	Farm 2	Farm 1	Farm 2	Farm 1
	Table 1	1	3	11	4
Table 2	5	1	3	7	>50
Table 3	3	-	7	-	>50
Knife 1	6	1	8	18	20
Knife 2	19	-	3	-	4
Hand 1	22	1	3	>50	8
Hand 2	18	-	24	-	11
Hand 3	22	-	19	-	>50

Farm 2 not tested after 15 crocs.

TABLE 2. EFFECT OF PROCEDURES EMPLOYED TO REDUCE CARCASS SURFACE CONTAMINATION (CFU/cm<sup>2</sup>).

Croc. no.	Skin		Tail meat			
	After Tank 1*		Before Tank 2**		After Tank 2	
	Farm 1	Farm 2	Farm 1	Farm 2	Farm 1	Farm 2
1	25	4	7	16	3	3
3	8	4	2	19	2	5
5	6	4	6	20	6	3
6	27	-	19	-	4	-
7	-	7	-	>50	-	3
8	7	-	2	-	4	-
9	-	1	-	12	-	1
10	9	-	2	-	22	-
11	29	-	30	-	12	-
14	>50	-	6	-	7	-
15	>50	-	4	-	10	-

- = Not tested; \* = Chlorine 100 ppm, 10 min; \*\* = Chlorine 30 ppm, 10 min. Skin surface samples taken before and after scrubbing and rinsing with water showed counts  $\leq$  50 CFU/cm<sup>2</sup>.



p.p.m. In this connection, it is interesting to note the increasing meat surface contamination of crocodile nos. 10, 11, 14 and 15 in Table 2.

In conclusion, the study showed that dipping in a chlorine solution appears to be an effective method to reduce surface contamination of dressed tail meat. Moreover, the handling of the product after dipping can very effectively ruin any hygienic achievements. The skin of crocodiles adheres more tenaciously to the underlying meat, which makes flaying a difficult dressing operation. If this is carried out on a table surface, opportunities for skin-hand-table-meat contacts become considerable. A system for skinning and dressing of crocodiles in a hanging position may prove useful.

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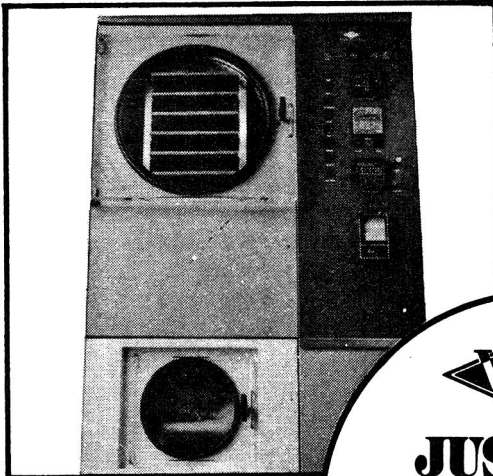
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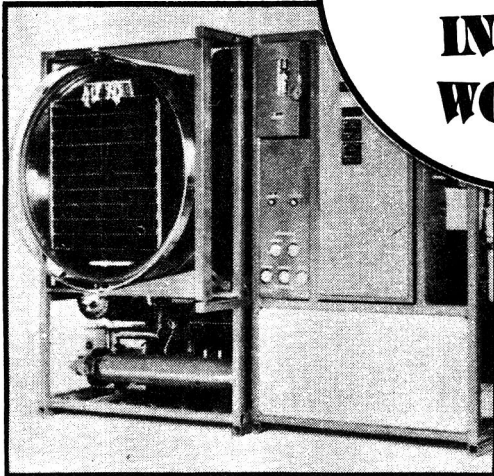


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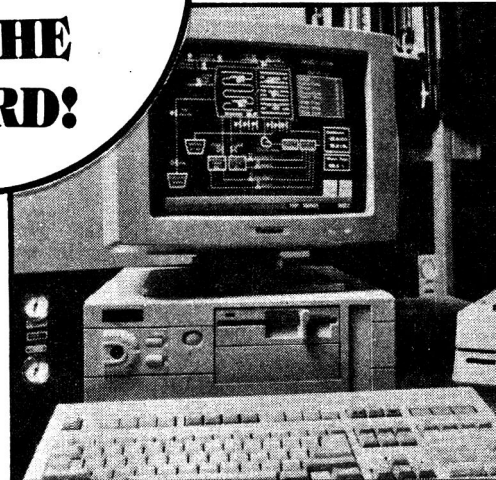


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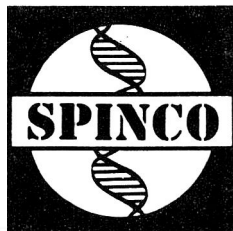
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## BOOK REVIEWS

*Fd. Sci. Technol*, 1992, Vol. 29, No. 4, 269—271

*Environmental Health Criteria No. 121; Aldicarb*; Published by the World Health Organisation, Geneva, 1991; pp:130; Price: Sw. fr.16, for developing countries: Sw: fr.11.20.

In this monograph, the available information on Aldicarb has been detailed under 13 main headings as follows: 1. Summary; 2. Identity, physical and chemical properties and analytical methods; 3. Sources of human and environmental exposure; 4. Environmental transport, distribution and transformation; 5. Environmental levels and human exposure, 6. Kinetics and metabolism; 7. Effects on laboratory mammals and *in vitro* test systems; 8. Effects on humans; 9. Effects on other organisms in the laboratory and field; 10. Evaluation of human health risks and effects on the environment; 11. Conclusions and recommendations; 12. Further research; and 13. Previous evaluations by International bodies.

Aldicarb is a heat sensitive and relatively unstable chemical. During gas chromatographic analysis, aldicarb nitrile interferes, thus necessitating time-consuming clean up procedure. Aldicarb nitrile cannot be detected by liquid chromatography with UV detector. It is a systemic pesticide that is applied to the soil to control certain insects, mites and nematodes. Residue levels greater than 1 ppm have been reported from potatoes. Ground water in USA has been reported to contain about 6.0 g/litre. Its residues have been detected in many crops. Exposure of general population to aldicarb and its toxic metabolites such as sulfones and sulfoxide occur mainly through food and water. Due to the high acute toxicity of aldicarb, both inhalation and skin contact under occupational exposure conditions may be dangerous for workers if preventive measures are inadequate. It is efficiently absorbed in gastrointestinal tract and to a lesser extent through skin. It is readily absorbed by the respiratory tract and distributes to all tissues including the developing foetus. But, aldicarb does not accumulate in the body as a result of long term exposure; half-life being 30-40 min. Non-fatal poisoning in men is rapidly reversible, recovery aided by administration of atropine.

Although hydrolysis destroys the insecticidal activity, the products both sulfoxide and sulfone are potent anticholine esterase inhibitors. The main route of excretion of aldicarb and its metabolites is through urine. Evidences show that it did not produce mutagenicity in any of the bacterial strains tested.

Owing to the potential for dermal absorption of carbamate, it is produced only in the granule form as Temik 5G, 10G, and 15G. The metabolite sulfone is also used as pesticide under the name aldoxycarb. Soil application rates are 0.56-5.6 kg ai/La. The effective life of it varies depending on the type of the soil, its mixture, temperature, irrigation conditions and presence of soil microorganisms. From the granules, water

is needed to release the active ingredient. In plants, it is metabolised by processes involving oxidation to the sulfoxide and sulfone as well as by hydrolysis to the corresponding oximes and ultimately to nitrile. The fate of aldicarb in the atmosphere has not received much attention, and also the fate in surface water has not been studied extensively. Since it is not transported to atmosphere to any greater extent, it is not a source of atmospheric contaminant.

Similar salient points about the aldicarb have been well documented in various chapters listed above. Finally, the monograph provides extensive references.

The WHO task group under the Chairmanship of Prof. W.J. Hayes has done a commendable job in bringing out the Environmental Criteria 121 on aldicarb.

J.R. RANGASWAMY  
CFTRI, MYSORE.

*Environmental Health Criteria: No. 120, Hexachlorocyclopentadiene*, WHO, Geneva, 1991, 126 pages, Price: Sw fr.15.

This monogram published under WHO's Environmental Health Criteria Series evaluates risks to human health and the environment posed by the production, use and disposal of hexachlorocyclopentadiene (HEX). The worldwide production of this chemical is around 15,000 tonnes per year, and is mainly used as an intermediate in the production of many other organochlorine pesticides including heptachlor, chlordane, aldrin, dieldrin, endrin, mirex, endosulfan, etc. HEX is also used in the manufacture of flame retardants, resins and dyes. During its production and processing, small amounts of HEX are released into the environment thus posing health risks. Some countries have restricted its use in the production of organochlorine pesticides.

The book begins with a chapter on the identity, physical and chemical properties, and analytical methods involved in HEX determination. The chapter on Sources of Human and Environmental Exposure evaluates data on quantities released during production, processing and use and during disposal of the waste. While exposure of the general population is known to be low, risk of exposure can be high in residential areas near HEX production, processing and disposal. The next chapter discusses environmental fate and transport of HEX. The chemical by and large is not persistent. While rapid degradation occurs in most environmental compartments, studies show that HEX sorbs strongly to organic matter and may persist in soil sediment and ground water following waste disposal.

The chapter on Kinetics and Metabolism discusses absorption, retention, distribution, metabolism and elimination of HEX in laboratory animals. The next chapter

concerns risks to the environment, and the report notes that HEX is toxic to many aquatic microorganisms at nominal concentrations. Low concentrations of HEX have also been shown to be toxic to aquatic life. Information on the effects of HEX on terrestrial vegetation and wild life is insufficient.

The chapter – 'Effects on Experimental animals and *in vitro* Test Systems' summarises the information on acute toxicity studies, short term and long term exposure effects, developmental and reproductive toxicity and mutagenicity/carcinogenicity. Toxicity studies reveal high toxicity for HEX vapour following oral, dermal and inhalation dosing in all species tested, with dosing via inhalation causing the most acute toxicity. The systemic effects of acute exposure, irrespective of the route of administration, include pathological changes in the lungs, liver, kidney and adrenal glands. Long term inhalation/oral exposure has revealed absence of teratogenicity and mutagenicity of HEX. A review of the limited data available on human health effects indicates evidence of severe irritation to the eyes, nose, throat and lungs with respiratory system being the major site of toxicity. A review of epidemiological studies notes the absence of reports on any increase attributable to HEX or its metabolites, in the incidence of neoplasms at any site. The monogram ends with a brief section on conclusions and recommendations for protection of human health and environment.

The monogram has listed 141 references. This comprehensive book on HEX is useful to those involved in pesticide regulation and to research workers engaged in pesticide toxicology.

K. SRINIVASAN  
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*Process Engineering in the Food Industry* Vol. 2, Convenience foods and quality assurance, Edited by R.W. Field & J.A. Howell, Published in Elsevier Applied Science, London/New York, 1990; pp: 213; Price: £38.

This book is the Proceedings of the Conference on 'Engineering Innovation in the Food Industry, its role in quality assurance' organized by the Food and Drink group of the Institutes of Chemical Engineering at the University of Bath in April 1990.

The volume contains totally 16 papers, arranged under two sections entitled 'Processing of Convenience foods' and 'Control, Related aspects and Quality assurance'.

The first section, on convenience foods contains three papers on Microwave ovens- Product design, reheating and pasteurization, one paper on ohmic heating and three papers on processing and handling of chilled foods. The section also includes a paper on foods thermally processed in rectangular containers.

The second section, on Controls and related aspects, has three papers on CIP systems, their integration with other quality assurance programmes and fouling of heat exchangers.

Three papers deal with quality assurance in fish, poultry and meat products, monitoring of quality and robotic applications in quality assurance. Rest of the papers are on the processing of wet particulate and viscoelastic fluid foods.

Though microwave heating is not of immediate importance to the third world, the papers themselves are useful as they discuss some vital aspects such as product design for microwave heating and temperature uniformity during microwave reheating. The paper on modelling of the ohmic heating process by A.A. de Alwis *et. al.* is useful in view of the increasing importance of the applications of ohmic heating – specially in HTST operations. Ohmic heating is an emerging technology which will make its impact soon in the Indian food/dairy industry also.

Two of the papers in this section describe in some detail the construction and operation of specific processing machinery/systems – rather a very uncommon practice. One of the systems is for single drop depositing of chilled ready meals while the other is for cooling ready meals. Both papers provide useful information to both technologists and engineers. Another interesting paper in this section is on consumer handling of chilled foods, which elaborates the measures needed for controlling the temperature of chilled foods, although – from the point of production to the point of final consumption.

The second section includes a useful paper dealing with the mechanisms of food fouling and their implications in heat exchanger design. The paper analyses in detail the types and nature of fouling layers, processes for their reduction and methodology to take into account the effect of fouling on heat transfer rates while designing heat exchangers. The information in the paper would be very useful to design and process engineers. The paper by B.O. Mills Lamptey *et. al.*, on processing of wet particulate solids, provides highly useful information on their flow properties of particulate foods, which could be useful in design of process and equipment for such foods. Though a good amount of published information is available on homogenous foods as well as dry granular foods, information on flow properties of wet particulate foods is rather scanty. Such information is needed in process and design engineering as more and more particulates are handled in canning and aseptic processing plants. The information in the paper is useful in this context.

The second section also includes interesting papers on monitoring the quality of meat, poultry and fish products. The paper on the processing of coated fish and meat products by George Hayes provides fairly detailed engineering information on both processes and machinery.

Overall, the book contains several papers covering topics of high interest and use to food engineers and technologists.

A. RAMESH  
CFTRI, MYSORE.

## AFST(I) News

### Annual General Body Meeting 1991-92

The Annual General Body Meeting of the Association for the year 1991-92 was held on 30th May 1992 at the New Auditorium, University Department of Chemical Technology, Bombay, under the Chairmanship of Dr. P.J. Dubash, the outgoing President. The Chairman, welcomed the members and conducted the Proceedings.

The Secretary's report and the Treasurer's report were read by the Secretary and the Treasurer respectively. After a brief discussion on the modification suggested by a senior member of the Association regarding Prof. V. Subrahmanyan Industrial Achievement Award, both the reports were approved.

Two new chapters started functioning at Kharagpur and Bhopal from this year. The Secretary reported that the membership of the Association (including all categories) at the end of the year 1991 stood at 2386.

### AFST(I) Fellows

The Association honoured three scientists, a consultant and an industrialist as AFST Fellows after reading out the citations pertaining to their contributions in their respective areas:

1. Dr. Richard Joseph, Scientist, CFTRI, Mysore
2. Dr. M. Mahadeviah, Scientist, CFTRI, Mysore
3. Dr. S.C. Basappa, Scientist, CFTRI, Mysore
4. Dr. V. Sreenivasamurthy, Past-President, AFST(I), Mysore
5. Mr. V.B. Verma, Parle Exports, Bombay.

### AFST(I) Awards for the year 1991

The various AFST(I) Awards were presented to the following persons in recognition of their meritorious work:

1. Prof. V. Subrahmanyan Industrial Achievement Award was jointly awarded to Dr. S.S. Arya, Additional Director, Defence Food Research Laboratory, Mysore and Dr. A.M. Nanjundaswamy, Scientist, CFTRI, Mysore.
2. Laljee Godhoo Smarak Nidhi Award was awarded to Dr. V. Prakash,
3. The Young Scientist Award was given to Mr. B S Sridhar, Scientist, CFTRI, Mysore.
4. The Best Paper Award was given to Drs. G.R. Patil, A.A. Patil, F.C. Garg, G.S. Rajorhia and S.K. Gupta of National Dairy Research Institute, Karnal for their Research Paper entitled 'Interaction between sensory instrumental data on texture of Khoa' published in the *Journal of Food Science and Technology*, 1990. Vol. 27, No. 3, pp.167-170.
5. The Best Student Award was presented to Ms. Monica Sharma, Haryana Agricultural University, Hissar.

### Publication of Journals:

The Association's two reputed Journals viz. (1) *Journal of Food Science and Technology* and (2) *Indian Food Industry* entered their 29th and 11th year of publication respectively. Original research papers and feature articles are being received from R&D workers from countries like, UK, USA, Europe, Japan and Africa for publication in these journals. The circulation of these journal reached 1800 per issue in the year 1991.

Dr. J.R. Rangaswamy relinquished as the Editor of the *Journal of Food Science and Technology* from June 1992 and Dr. B.K. Lonsane, Scientist, Fermentation Technology and Bio engineering Division, CFTRI, Mysore has taken over as the new Editor from July 1992 with a newly constituted Editorial Board. The Association places on record the services rendered by Dr. J.R. Rangaswamy for improving the quality of the Journal and wishes Dr. B.K. Lonsane, the New Editor all success.

The *Indian Food Industry* journal is appearing with a new look and format from Volume II. It is hoped that both these journals will continue to make steady progress in achieving the objectives of the Association.

### AFST(I) Education and Publication Trust

The Trust met twice during the year 1991. The Scholarship Award Committee, constituted to recommend award of scholarships to deserving students, selected 4 students for the year 1991. They are; (i) Mr. B.B. Borse, Marathwada Agricultural University, Parbhani. (ii) Ms. V. Hemavathy, Man Power Development Discipline, CFTRI, Mysore (iii) Mr. Y. Prabhakar, Agricultural College and Research Institute, Madurai and (iv) Mr. P.P. Roy, Man Power Development Discipline, CFTRI, Mysore.

### New office bearers of the Association for the year 1992-93.

President	: Dr. A.M. Nanjundaswamy
Past President	: Dr. P.J. Dubash
Vice-President (HQ)	: Dr. P. Narasimham
Vice-Presidents	: Dr. S.K. Berry (Ludhiana) Mr. N. Ibrahim (Madras) Dr. S.R. Padwal Desai (Bombay) Dr. P.C. Panda (Hissar)
Hon. Exec. Secretary	: Dr. M.N. Krishnamurthy
Past Hon. Exec.	
Secretary	: Dr. M.S. Prasad
Hon. Jt. Secretary	: Mr. G.A. Krishna
Hon. Treasurer	: Mr. N.S. Singh

The meeting ended with a Vote of Thanks to the Chair.

# INSTRUCTIONS TO AUTHORS

Manuscript, in triplicate should be typed/printed in double-space on one side of A<sub>4</sub> size/bond paper, leaving 2.5 cm margin on all four sides of the page. The data reported in the manuscript must be original with clear definition of objectives, materials used, methods employed and without repetition. It should not have been published or offered for publication elsewhere. The manuscript must be as per format of the journal and authors should consult a recent issue of the journal for style and layout. The manuscript will be returned to authors, if it departs in any way from the required format and style. Papers essentially of an advertising nature will not be accepted. Footnotes for text are to be avoided. All submissions will be reviewed by two referees and an appropriate editorial board member.

Three different types of papers are published:

1. Research Papers with a maximum length of 14 manuscript pages, including figures, tables and references.
2. Research Notes, Limited to a maximum length of 6 manuscript pages, all inclusive.
3. Rapid Communication of the size of the maximum length of 8 manuscript pages (all inclusive) will be published rapidly, out of order of submission. Such communications must be based on new results of impact making quality. The authors have to append a note, indicating novelty, implications of the results and urgency in publication. The editor reserves the right to decide, what constitutes a Rapid Communication.

Materials and Methods must give sufficient details for the work to be repeated. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Any hazard must be mentioned and the relevant safety precautions described or reference made to safety procedures. The use of proprietary names should be avoided.

Each paper should be provided with an abstract of maximum ten line length in the manuscript, reporting concisely on the principal findings of the paper and in the form acceptable to abstracting agency.

The manuscript of the research paper should be divided into sections viz. Abstract, Introduction, Materials and Methods, Results and Discussion and References. The research notes will be without these sections, except for abstract and references. The chemicals are to be referred by names and not by formula in the text.

The title of the paper is to be typed in capital and small letters for all types of papers. Authors' names should be in capitals and the affiliation in capital and small letters. This should be followed by Abstract and Introduction (without heading).

Tables, numbered consecutively with Arabic numerals are to be typed on separate sheet and placed after references section. No vertical lines be used and the table should not have more than nine columns. Nil results should be indicated by using zero, while absence of data by the sign—.

Graphs and line drawings must be in a style and standard of draughtsmanship. These should be drawn in Indian ink, with stencilled lettering, on tracing paper or white drawing paper or preferably art paper. The lettering should be twice the size of the printed letter. Photographs should be submitted as clear black-and-white prints on glossy paper and must have good contrast. High quality computer generated line diagrams or glossy prints are also acceptable. Legends for all the figures are to be typed on separate page with details of symbols. The graphs, line drawings and photographs must be protected adequately against damage and bending of the envelop during transit. The manuscript will be returned to authors, if these requirements are not satisfied.

References should be cited at the appropriate point in the text by a superscript numeral in the order of their citing in the text. A list of references, in numerical order, should appear at the end of Results and Discussion section, maintaining the same order of number. Abbreviations such as *et. al.*, *ibid*, *idem* must be avoided. The titles of all scientific periodicals should be abbreviated in conformity with the World List of Scientific Periodicals, Butterworth Scientific Publications, London, 1962. Unpublished data or private communications should not appear in the list, but can be indicated in the text. The examples of layout of typical references are as below (please note the 4 italic portions and bold figures):

- a) Tairu A O, Omotosu M A, Oderinde R A and Bamiro F O, Studies on oxidative stability of crude and processed yellow nutsedge tuber and almond seed oil. *J Fd Sci Technol*, 1991, **28**, 8.
- b) Hacking A J, *Economic Aspects of Biotechnology*, Cambridge University Press, Cambridge, 1986, 306.
- c) Kurtzman C P, Phaff H J and Meyer S A, in *Yeast Genetics, Fundamental and Applied Aspects*, by Spencer J F T, Spencer D M and Smith A R W, Springer-Verlag, New York, 1983, 139.
- d) Nambudiri E S and Lewis Y S, Cocoa in Confectionery, *Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India*, Mysore, May 1979, 27.
- e) Ramesh M V, Production of Heat Stable Bacterial Alpha-Amylase, *Ph.D. Thesis*, University of Mysore, Mysore, India, 1989.
- f) Sreekantiah K R, Jaleel S A and Ramachandra Rao T N, *Indian Patent* No. 115537, 1968.
- g) Srihari K A, Vijaya Rao D and Siddaiah C H, Microbiological quality of spice mixtures as evidence of safe manufacturing capabilities, Paper Presented at 32nd Annual Conference, Association of Microbiologists of India, Madurai, India, 10-12, January 1992.

There are no page charges. One off-print of the paper will be provided free of charge to first author of each published paper. Additional off-prints can be ordered at current printing prices at the time of submission of the manuscript.



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