

ISSN-0022-1165

**JOURNAL  
OF  
FOOD SCIENCE  
AND  
TECHNOLOGY**



**ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS, INDIA**

**VOL. 23. NO. 1.**

**JAN./FEB. 1986**



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Number 1

Jan./Feb. 1986

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# Thermonuclease as an Indicator of Growth of *S. aureus* and Production of Enterotoxin

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*Manuscript received 18 December 1983; revised 23 May 1985*

Enterotoxin and thermonuclease production by *S. aureus* (FRI-100) was studied in ham, bacon, sausage and brain heart infusion broth medium. Correlation between the production of thermonuclease and enterotoxin was observed in all the four substrates, irrespective of incubation temperature and level of inoculum, as both could be detected simultaneously in 95% (122/128 samples) of the samples analysed. In the remaining samples, thermonuclease was detected earlier than enterotoxin. It is suggested that detection of thermonuclease can be used as an indicator for the production of enterotoxin of *S. aureus* in ham, bacon and sausages.

Preformed enterotoxins in the food due to *Staphylococcus aureus* can be detected through an indicator which is stable under various processing and storage conditions. Studies on *S. aureus* thermonuclease (TDNase) indicates the possibility of its use as an indicator<sup>1,2</sup>.

The present study was conducted using four different substrates, namely ham, bacon, sausage and brain heart infusion (BHI) broth to assess the production of thermonuclease at two different temperatures at different doses of initial inocula of *S. aureus* strain FRI-100.

## Materials and Methods

Strain FRI-100 of *S. aureus* known for producing enterotoxin A was used to study the growth, thermonuclease and enterotoxin production in ham, bacon, sausages and BHI broth at 25 and 37°C at different growth periods. The first three substrates were autoclaved at 1.1 kg/cm<sup>2</sup> pressure for 10 min to eliminate contaminants. One hundred gm or 100 ml of substrate was utilised to study the production of toxin and thermonuclease; 10 g (10ml of BHI) was used to study the growth pattern. Four samples of each substrate were inoculated with  $5 \times 10^2$ ,  $1 \times 10^3$ ,  $1 \times 10^4$  and  $1 \times 10^5$  organisms per g/ml. Inoculum was obtained by centrifuging (27000 × g for 15 min) 18 hr growth culture at 37°C of *S. aureus* FRI-100 in BHI. The cells obtained were washed twice with 0.85 per cent saline, and diluted in 0.85 per cent normal saline solution (N.S.S.) to give the required level of inoculum in 0.5 ml total volume. Inoculum was spread uniformly with a bent glass rod on the surface of bacon and ham, whereas in sausages it was inoculated as uniformly as possible with needle and syringe at several places.

Incubation period in case of BHI was 2,5,10 and 24

hr at 25 and 37°C. In case of bacon, ham and sausages it was 15, 24, 48 and 72 hr at both the temperatures.

*Estimation of growth:* Homogeneous suspensions (10% w/v) of the 10 gms of each substrate stored at above two temperatures were made in 0.85 per cent saline. Serial decimal dilutions were prepared and 0.1 ml of each dilution in duplicate was inoculated into Carter's medium<sup>3</sup>. Colonies with fried egg appearance appearing after 48 or 72 hrs of incubation at 37°C were considered as those of *S. aureus* and were counted. Numbers of *S. aureus* per gram of the substrate were calculated arithmetically.

*Detection of thermonuclease (TDNase) and enterotoxin:* Procedure described by Niskanen and Lindroth<sup>4</sup> was adopted for the isolation of enterotoxin while the method of Tatini *et al.*<sup>5</sup> was followed to estimate TDNase activity. Isolated TDNase was heated for 15 min in boiling water and qualitatively estimated in assay system of Lachica *et al.*<sup>6</sup> using toluidine blue O-DNA agar. Development of pink zones indicated the presence of TDNase. For quantitative estimations the diameter of the pink zones formed were compared with the standard curve, obtained by using micrococcal DNase (Sigma) following the method of Cords and Tatini<sup>2</sup>.

Isolated enterotoxin was identified by gel diffusion test using standard enterotoxin A antiserum. Standard enterotoxin A was used as a positive control. The standard antiserum and toxin were obtained from Professor M. S. Bergdoll, Food Research Institute, Wisconsin University, U.S.A.

## Results and Discussion

*Relationship between growth and TDNase production:* *S. aureus* growth and TDNase production at two temp

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TABLE 1. GROWTH, THERMONUCLEASE AND ENTEROTOXIN PRODUCTION IN BACON, HAM AND SAUSAGE AT DIFFERENT LEVELS OF INOCULUM AT 25°C INCUBATION

	Thermonuclease and <i>S. aureus</i> at indicated levels of inoculum/g							
	5 × 10 <sup>2</sup>		10 <sup>3</sup>		10 <sup>4</sup>		10 <sup>5</sup>	
	T.U./50 g	<i>S. aureus</i> (CFU/g)	T.U./50 g	<i>S. aureus</i> (CFU/g)	T.U./50 g	<i>S. aureus</i> (CFU/g)	T.U./50 g	<i>S. aureus</i> (CFU/g)
<b>Fifteen hours</b>								
Bacon	ND	1.2 × 10 <sup>4</sup>	ND	2.3 × 10 <sup>4</sup>	ND	4.8 × 10 <sup>4</sup>	Ab	5.6 × 10 <sup>5</sup>
Ham	Ab	3.1 × 10 <sup>3</sup>	Ab	1.5 × 10 <sup>4</sup>	Ab	5.1 × 10 <sup>4</sup>	Ab	6.3 × 10 <sup>5</sup>
Sausage	ND	5.6 × 10 <sup>3</sup>	ND	2.5 × 10 <sup>4</sup>	ND	6.5 × 10 <sup>4</sup>	Ab	6.5 × 10 <sup>5</sup>
<b>Twenty four hours</b>								
Bacon	ND	6.1 × 10 <sup>4</sup>	ND	4.6 × 10 <sup>5</sup>	0.1	2.6 × 10 <sup>7</sup>	0.7*	5.4 × 10 <sup>7</sup>
Ham	ND	1.0 × 10 <sup>5</sup>	Ab	2.5 × 10 <sup>5</sup>	+	2.1 × 10 <sup>6</sup>	1.6*	1.8 × 10 <sup>8</sup>
Sausage	Ab	4 × 10 <sup>5</sup>	ND	7 × 10 <sup>5</sup>	0.3	4.0 × 10 <sup>6</sup>	20.0*	2 × 10 <sup>8</sup>
<b>Forty eight hours</b>								
Bacon	Ab	6.3 × 10 <sup>5</sup>	0.1*	1.6 × 10 <sup>7</sup>	2.5*	2.9 × 10 <sup>7</sup>	0.7	2.5 × 10 <sup>8</sup>
Ham	Ab	2.3 × 10 <sup>5</sup>	ND	1.8 × 10 <sup>7</sup>	1.6*	3.2 × 10 <sup>8</sup>	7.1	4.5 × 10 <sup>9</sup>
Sausage	0.3	1.0 × 10 <sup>7</sup>	2.1*	6.3 × 10 <sup>7</sup>	7.1*	4.4 × 10 <sup>8</sup>	44.9	3.3 × 10 <sup>9</sup>
<b>Seventy two hours</b>								
Bacon	1.6*	6.1 × 10 <sup>7</sup>	ND	6.3 × 10 <sup>7</sup>	ND	3.1 × 10 <sup>7</sup>	20.0	3.7 × 10 <sup>7</sup>
Ham	ND	4.5 × 10 <sup>6</sup>	ND	7.3 × 10 <sup>7</sup>	+	4.1 × 10 <sup>8</sup>	20.0	5.1 × 10 <sup>9</sup>
Sausage	2.5*	2.7 × 10 <sup>6</sup>	ND	2.3 × 10 <sup>9</sup>	ND	1.5 × 10 <sup>9</sup>	63.4	3.9 × 10 <sup>9</sup>

\*Enterotoxin first detected; Ab=Absent; ND=Not done; + Qualitatively found present; quantitative estimation not done.

T.U. Thermonuclease units/50 g of substrate.

TABLE 2. GROWTH, THERMONUCLEASE AND ENTEROTOXIN PRODUCTION IN BACON, HAM AND SAUSAGES AT DIFFERENT LEVELS OF INOCULUM AT 37°C INCUBATION

	Thermonuclease and <i>S. aureus</i> at indicated levels of inoculum/g							
	5 × 10 <sup>2</sup>		10 <sup>3</sup>		10 <sup>4</sup>		10 <sup>5</sup>	
	T.U./50 g	<i>S. aureus</i> (CFU/g)	T.U./50 g	<i>S. aureus</i> (CFU/g)	T.U./50 g	<i>S. aureus</i> (CFU/g)	T.U./50 g	<i>S. aureus</i> (CFU/g)
<b>Fifteen hours</b>								
Bacon	ND	2.1 × 10 <sup>4</sup>	Ab	5.5 × 10 <sup>4</sup>	Ab	3.7 × 10 <sup>6</sup>	0.7*	4.9 × 10 <sup>7</sup>
Ham	ND	2.1 × 10 <sup>4</sup>	Ab	4.5 × 10 <sup>4</sup>	Ab	1.9 × 10 <sup>6</sup>	1.1*	2.8 × 10 <sup>6</sup>
Sausage	ND	2.6 × 10 <sup>4</sup>	Ab	5.3 × 10 <sup>4</sup>	0.3	2.3 × 10 <sup>6</sup>	1.6*	3.3 × 10 <sup>7</sup>
<b>Twenty four hours</b>								
Bacon	0.7*	4.5 × 10 <sup>7</sup>	1.6*	6.0 × 10 <sup>7</sup>	4.0*	9.2 × 10 <sup>8</sup>	63.4	7.9 × 10 <sup>9</sup>
Ham	Ab	4.3 × 10 <sup>5</sup>	7.1*	6.8 × 10 <sup>6</sup>	20.0*	2.2 × 10 <sup>8</sup>	63.4	2.5 × 10 <sup>9</sup>
Sausage	0.70*	3.7 × 10 <sup>8</sup>	44.9*	2.9 × 10 <sup>8</sup>	100.5*	1.3 × 10 <sup>9</sup>	142.0	3.3 × 10 <sup>9</sup>
<b>Forty eight hours</b>								
Bacon	1.1	4.5 × 10 <sup>7</sup>	2.5	3.1 × 10 <sup>8</sup>	44.9	2.3 × 10 <sup>9</sup>	142.0	1.5 × 10 <sup>10</sup>
Ham	+	3.1 × 10 <sup>7</sup>	ND	3.3 × 10 <sup>8</sup>	ND	6.7 × 10 <sup>9</sup>	225.0	1.5 × 10 <sup>10</sup>
Sausage	7.1	5.1 × 10 <sup>7</sup>	ND	3.3 × 10 <sup>8</sup>	ND	2.5 × 10 <sup>9</sup>	315.0	5.3 × 10 <sup>9</sup>
<b>Seventy two hours</b>								
Bacon	45.0	3.8 × 10 <sup>7</sup>	ND	3.5 × 10 <sup>7</sup>	ND	5.7 × 10 <sup>8</sup>	225.0	6.3 × 10 <sup>8</sup>
Ham	1.6	2.5 × 10 <sup>7</sup>	ND	2.3 × 10 <sup>8</sup>	100.5	6.1 × 10 <sup>9</sup>	353.0	9.1 × 10 <sup>9</sup>
Sausage	64.9	1.9 × 10 <sup>8</sup>	ND	5.8 × 10 <sup>8</sup>	ND	5.1 × 10 <sup>9</sup>	504.0	8.3 × 10 <sup>9</sup>

T.U. Thermonuclease units per 50 g of the substrate

Ab=absent; ND = Not done; +=Qualitatively found present; quantitative estimations not done.

ratures of incubation and various doses of inoculum in BHI broth, bacon, ham and sausages are recorded in Tables 1, 2 and 3.

Minimum cell population at which TDNase could be detected varied between  $2.1 \times 10^6$  to  $4.9 \times 10^7$ /ml (Tables 1,2,3). Other workers<sup>1</sup> could detect TDNase in cheese when the cell population was atleast  $1.5 \times 10^6$ /g. It was however, evident that only the attainment of required population did not produce detectable TDNase, unless it was coupled with sufficient duration of incubation. Incubation for 24 hr at 25°C of BHI broth inoculated with  $10^3$  cells/ml of *S. aureus* resulted in detectable TDNase production with growth level of  $2.4 \times 10^7$  cells/ml, but no detectable TDNase was observed at 10 hr of incubation at 25°C in BHI broth inoculated with  $10^5$  cells/ml even though the cell population attained was slightly higher i.e.,  $3.1 \times 10^7$ /ml (Table 3).

Substrates had a definite effect on TDNase production. Of the three substrates, sausages supported more TDNase production at all the doses of inoculum, at both

incubation temperatures with different initial inocula (Table 1 and 2).

Temperature also exerted great influence on the growth of *S. aureus* and on TDNase production. Maximum recorded was at 37°C.

*Growth cycle and TDNase production:* Growth curve of *S. aureus* and TDNase production in bacon, ham and sausage substrates at 37°C with  $10^5$  organisms/ml initial inoculum is plotted in Fig 1. Fig 2 shows the growth cycle and TDNase production by *S. aureus* at 37°C with various initial inocula in BHI broth.

In ham, bacon and sausages, *S. aureus* population showed a steep rise upto 24 hr. Thereafter in ham and sausages, it was maintained until 72 hr of incubation, the end of observation period. In bacon however, the stationary per od seemed to prolong from 24 to 48 hr followed by a sharp decline (Fig 1). Production of TDNase in all these three substrates ran more or less parallel to the log phase of growth curves. It also showed

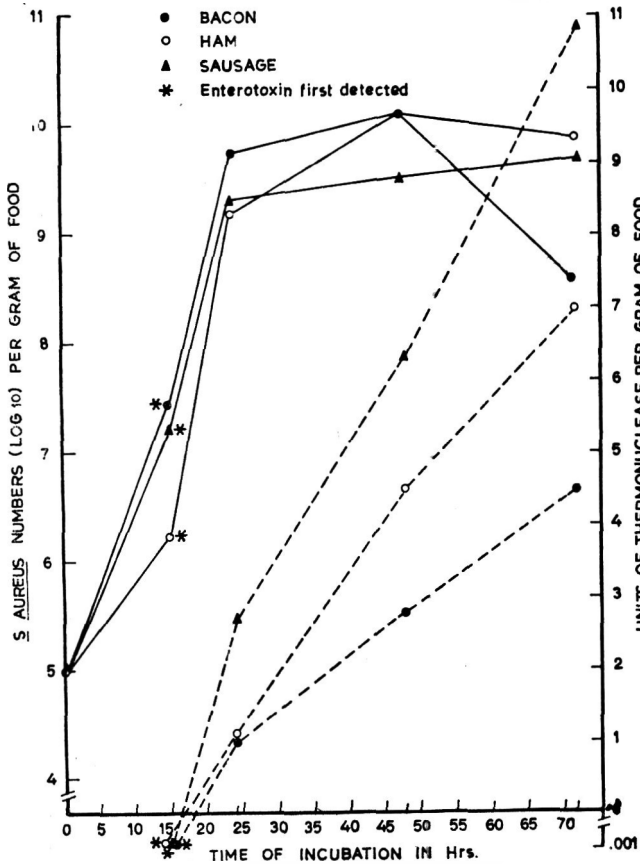


Fig 1. Growth, thermonuclease and enterotoxin production by *S. aureus* FRI-100 at 37°C in bacon, ham and sausages.

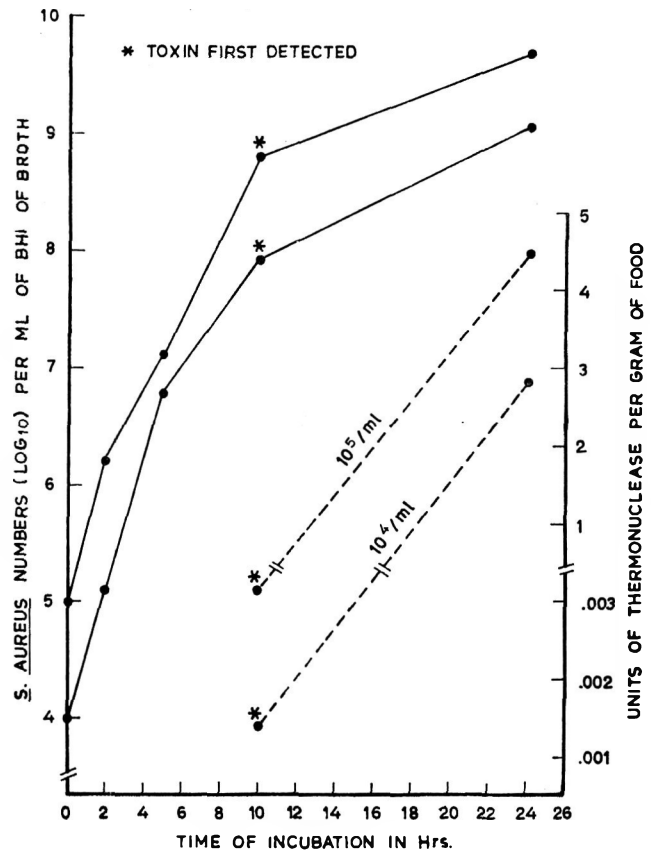


Fig 2. Growth, thermonuclease and enterotoxin production by *S. aureus* FRI-100 at 37°C in BHI,

TABLE 3. GROWTH, THERMONUCLEASE AND ENTEROTOXIN PRODUCTION IN EXPERIMENTALLY INOCULATED BHI BROTH WITH *S. aureus* STRAIN FRI-100

Incubation period <sup>d</sup> (hr)	Thermonuclease and <i>S. aureus</i> at indicated levels of inoculum/g.							
	$5 \times 10^2$		$10^3$		$10^4$		$10^5$	
	T.U./50ml	<i>S. aureus</i> (CFU/ml)	T.U./50ml	<i>S. aureus</i> (CFU/ml)	T.U./50ml	<i>S. aureus</i> (CFU/ml)	T.U./50ml	<i>S. aureus</i> (CFU/ml)
37°C								
2	Ab	$4.5 \times 10^3$	Ab	$8.2 \times 10^3$	Ab	$1.6 \times 10^5$	Ab	$2.2 \times 10^6$
5	Ab	$1.4 \times 10^4$	Ab	$8.0 \times 10^4$	Ab	$7.9 \times 10^6$	Ab	$1.5 \times 10^7$
10	Ab	$2.1 \times 10^6$	Ab	$4.3 \times 10^6$	0.7*	$9.1 \times 10^7$	1.6*	$8.3 \times 10^8$
24	100.5*	$4.1 \times 10^7$	121.0*	$5.7 \times 10^7$	141.9*	$1.1 \times 10^9$	224.9*	$3.7 \times 10^9$
25°C								
2	Ab	$2.5 \times 10^3$	Ab	$5.9 \times 10^3$	Ab	$5.0 \times 10^4$	Ab	$1.1 \times 10^6$
5	Ab	$5.3 \times 10^3$	Ab	$3.0 \times 10^4$	Ab	$4.0 \times 10^5$	Ab	$3.3 \times 10^6$
10	Ab	$1.2 \times 10^5$	Ab	$2.0 \times 10^5$	Ab	$4.3 \times 10^6$	Ab	$3.1 \times 10^7$
24	Ab	$1.4 \times 10^7$	0.7*	$2.4 \times 10^7$	1.6*	$3.3 \times 10^7$	7.1*	$3.9 \times 10^8$

T. U. Units of thermonuclease per 50 ml; \*Enterotoxin first detected; Ab: Absent.

steep rise in all the three, upto 24 hr of incubation as in case of growth from the point of its first detection which incidently was at 15 hr (starting point for taking observations).

In BHI broth also, production of TDNase whenever detected was found parallel to the log phase of growth of *S. aureus*. These results also indicate that maximum TDNase is produced during log phase.

*Relationship between TDNase and enterotoxin production:* Tatini *et al.*<sup>1</sup> reported the detection of enterotoxin A and D only subsequent to the detection of TDNase in BHI, meat and milk and meat products. In our studies, however, both TDNase and enterotoxin could be detected simultaneously in nearly 90 per cent of the samples comprising ham, bacon and sausages and BHI broth. Results obtained with TDNase production, growth and enterotoxin are shown in Table 1, 2 and 3. A good correlation was found between enterotoxin and TDNase production in the samples of BHI, bacon, ham and sausages. Out of the 32 samples obtained from substrates inoculated with *S. aureus* at different initial levels of inoculum and incubated at 25 and 37°C, 27 showed TDNase and enterotoxin. Only in five samples (10.5 per cent) TDNase was detected earlier than the enterotoxin. All these five samples were from the batch in which initial inoculum was  $10^4$  cells/ml. Of these five samples four (one each of ham, bacon, sausages and BHI) were incubated at 25°C. In the former three samples TDNase was first detected at 24 hr of incubation at the growth level varying between  $2 \times 10^6$

and  $3 \times 10^7$  cells/ml while enterotoxin could be detected only at 48 hr of incubation at the growth levels between  $3 \times 10^7$  and  $4 \times 10^8$  cells/ml (Table 1). In BHI sample, 10 hr incubation yielded TDNase while enterotoxin could be isolated subsequently at 24 hr of incubation (Table 3). The fifth sample in which TDNase was detected earlier was of sausages and was incubated at 37°C (Table 2). Reaction for TDNase could be seen at 15 hr of incubation (the first point of observation) while enterotoxin was detected later at 24 hr of incubation.

It was not possible to mark the minimum level of TDNase production which would be associated with detectable enterotoxin production as the similar amount of TDNase produced under all the circumstances was not always accompanied with detectable enterotoxin. It was observed that the length of incubation played a significant role. In bacon ( $10^3$  cells/g at 25°C) enterotoxin production could be detected at TDNase level of 0.1 unit/50g at 48 hr of incubation period (Table 1). However, the production of same level of TDNase in the same substrate but at 24 hr of incubation with  $10^4$  cells/g initial inoculum, was not associated with detectable levels of enterotoxin. In sausages ( $10^4$  cells/g initial inoculum at 25°C for 24 hr) though a different substrate, production of 0.3 units/50 g, more than that produced in bacon, did not yield detectable enterotoxin. Cords and Tatini<sup>2</sup> however observed that TDNase could be detected in cheeses containing more than  $1.0 \mu\text{g}$  of TDNase.

Considering the results obtained above it may be said



that TDNase is a useful test in screening foods for likely presence of enterotoxins and for certain, the growth of *S. aureus* in them. It may further be added that TDNase detection in substrates like ham, bacon and sausages is a reflection of growth of *S. aureus* to at least  $1 \times 10^6$  to  $1 \times 10^7$  organisms/g. Cords and Tatini<sup>2</sup>, Chesbro and Auburn<sup>7</sup>, Lachica *et al.*<sup>8</sup> Tatini *et al.*<sup>1</sup> had earlier reported the usefulness of TDNase test in screening of foods for likely presence of enterotoxin, despite the fact that conditions of *S. aureus* growth and growth substrate influences the quantitative production of TDNase.

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## Efficiency of Ethanol Production by Coconut Toddy Yeasts

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*Manuscript received 3 August 1984; revised 7 June 1985*

Yeast flora present during natural fermentation of coconut inflorescence sap was tested for their ability to produce ethanol in pure cultures. A total of 144 yeast isolates were characterized. Of these 75 (52%) isolates converted sugars into 9% ethanol. Medium ethanol concentrations of (4 to 6%) were produced by 17 (12%) of the isolates. The remaining 52 (36%) isolates produced either traces or no ethanol. The average ethanol production for all the isolates tested was 4.4% and for the high ethanol producers was 9%. The low ethanol concentrations of 6 to 7% observed in the coconut sap fermentation industry could probably be increased to 9% if the low and medium ethanol producing yeasts are suppressed.

Toddy is an alcoholic beverage obtained by the natural fermentation of coconut palm sap. The coconut inflorescence sap is traditionally collected in clay pots sterilized by inverting over a flame for 5 min and allowed to ferment in open pots for upto 2 days<sup>5</sup> during collection of sap. During this period microorganisms from the atmosphere enter the clay pots and multiply in the palm sap which contains (15-18) per cent sucrose<sup>5</sup>, transforming the sugar into ethanol and other products.

The resulting liquid containing about 7 per cent ethanol is known as toddy.

Natural fermentation of coconut sap is brought about by a succession of heterogenous microorganisms consisting of yeasts and bacteria<sup>1</sup>. Some of them transform sugars in the coconut sap into ethanol while others may merely survive or bring about other biochemical changes in the sap.

In this study yeasts were isolated during various

stages of fermentation and their capacity to produce ethanol was examined with a view to understand the role of yeast population in the fermenting sap.

**Materials and Methods**

**Yeasts:** The sap was collected in a pot sterilized by inverting over a flame for 5 min. Yeasts collected in this during traditional fermentation process of coconut inflorescence sap were isolated by sampling the sap at 4, 7, 10, 13, 18, 28, 39, 54, 61, 79, 103 and 157 hr from the time of introducing the pot. The samples were plated in Bacto-wort agar, Bacto-nutrient agar, Bacto-sabraud dextrose agar and 2 per cent Bacto-agar in unfermented coconut sap. The pure cultures were isolated and purified by streak plate technique.

The yeasts were identified by the following tests<sup>2-4</sup>

- (a) Fermentation of glucose, galactose, lactose, sucrose, maltose and raffinose when supplied as the sole source of carbon in Bacto-phenol red broth base.
- (b) Assimilation of glucose, galactose, lactose, sucrose, maltose, raffinose, melibiose, xylose, inulin, salicin, cellobiose, trehalose, mannitol, erythritol and rhamnose when supplied as the sole source of carbon.
- (c) Assimilation of nitrate and ammonium ions when supplied as sole source of nitrogen.
- (d) The shape and size after one week of growth in Bacto-yeast morphology agar at 25°C, growth in the same medium at 37°C, pseudomycelium formation under aerobic and anaerobic conditions in Bacto-corn meal agar and sporulation in Gorodkova agar and acetate agar.

**Fermentation of coconut sap:** In each experiment 12 hr old unfermented sap (200 ml) in 500 ml conical flasks was sterilized by autoclaving at 115°C for 10 min and inoculated separately with isolated pure cultures of yeasts. They were incubated at 30°C for 12 days. The conical flasks containing incubated cultures were shaken and weighed twice daily. The ethanol production in replicate cultures was estimated on the third and fifth day of fermentation using an ebulliometer.<sup>6</sup>

**Results and Discussion**

Loss in weight of the sap against ethanol production on the fifth day by the yeasts was plotted to find out the relation, if any, between the sugar utilization and ethanol production. Such a plot showed a linear relationship and three distinct groups of micro organisms (Fig 1). The first group converted the sugars producing about 9 per cent ethanol (Table 1). This group consisted mainly of *Saccharomyces chevalieri*. The other yeasts of

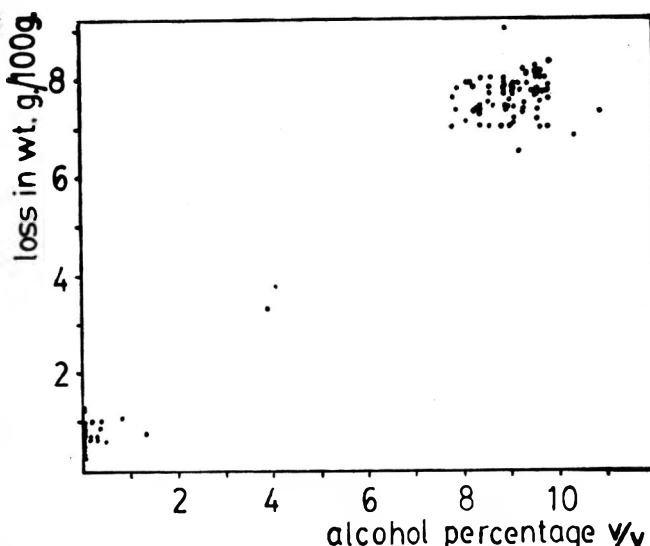


Fig 1. Relationship between loss in weight and ethanol production when sterile coconut sap is fermented with pure yeast cultures isolated from toddy.

high ethanol producing capacity were *Schizosaccharomyces pombe*, *Pichia ohmeri* and *Kloeckera javanica*. The second group produced medium ethanol concentrations

TABLE 1. ETHANOL PRODUCING CAPACITY OF YEAST ISOLATES WHEN INCUBATED IN UNFERMENTED STERILE SAP

Species identified	No. of isolates tested	Ethanol (%) on 3rd day	Ethanol (%) on 5th day
		Mean ± S.D.	Mean ± S.D.
<b>Group I</b>			
<i>Schizosaccharomyces pombe</i>	5	5.9 ± 0.8	9.3 ± 0.9
<i>Pichia ohmeri</i>	11	8.5 ± 0.5	9.2 ± 0.3
<i>Saccharomyces chevalieri</i>	57	8.9 ± 0.5	9.0 ± 0.6
<i>Kloeckera javanica</i>	2	6.5 ± 0.7	8.7 ± 1.3
<b>Group II</b>			
<i>Saccharomycodes ludwigii</i>	5	2.7 ± 0.8	6.3 ± 2.5
Yeast like fungi	6	2.1 ± 0.1	3.9 ± 0.3
<i>Candida tropicalis</i>	6	1.4 ± 1.2	3.9 ± 0.7
<b>Group III</b>			
<i>Candida parapsilosis</i>	10	0.0 ± 0.1	0.4 ± 0.4
<i>Candida guilliermondii</i>	7	0.1 ± 0.2	0.1 ± 0.2
<i>Candida valida</i>	11	0.1 ± 0.1	0.1 ± 0.1
Unidentified B	4	0.1 ± 0.1	0.1 ± 0.1
<i>Pichia membranaefaciens</i>	9	0.0 ± 0.0	0.1 ± 0.1
<i>Torulopsis candida</i>	4	0.0 ± 0.0	0.0 ± 0.0
Unidentified A	7	0.0 ± 0.0	0.0 ± 0.0

of 3.9 to 6.7 per cent. The third group produced traces or no ethanol, but utilized upto 1 per cent of the sugars in the sap. The main fermenting organism is *Saccharomyces chevalieri* which was isolated more frequently than the other yeasts.

Of the 144 isolates tested, 75 (52 per cent) belonged to the first group; the average ethanol production being 9 per cent. However, the average ethanol production for all the isolates tested, assuming all of them to be of equal distribution and activity, is 4.4 per cent. As against the above observations, the ethanol yields of 6-7 per cent observed in the industry probably indicate only a partial dominance of the high ethanol producing micro organisms. It may be possible to increase the ethanol yields to 9 per cent, in the industry, if the low ethanol producing micro organisms could be suppressed permitting the total dominance by high ethanol producing yeasts.

The toddy fermented by *Saccharomyces chevalieri* did not emit the sulphurous smell normally found in toddy and was more like dry wines after complete fermentation. It was also less susceptible to acetification than the naturally fermented toddy

### Acknowledgement

The authors wish to thank the toddy tapper Mr. M. A. P. Perera for his assistance in this project.

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## Sorghum Quality Studies. Part II. Suitability for Making Dumpling (*Mudde*)

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*Manuscript received 23 November 1984; revised 22 April 1985*

Nineteen varieties of sorghum were tested for *mudde* preparation. Large varietal differences were found in the quality of *mudde* prepared from them. *Mudde* from H-2259, M-3265, E-35-1 and M-7777 were found to be less sticky when tested objectively and subjectively. They showed higher grain hardness, reduced swelling in the Brabender Visco-graph and lower amylase activity. Retrogradation was shown to be important in preparing good quality *mudde*. Polishing of sorghum improved the quality of *mudde* by increasing retrogradation and decreasing  $\alpha$ -amylase activity.

*Mudde* is a dumpling prepared from whole meal or partially refined sorghum flour. It is also known as *ugali* or *to* in Africa and may be made with water, acid or alkali. A few recent reports have dealt with quality of sorghum needed for making *to*<sup>1</sup>, *mudde*<sup>2</sup> and *roti*<sup>3</sup> and varietal differences have been noted. The present work describes our studies on the physicochemical basis for varietal differences in respect of suitability for making *mudde* or dumpling.

### Materials and Methods

Nineteen varieties of pure bred cultivars used in the experiments were obtained from the ICRISAT, Hyderabad, India.

*Mudde preparation.* Twenty grams of flour from each variety were taken in a beaker, 40 ml of water was added mixed well, and steamed for 30 min. The cooked dumpling was cooled for one hour and again uniformly mixed and evaluated subjectively and objectively for textural differences.

**Subjective evaluation:** The cooked *mudde* was evaluated for its ability to be rolled into balls without sticking to the fingers by a panel. Varieties that could be easily rolled into balls were labelled as good *mudde* varieties. Varieties that produced *mudde* that could be rolled but left some residue on the fingers were considered moderately good varieties. The poor *mudde* varieties could not be rolled into balls. There were clear differences between the three groups.

**Objective evaluation:** The tachymeter used by Kumar *et al.*<sup>4</sup> for measurement of stickiness in rice was employed. This consists essentially of a physical balance with one pan replaced by a balancing weight of 250 g. This weight is placed on and in contact with 10 g of *mudde* contained in a cup for a period of 60 sec. The weight sticks to the *mudde* surface. The weight of sand added to the pan to release the weight in grams is taken as a measure of the stickiness of the *mudde*. An average of ten replicates was taken to measure stickiness.

**Analysis:** Total, soluble and insoluble amylose and Brabender paste viscosities were determined as described earlier<sup>4</sup>, noting gelatinization temperature, peak viscosity and setback. The latter two are expressed as Brabender units (B.U.)

**$\alpha$ -amylase activity:** Ten grams of flour was blended with 100 ml of water in a Waring blender for 90 sec, and filtered.  $\alpha$ -amylase activity was measured in the filtrate at three pH levels in 0.05 M acetate buffer (pH 4.5), 0.05 M sodium carbonate (pH 8.5) or in water (pH 5.6). Soluble starch (Schmidt and Berger) was the substrate, and the enzyme reaction was carried out at 37°C for 15 min. The reducing sugars released were measured using the dinitrosalicylic acid method of Bernfeld<sup>5</sup>, maltose being used as standard.

**Kiya hardness:** The average crushing strength of fifty grains from each variety was measured using the Kiya hardness tester<sup>6</sup> while the grains were placed with germ end on the platform.

**Effect of polishing on retrogradation,  $\alpha$ -amylase activity and *mudde* quality:** A commercial variety containing high  $\alpha$ -amylase activity was selected and polished in a McGill rice polisher according to the method of Raghavendra Rao and Desikachar<sup>7</sup>.

$\alpha$ -amylase activity was determined as described above. Starch retrogradation during overnight storage, in the cold, of cooked flour slurry (2.5 per cent) of polished and whole meal flour in one variety was measured using slight modification of the procedure described by Watson<sup>7</sup>. Starch was estimated by hydrolysis with glucoamylase. Reducing sugar in the supernatant was determined with dinitrosalicylic acid reagent.

## Results and Discussion

Table 1 gives the grouping of the sorghum varieties

into good, moderate and poor *mudde* types based on sensory evaluation and texture. The tachymeter values for stickiness were  $194 \pm 45$  g for the good *mudde* group,  $226 \pm 43$  g for the moderate *mudde* group and  $253 \pm 34$  g for the poor *mudde* group. The values for stickiness obtained from the tachymeter were higher when more than 2 parts water were used for making *mudde*, but the varietal differences were in the same order as obtained with *mudde* made with less water. Total and insoluble amyloses were slightly higher for the good *mudde* group but differences between groups were not significant. The gelatinization temperature (GT) was definitely higher ( $76.5 \pm 1.6^\circ\text{C}$ ) and peak viscosity tended to be generally lower for the good *mudde* groups ( $322 \pm 143$  B.U.). The moderate *mudde* groups had a GT of  $71.0 \pm 3.6^\circ\text{C}$  and a peak viscosity of  $367 \pm 76.4$  B.U. Poor *mudde* varieties had average values of  $70.5 \pm 1.6^\circ\text{C}$ , and  $475 \pm 157$  B.U. for gelatinization temperature and peak viscosity respectively. The difference between setback and peak, viscosity (a measure of retrogradation) was higher for the moderate and good *mudde* groups than for the poor *mudde* group.

The Kiya hardness values of grains were highest for the good *mudde* varieties  $7.8 \pm 2.0$  kg/cm<sup>2</sup>. The corresponding values being  $4.8 \pm 1.3$  kg/cm<sup>2</sup> for the poor *mudde* group, and  $6.0 \pm 1.7$  kg/cm<sup>2</sup> for the moderate group. It has been shown by us earlier that hardness in sorghum reduces granule swelling during cooking<sup>8</sup>. A recent study reported low stickiness values for porridges made from hard, corneous sorghum as compared with those made from soft and floury sorghums<sup>9</sup>.

The  $\alpha$ -amylase activity was definitely lower for the good *mudde* group ( $0.38 \pm 0.24$  mg maltose) than for the moderate ( $0.43 \pm 0.37$  mg maltose) or the poor ( $0.79 \pm 0.03$  mg maltose) *mudde* group.

The results clearly indicate that hard grains with low  $\alpha$ -amylase activity are to be preferred for making good *mudde* possessing the desired cohesiveness. Flours from hard grains tend to be coarser, contain less damaged starch, and hence would swell less on cooking. High  $\alpha$ -amylase activity will decrease retrogradation and increase stickiness.

Data on the effect of polishing sorghum on  $\alpha$ -amylase, starch retrogradation and *mudde* quality are presented in Table 2. *mudde* was prepared from polished and unpolished sorghum flour in water and alkali solutions. Retrogradation was 44 per cent with unpolished flour and 52 per cent with polished flour in aqueous medium.  $\alpha$ -amylase activity was considerably higher in the unpolished flour and this resulted in *mudde* with lower stickiness. The texture of *mudde* made from polished grain was considered more acceptable by the panel. Polishing of sorghum reduced  $\alpha$ -amylase activity and increased starch retrogradation. The cohesive

TABLE 1. RELATION OF AMYLOSE CONTENT, PASTE VISCOSITY (PV), GRAIN HARDNESS AND AMYLASE ACTIVITY TO *MUDDE* QUALITY IN SORGHUM VARIETIES

Sorghum variety	Amylose			G.T. (°C)	P.V. (BU)	Setback (BU)	Kiya hardness (kg/cm <sup>2</sup> )	Tachymeter reading (g)	Amylase units**
	Total (%)	Soluble (%)	Insoluble (%)						
<b>Bad Quality <i>Mudde</i></b>									
BMP 53	30.1	20.3	9.9	72.0	220	500	6.5	210	0.78
Patchajuna	—	—	—	72.0	400	600	5.1	280	0.95
Patencheri	31.6	19.6	12.1	67.5	430	760	5.7	244	0.19
P721	25.8	14.8	11.1	72.0	400	600	6.0	280	0.95
IS2176	26.5	15.3	11.2	69.0	710	350	4.7	—	—
IS1122	26.3	14.1	12.3	70.5	670	360	3.2	—	0.49
IS6461	26.4	14.7	11.7	70.5	500	210	3.2	—	0.38
Mean	27.2±2.7	16.1±2.7	11.7±1.1	70.5±1.6	475±157	468±179	4.8±1.3	253±22	0.79±0.53
<b>Moderate Quality <i>Mudde</i></b>									
GPR320	29.9	17.4	12.5	69.0	460	540	5.3	250	0.19
GPR148	32.2	12.6	10.6	78.0	320	580	5.8	206	1.07
CSH8	33.4	16.4	17.0	70.5	390	700	8.6	240	0.17
M647	—	—	—	73.5	440	780	6.6	185	0.17
CSH1	31.6	17.8	13.8	67.5	245	490	6.6	160	0.17
CSH6	31.6	13.8	17.8	69.0	320	555	6.4	280	0.80
A2283	32.0	16.1	16.1	69.0	400	650	3.0	260	0.46
Mean	30.8±3.6	15.7±2.0	14.6±2.8	71.0±3.6	367±76	613±101	6.0±1.7	226±43	0.43±0.38
<b>Good Quality <i>Mudde</i></b>									
H2259	29.8	17.5	12.3	81.0	260	360	7.2	193	0.33
M3265	29.7	15.8	13.9	78.0	150	240	7.2	250	0.72
E35-1	29.9	15.6	14.3	73.5	440	780	10.8	193	0.25
M7777	32.8	18.6	14.2	73.5	440	790	6.2	140	0.20
Mean	30.6±1.5	16.9±1.4	13.7±0.9	76.5±3.7	322±143	540±281	7.8±2.0	194±45	0.38±0.24

\*All mean values are expressed as Mean ± S.D.

\*\*Amylase units are expressed as mg maltose produced by 10g flour in acetate buffer, pH 4.5, 37°C, in 15 min.

TABLE 2. CHARACTERISTICS OF *MUDDE* MADE FROM POLISHED AND WHOLE MEAL SORGHUM FLOURS

	Unpolished	Polished
Tachymeter values (g)	320	260
Retrogradation % in water	44	52
Retrogradation % in alkali	40	20
Amylase activity* in water (pH 4.6)	2.90	0.56
Amylase activity* in alkali (pH 8.5)	0.9	0.39
Subjective evaluation	NS	S

\*Amylase units are expressed as mg maltose produced by 10 g of flour at 37°C in 15 min.

NS: Not satisfactory. S: Satisfactory.

characteristic of *mudde* as measured subjectively or by a tachymeter was more in the *mudde* made from polished material than those made from whole meal flours. Both retrogradation of starch and  $\alpha$ -amylase activity were less in the alkaline medium than in the aqueous medium, for the whole meal as well as for polished flours. The levels of  $\alpha$ -amylase activity in acetate buffer and in water were found to be the same.

It may be submitted that good *mudde* is made from hard grains with a Kiya value of 7-8 kg cm<sup>2</sup>, with a gelatinization temperature above 73.5°C and possessing low  $\alpha$ -amylase activity. High stickiness in *mudde* made from a bad *mudde* variety of sorghum or weathered grain may be improved by admixture with flour from pearled sorghum.

It may be pointed out that the quality characteristics of grain and flour that are indicative of optimal *mudde* quality are opposite of those required for optimal *roti* rolling. Good *Roti* varieties tend to have a low gelatinization temperature, high paste viscosity, low grain hardness and high stickiness of dough as shown previously<sup>3</sup> and the varieties best for *mudde* tend to give *roti* doughs with low stickiness. The same test (such as grain hardness and paste viscosity) may therefore be used to identify varieties suitable for either *roti* or *mudde* making.

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## Studies on the Functional Characteristics of Differently Milled Whole Wheat Flour (*Atta*)

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*Manuscript received 5 November 1984; revised 12 June 1985*

The chemical composition and dough forming characteristics and chapati making quality of whole wheat flour (*atta*) milled in (i) stone, (ii) disc (*chakki*), (iii) hammer, (iv) pin and (v) roller mills have been studied. *Atta* samples milled in disc, stone and pin mills had significantly higher diastatic activity (365-505 mg/10g flour) and damaged starch (12.0-18.8%) as compared to hammer and roller milled flours (200-220 mg/10g flour 8-10%). The water requirement of a chapati dough varied from 68 to 82%.

Among the differently milled *atta* samples, considerable variations were observed in dough development time (3.0-5.5 min) and dough stability (2.0-4.5 min) as indicated by the farinograms and resistance to extension (410-590 BU) and extensibility (50-65 mm) as indicated by the extensograms. The chapati made from stone milled *atta* was superior with soft texture and better flavour, while those made from hammer milled or roller milled *atta* were inferior with somewhat hard texture and bland flavour.

In India, more than 75 per cent of the total wheat production of 44.6 million tonnes is milled into whole wheat flour (*atta*) in stone mill, disc mill (*chakki*), hammer mill, roller mill, etc. for use in the preparation of chapati or *roti*. It has been reported<sup>1</sup> that the functional

characteristics of *atta* milled in different *chakkies* varied considerably and depended mainly on the severity of milling, which varies for different types of mills. Evidently the functional characteristics of *atta* obtained from different mills are also likely to vary. So far no scientific informa-

tion is available on the functional characteristics of *atta* as affected by the use of different types of mills for grinding wheat. Such information will be helpful in determining the suitability of differently milled *atta* for chapati making. The results of studies on chemical, rheological and chapati making quality of *atta* are presented in this paper.

### Materials and Methods

**Wheat:** A commercially available medium hard wheat, variety 'Bhojan Samrat,' suitable for chapati making was procured from the local market.

**Whole wheat flour:** *Atta* was obtained by milling 5 kg lots of cleaned wheat in stone mill (Bemco), pin mill (mini Kek mill), hammer mill (Apex) and disc mill (*chakki*). Sieve of 60 mesh size (250  $\mu$ ) was used in hammer and pin mills. For comparison, whole wheat flour was also milled in a Buhler laboratory roller flour mill (Model—MLU 202) by passing wheat successively through break and reduction rolls without using any sieves. The distance between the rolls was so adjusted that over 65 per cent of *atta* passed through 80 mesh sieve. The temperature of the whole wheat flour delivered at the outlet of different mills was recorded.

**Sieve analysis:** This was carried out in replicates in a Buhler laboratory plansifter using 200g samples. The overtailings on each sieve were weighed after running the sifter for 5 min and the percentages of each of the fractions were calculated.

**Chemical analysis:** Moisture, diastatic activity and the damaged starch content in different *atta* samples were determined by standard AACC methods<sup>2</sup>. Iron was estimated according to the AOAC method<sup>3</sup>.

**Rheological characteristics:** Farinograph and extensograph characteristics of *atta* samples were determined according to the method standardized by Haridas Rao<sup>4</sup>. Farinograph characteristics were determined at the optimum chapati dough consistency of 450-480 BU at a lever position of 1:3, while the extensograph characteristics were determined for chapati dough made in a Hobart mixer using the required quantity of water as indicated by farinograph. As this chapati dough was relatively stiff, only 75g dough was taken for stretching instead of 150g as recommended normally in the standard procedure for obtaining the curve within the recording chart. The remaining weight of 75 g was compensated by keeping 75g weight on the dough holder of the extensograph. The evaluation of farinograms and extensograms was carried out according to the standard procedure of AACC<sup>2</sup>.

**Determination of chapati water absorption:** For chapati making trials, water absorption capacity of whole wheat flour used to obtain a chapati dough of optimum consistency was determined by using the

Research Water Absorption Meter<sup>1</sup>. The water required to obtain a dough having an extrusion time of 60-65 sec under a force of 4.4 kg was considered as the chapati water absorption.

**Preparation of chapati:** Chapati dough was made by mixing 200g flour and water, equivalent to chapati water absorption in a Hobart mixer (Model N-50) for 3 min. About 40 g of the dough was sheeted to a thickness of 2 mm on a specially designed platform<sup>4</sup>. The sheeted dough was then cut into a circular shape of 15 cm diameter using a die with a sharp edge. It was then baked on a hot plate maintained at 205°C for 45 sec followed by baking the other side for 105 sec and puffing for 25 sec in a gas *tandoor* maintained at 371°C. The height of puffed chapati was immediately measured using a meter scale. The puffed chapati was then cooled for 15 min before evaluation.

**Evaluation:** The sensory evaluation of chapati was conducted by a panel of 6 judges for its appearance (colour of the spots and their uniformity), hand feel (rough, smooth, pliable) eating quality (soft, hard, leathery), and taste (sweetish, wheaty, bland) according to the method of Shurpalekar and Prabhavathi<sup>5</sup>.

The texture of chapati was evaluated by using Warner Bratzler shear press. Each chapati was folded to have 4 layers and placed in the centre of a conical blade provided on the instrument. The maximum force in pounds, as indicated on the dial, for shearing the chapati was recorded. The average of values was recorded for 3 chapaties. The pliability was measured for a chapati strip of 7×2 cm using a simple device developed by Haridas Rao<sup>4</sup>.

All analyses were carried out in duplicate and averages are given in the tables.

### Results and Discussion

**Sieve analysis:** Sieve analysis of whole wheat flours ground in different types of mills are given in Table 1. It is observed that in most cases, less than 10 per cent of *atta* was retained over the 60 mesh sieve. Maximum amount of *atta* was found over 12 XX (112  $\mu$ ) sieve in all the cases. The stone milled or roller milled *atta* was slightly finer than others.

**Physico-chemical characteristics:** The physico-chemical characteristics of *atta* are given in Table 2. The temperature developed during grinding varied from 31 to 92°C. Maximum temperature was observed in case of stone milled *atta* while negligible rise in temperature was observed in case of roller mill.

The loss of moisture from grains during grinding appeared to be a function of the frictional heat developed and resultant temperature of *atta* and that the loss of moisture in *atta* samples was greater, if its resultant temperature was higher. Thus, the roller milled *atta*

TABLE 1. SIEVE ANALYSIS (%) OF WHOLE WHEAT FLOURS (*ATTA*) GROUND IN DIFFERENT MILLS

Sieve used	Particle size ( $\mu$ )	Disc mill ( <i>chakki</i> )	Pin mill	Hammer mill	Stone mill	Roller mill
32	670	1.8	5.5	—	0.7	0.1
45	480	3.2	3.6	0.1	1.3	2.2
60	340	6.0	5.8	6.9	3.4	6.4
6 XX	219	9.1	12.8	21.5	6.7	4.5
10 XX	129	13.6	9.7	14.0	12.7	3.6
12 XX	112	38.1	32.0	24.5	35.5	31.7
15 XX	85	7.0	19.8	10.9	9.5	18.3
25 P	62	10.5	5.0	14.1	17.5	19.8
Pan	—	8.3	4.6	7.7	11.8	12.1

TABLE 2. SOME QUALITY CHARACTERISTICS§ OF WHOLE WHEAT FLOUR GROUND IN DIFFERENT MILLS

Type of mill	Temp* (°C)	Moisture‡ (%)	Diastatic activity (mg/10g)	Damaged starch (%)	Iron (mg %)	Water absorption** (%)
Disc ( <i>chakki</i> )	78	7.8	370	12.9	16.4	77
Pin	61	8.8	365	12.0	11.8	74
Hammer	50	9.5	220	9.9	10.3	70
Stone	92	7.4	505	18.8	7.5	82
Roller	31	9.9	200	8.2	10.1	68

§Values (other than moisture) expressed on 14% moisture basis.

\*Recorded at discharge outlet in the respective mills.

‡Initial moisture content in wheat—10.2%.

\*\*As determined in a Research water absorption meter.

which had the minimum increase in temperature (4°C) had the lowest loss of moisture (0.3 per cent), while the loss was 2.8 per cent in case of stone mill.

Though the particle size distribution of different *atta* samples did not vary considerably, the damaged starch content in these flours varied widely; highest was in flour ground in stone mill (18.8 per cent) while the lowest was in roller mill flour (8.2 per cent). The flours from disc mill, and pin mill had a starch damage of about 12.0 per cent. The results indicated a positive correlation between resultant temperature, loss of moisture and starch damage in *atta* samples during grinding.

Diastatic activity, which is influenced by the content of amylolytic enzymes as well as the damaged starch followed a pattern similar to that of damaged starch. Hence, it is likely that the chapati made from stone milled *atta* will be relatively more sweetish as more sugar will be formed

during mixing and resting of the chapati dough.

The water required to make the chapati dough of optimum consistency varied widely among different flours and it depended mainly on the damaged starch<sup>6</sup>. Highest water absorption of 82.0 per cent was found for stone mill flour, while minimum water absorption of 68.0 per cent was for roller milled *atta*.

As expected, disc mill *atta* had the maximum iron content of 16.4 mg per cent, whereas stone mill flour had a minimum iron content of 7.5 mg per cent. However, the digestibility and absorption of such iron which is in ferric form coming from the disc is less as compared to iron in ferrous form<sup>7</sup>.

*Dough characteristics:* Farinograph water absorption ranged from 69.0 to 81.5 per cent and like the chapati water absorption determined by using Research Water Absorption Meter, maximum absorption was



TABLE 3. RHEOLOGICAL CHARACTERISTICS OF WHOLE WHEAT FLOUR GROUND IN DIFFERENT MILLS

Type of mill	Farinograph*			Extensograph		Ratio figure R/E
	Water absorption (%)	Dough develop- ment time (min)	Stability (min)	Resistance to extension (R) (BU)	Extensibility (E) (mm)	
Disc ( <i>chakki</i> )	76.0	4.5	3.5	480	50	9.6
Pin	74.0	4.0	3.5	460	55	8.4
Hammer	71.0	3.0	2.0	410	65	6.3
Stone	81.5	5.5	4.5	590	50	11.8
Roller	69.0	3.5	2.5	480	60	8.0

\*Determined at 450 BU consistency at level position of 1:3.

found for stone mill *atta* (Table 3). On the other hand, the minimum water absorption was found for roller mill *atta*. Dough development time as well as the stability varied from 3.0 to 5.5 min and 2.0 to 4.5 min respectively in flours milled in different mills. Stone mill flour had maximum dough development time as well as the stability while these values were minimum for *atta* from hammer mill.

Extensograph characteristics of *atta* milled in different types of mills also showed marked variation. The resistance to extension varied from 410 to 590 BU and the extensibility from 50 to 65 mm. Stone mill *atta* had the maximum resistance to extension while *atta* milled in hammer mill had the minimum value. However, the *atta* samples milled in hammer mill and roller

mill showed higher extensibility. Ratio figure which indicates the stiffness of the dough showed that stone milled *atta* forms stiffer dough than the *atta* samples from other mills.

*Chapati making quality:* Data presented in Table 4 show that chapati making quality of *atta* ground in different mills varied considerably. The height of puffed chapati, an important quality parameter, was found to be maximum for stone milled *atta*. Next in order were chapatis made from flours milled in disc mill, pin mill, hammer mill and roller mill respectively. The puffed height was dependent on starch damage<sup>1</sup>, the greater the starch damage, the greater is the water absorption and greater the puffed height.

The pliability of chapati, another quality parameter

TABLE 4. QUALITY OF CHAPATI MADE FROM WHOLE WHEAT FLOUR GROUND IN DIFFERENT MILLS

Type of mill	Instrumental measures			Sensory parameters				Overall quality
	Ht. of puffed chapati (cm)	Pliability (cm)	WB Shear value (lb)	Spots	Hand feel	Texture	Taste	
Disc ( <i>chakki</i> )	7.2	2.4	6.8	Light brown, uniform	Smooth, pliable	Soft	Sweetish	Good
Pin	6.9	2.0	7.0	"	"	"	"	"
Hammer	6.4	1.7	7.8	Dull grey, uniform	Slightly rough, somewhat pliable	Slightly hard	Bland	Fair
Stone	7.5	3.1	5.5	Slightly dark brown, uniform	Smooth, highly pliable	Very soft	Highly sweetish	Excellent
Roller	6.0	1.7	8.1	Dull grey, uniform	Rough somewhat pliable	Slightly hard	Bland	Fair

of acceptability varied from 1.7 to 3.1 cm and the maximum pliability was recorded for chapati made from *atta* ground in stone mill. This is possibly due to the higher water absorption capacity of the *atta* sample, which renders the chapati soft. However, the chapati made from *atta* milled in hammer mill or roller mill had the lowest pliability value of 1.7 cm.

Shear value showed that chapati made from stone milled *atta* was quite soft as indicated by the low shear value (5.5 lb) followed by *atta* samples from disc mill, pin mill, hammer mill and roller mill. The chapati made from roller mill flour was relatively tough, as indicated by the high shear value of 8.1 lb.

Stone mill *atta* gave superior quality chapati as judged by hand feel, eating quality and taste, the next in order being chapatis made from *atta* milled in disc, pin, hammer and roller mills. The soft texture of chapati made from stone milled *atta* could be attributed to its higher water absorption capacity, whereas the chapati from *atta* milled in hammer mill and roller mill were somewhat hard in texture and also lacked the typical sweetish taste. Chapatis made from disc and pin mill *atta* were similar and the overall quality was quite good when compared with chapatis from stone milled *atta*.

It can be inferred that the *atta* obtained from stone mill gave superior quality chapati than those obtained from other mills. On the other hand *atta* from hammer mill and roller mill were not found to be quite suitable for chapati making.

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## Pressure-Parboiled Rice: A New Base for Making Expanded Rice

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*Manuscript received 3 December 1984; revised 18 June 1985*

Expanded rice (*puri*) is prepared by high-temperature short-time (HTST) treatment of milled parboiled rice. Normal parboiled rice (paddy fully soaked, then steamed) or dry-heat parboiled rice (paddy fully soaked, then roasted with sand) is at present used for making the product. The present work shows that pressure-parboiled rice (paddy partially soaked, then pressure steamed) is best for expansion. However, white belly or starch retrogradation in the rice are both to be avoided. What is needed is full parboiling upto the grain centre, but at a low enough grain moisture to inhibit starch retrogradation. The high discolouration of such rice can be eliminated using 2 per cent sodium bisulphite solution instead of water to wash the paddy before steaming. This process would also lend itself to large-scale production of expanded rice.

Expanded rice (*puri*, *muri*, *murmura*) is a popular rice product widely consumed in India. It is made by high-temperature short-time (HTST) treatment of milled parboiled rice, when the rice expands into the familiar fluffy crisp product. The process is an established cottage industry in India. A flow diagram of the process

as practised by the cottage industry in India is shown in Fig. 1 (left).

An important aspect of the process is that neither all rice varieties nor all parboiling treatments yield an equally good product. Specific varieties are used for processing in the trade and, moreover, the paddy is

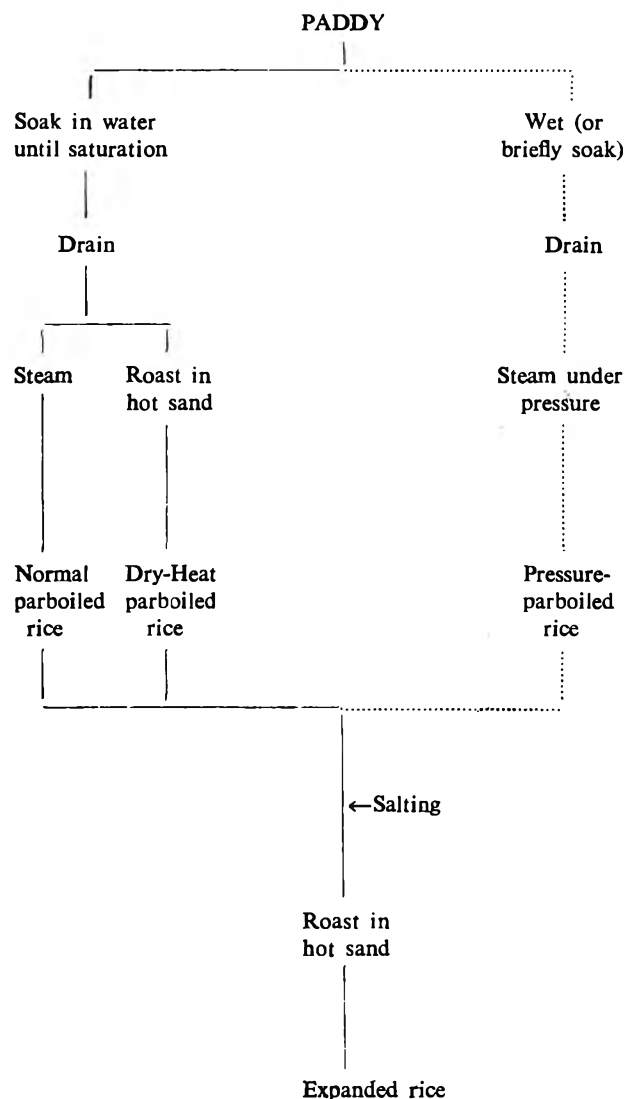


Fig 1. Flow diagram of making expanded rice in cottage industries in India. The existing systems are shown in continuous lines on left. The proposed pressure-parboiling system is shown in broken lines on right.

parboiled in specific ways. These aspects were investigated by us earlier<sup>1,2</sup>.

As far as parboiling is concerned, it was observed<sup>2</sup> that in the normal steam-parboiling process (paddy soaked in water until saturation, drained, and steamed), the degree of expansion of the resulting parboiled rice increased with increasing severity of steaming during parboiling of the paddy. However dry-heat parboiling (paddy soaked in water, drained and then, instead of steaming, subjected to HTST treatment with hot sand) gave still better expansion, as is indeed usually practised in the trade (Fig 1).

Recently pressure parboiling has been devised<sup>3,4</sup>. In the normal steam-parboiling process, the paddy is

soaked in water until it is saturated and then steamed either at atmospheric or elevated pressure. In pressure-parboiling, paddy is partially soaked and subjected to high-pressure steaming which brings out the gelatinization. This process yields a product having distinct properties which have been investigated and discussed by us elsewhere<sup>5</sup>.

Preliminary experiments suggested that pressure-parboiled rice under certain circumstances could yield a very satisfactory expanded rice product, and hence this aspect was investigated in detail.

### Materials and Methods

**Paddy:** 'Intan' variety of paddy, normally used by the local cottage industry for producing expanded rice, was used for the experiments. The paddy was procured from the local market in bulk, cleaned, dried (11-12 per cent moisture content, wet basis, w.b.) and stored in metal containers for about a year at room temperature in the laboratory.

**Parboiling, milling and expanding:** Paddy was soaked in water at room temperature, portions being removed at various intervals and tempered in closed bottles for about 30 hr to obtain various initial moisture contents. Four moisture levels were selected, viz. 11.3 (original unsoaked paddy), 17.4, 20.1 and 22.3 per cent w.b. (nominally referred to as 11, 17, 20 and 22 per cent) moisture respectively. This moisture-adjusted paddy was taken in a wire mesh tray and steamed under various pressures (0.5-3.0 kg/cm<sup>2</sup>) and for various time intervals (5-30 min) each in an autoclave connected to a steam line. Fully soaked paddy (about 30 per cent moisture) was used for normal steam parboiling and dry-heat parboiling.

To study the effect of bisulphite on the brown colour of parboiled rice, another set of paddy samples was soaked in various concentrations (0.1-10 per cent) of sodium bisulphite solution for 1-2 min and drained. It was then divided into two equal portions, one being steamed immediately at 2.5 kg/cm<sup>2</sup> steam pressure for 15 min, and the other steamed similarly after tempering in a closed bottle for 30 hr.

To produce dry-heat parboiled rice (DH-parboiled rice), fully soaked paddy was roasted with hot sand (250°C) for about 2.5 min in a laboratory coffee roaster as described<sup>2</sup>.

All samples were dried in shade and milled in laboratory McGill equipment to full milling. The milled rice was exposed to the atmosphere in the room to adjust the moisture content to about 10.5 per cent, which was optimum for best expansion<sup>2</sup>.

For expanding, 20g of milled parboiled rice was heated with hot sand (250°C) in a laboratory coffee roaster for 10-11 sec. Expansion ratio was calculated

as the ratio of bulk volume of expanded rice to that of original rice.<sup>2</sup> For expansion of rice with salt, 0.5 ml of saturated sodium chloride solution was sprinkled on 20g of milled parboiled rice, mixed well, and the rice was immediately expanded as above.<sup>2</sup>

**Analytical:** 'White belly' (ungelatinized opaque central core) area in milled parboiled rice was estimated visually and the translucence index was calculated as 100 minus mean per cent area covered by white belly. The colour score (0-10) of the milled parboiled rice was also determined visually; score zero corresponded to that of raw milled rice, 0.5 to that of normal mild steam-parboiled rice (steamed at atmospheric pressure for 10 min), 1 to that of normal severe steam-parboiled rice (steamed at atmospheric pressure for 60 min), and score 10 to dark amber colour. Head rice yield (per cent unbroken grains in milled rice) was determined by a standard sizing device. The equilibrium moisture content attained by rice upon soaking in water at room temperature (EMC-S, expressed as per cent dry basis, d.b.) was determined as described by Indudhara Swamy *et al.*<sup>6</sup>

The colour of the bisulphite treated rice was determined with an Elico reflectometer. Fifty grams of milled parboiled rice was taken in a 4.5 cm dia × 1.5 cm petri dish and packed by tapping, and readings were taken at two different positions. The process was repeated, and the average of four readings are reported. Magnesium oxide white block was used as reference (reading 100).

All determinations were performed in duplicate and the means are reported.

## Results and Discussion

**Effect of parboiling conditions:** Preliminary experiments showed that the conditions of pressure parboiling had a profound effect on the degree of expansion of the resulting milled rice when subjected to HTST treatment. Paddy was therefore pressure parboiled under diverse conditions and the resultant products were assessed with respect to translucence index, colour, grain breakage and moisture absorption capacity as indicators of inherent quality<sup>5</sup>, and these properties were compared with the expansion volume of the respective samples. The results are schematically presented in Fig 2, which, by virtue of the symbols chosen, immediately gave a visual idea of the trend of the results (from white to increasing blackness).

As observed earlier<sup>5</sup>, translucence index (an index of the degree of parboiling), colour and head rice yield increases from left to right and from top to bottom (Fig 2), i.e., with increasing moisture content, steaming pressure and time of steaming. In other words, while low-moisture samples were not fully parboiled upto the centre of the kernel when steamed under low pressure, as is to be expected, the deficiency in moisture

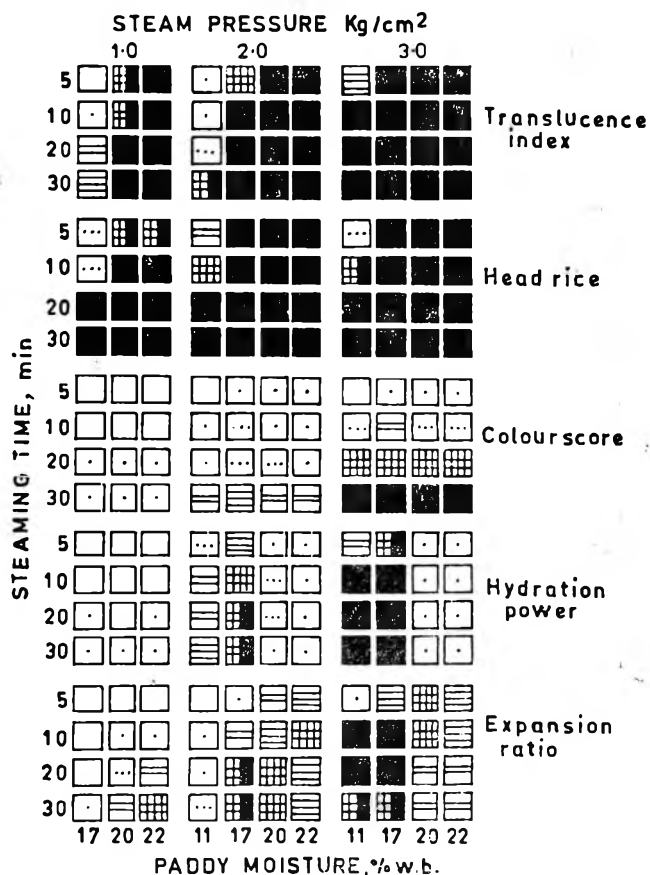


Fig 2. Effect of pressure-parboiling conditions (moisture content and pressure and time of steaming of paddy) on quality of resulting rice and its heat expansion capacity. Meaning of symbols is as follows:

Symbol	Translucence index (% area)	Head rice (%)	Colour score	Hydration power (EMC-S, % d.b)	Expansion ratio
□	0-15	0-20	0-0.5	61-70	2.0-3.0
◻	16-30	21-40	0.6-1.0	71-80	3.1-4.0
◻◻	31-45	41-60	1.1-1.5	81-100	4.1-5.0
◻◻◻	46-60	61-80	1.6-2.0	101-120	5.1-6.0
◻◻◻◻	61-75	81-85	2.1-2.5	121-140	6.1-6.5
◻◻◻◻◻	76-90	86-90	2.6-5.0	141-160	6.6-7.0
◻◻◻◻◻◻	91-95	91-95	5.1-7.5	161-180	7.1-7.5
◻◻◻◻◻◻◻	96-100	96-100	7.6-10	181-200	7.6-8.0

content could be made good by increasing pressure and time of steaming. Paddy having higher moistures naturally required less pressure or time of steaming to be fully parboiled. The colour of the samples increased drastically with pressure of steaming and less so with

steaming time. Head rice yield was satisfactory under all conditions except in samples which were steamed under low pressures at a low moisture.

The equilibrium moisture content attained after soaking (EMC-S) of the samples did not show a similar trend as above but showed certain optima. This was because EMC-S represents the net effect of gelatinization and retrogradation of starch in the rice<sup>5</sup>. High moisture contents in parboiled rice favour starch retrogradation while moisture contents below 20 per cent (w.b.) inhibit starch retrogradation<sup>7</sup>. For this reason samples with high moisture contents—even if steamed under high pressures, which would otherwise increase the gelatinization and hence favour a high EMC-S—underwent retrogradation and hence showed a relatively low EMC-S. Samples steamed under low pressures also showed low EMC-S, but this time due to low starch gelatinization. On the other hand low-moisture samples fully parboiled by high-pressure steaming showed high EMC-S, because on the one hand they were well gelatinized by high-pressure steaming and on the other hand they did not suffer starch retrogradation due to their inherent low moisture.

A comparison of the expansion ratio data with the EMC-S data in Fig 2 shows that these were interrelated. These expansion results too may therefore be due to the opposing effects of starch gelatinization and starch retrogradation. Earlier studies<sup>2</sup> showed that expansion ratio increased with increasing degree of starch gelatinization but decreased with starch retrogradation. This would explain why high-moisture pressure-steamed samples, despite increasing gelatinization, showed decreased expansion, viz. due to starch retrogradation. Incompletely parboiled samples, namely with white belly, too showed low expansion; in fact these samples after expansion showed a clear hard centre; this was due to low gelatinization. But the samples which were completely parboiled under a low moisture content by high-pressure steaming showed the best expansion, evidently due to high gelatinization but low retrogradation, and the same samples for similar reasons showed the highest EMC-S also.

These overall results are brought out in Fig 3, where the effects of paddy moisture, pressure of steaming and time of steaming are compared. It is clear that while expansion ratio increased with increasing moisture content under mild conditions of steaming due to increasing gelatinization, at high steam pressures or long steaming times, it later decreased due to starch retrogradation. High-pressure treatment at a low-moisture gave the maximum expansion ratio, because these conditions corresponded to a high degree of gelatinization without accompanied retrogradation. However white belly had to be avoided in the samples, because of its high

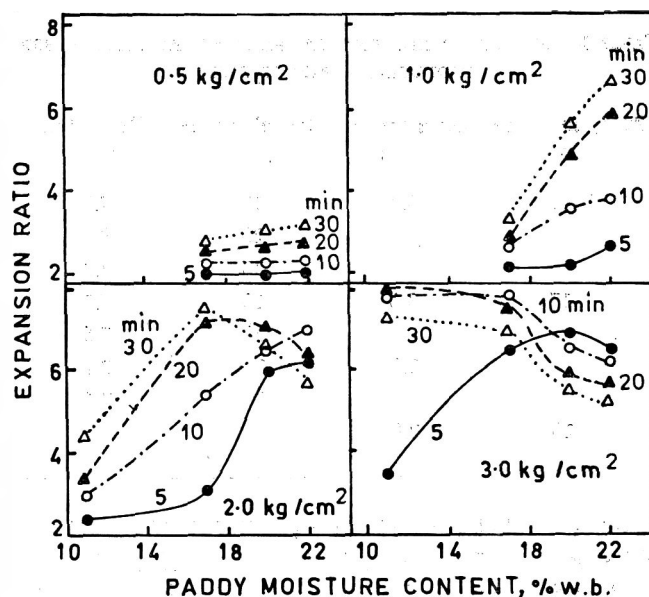


Fig 3. Relation between moisture content (abscissa), steaming pressure (box figures, kg/cm<sup>2</sup>) and steaming time (figures beside the curves, min) of paddy on expansion ratio of resulting rice.

The relationship between expansion ratio and EMC-S are shown in Fig 4. While expansion ratio increased with increasing EMC-S under all processing conditions, the data could be clearly divided into two separate sets, one with moisture contents below 20 per cent and the other with moisture contents of 20 per cent and above. The former showed highest EMC-S as well as expansion, while the latter showed low values. The main difference between the two sets is evidently due to starch retrogradation.

Best expansion was therefore given by parboiled rice produced by a combination of the lowest grain moisture and a high pressure of steaming, the latter being for such

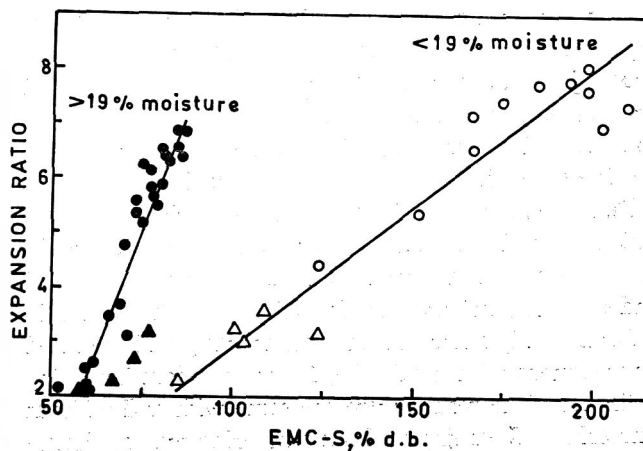


Fig 4. Relation of expansion ratio to hydration capacity (EMC-S) of pressure-parboiled rice.  $\Delta$ ,  $\bullet$  = parboiled rices with white belly.

TABLE 1. OPTIMAL CONDITIONS OF PRESSURE PARBOILING FOR PRODUCING EXPANDED RICE

Paddy moisture (% w.b.)	Steam pressure (kg/cm <sup>2</sup> )	Steaming time (min)	Expansion ratio
11	3.0	10	7.7
		20	8.0
		30	7.3
17	3.0	10	7.7
		20	7.6
		2.0	7.1
20	2.0	30	7.4
		30	7.4

time that yielded a completely parboiled rice (i.e. without white belly). These conditions can be visualized from Fig 3 and are summarized in Table 1. It can be concluded from the data that paddy with a moisture content of 11 per cent (i.e. without soaking or wetting) and steamed at 3 kg/cm<sup>2</sup> for 10-20 min, or paddy at a moisture content of 17 per cent (i.e. just wetted) and steamed at 2.5-3 kg/cm<sup>2</sup> pressure for 10-15 min would yield the best product for expansion. Head rice yield of these rices would also be satisfactory (Fig 2).

It is of interest that the expansion ratio achieved by the above process (7.5-8) was greater than even that by dry-heat parboiling (about 6-6.5). The advantage of pressure parboiling for producing expanded rice is thus obvious.

*Elimination of excessive colour:* However a great disadvantage of the above process is the high colour of the rice. Optimum process conditions as stated above yielded a strongly amber rice. Although the colour was much reduced after expansion, the final product was still unacceptable. Means of reducing or eliminating the colour by treatment with bisulphite<sup>8</sup> was therefore explored. Treatment of unsoaked paddy as a method of processing had therefore to be abandoned as it would preclude any treatment with bisulphite. As the moisture content had at the same time to be kept low, a brief wetting with bisulphite solution was tried.

Paddy was soaked in solutions of various concentrations of sodium bisulphite for 1-2 min, with and without tempering in a closed bottle, and was then steamed under 2.5 kg/cm<sup>2</sup> pressure for 15 min. The results in Table 2 clearly show that the colour was greatly reduced by treatment with bisulphite, 2 per cent solution being optimum. The results were the same with and without tempering. Steaming without tempering was obviously more convenient in practice. This treatment reduced the the colour of parboiled rice to the level obtained in normal steam parboiling. The colour of expanded rice

TABLE 2. EFFECT OF SOAKING PADDY IN SODIUM BISULPHITE SOLUTION BEFORE PRESSURE PARBOILING<sup>a</sup> ON THE COLOUR AND HEAT EXPANSION CAPACITY OF THE RESULTING RICE

Equilibrated			Unequilibrated		
Sodium bisulphite conc. (%)	Reflectometer reading	Expansion ratio	Sodium bisulphite conc. (%)	Reflectometer reading	Expansion ratio
<b>Sodium bisulphite treated</b>					
Nil	30	8.0	Nil	31	8.1
0.1	35	7.9	1.0	35	7.8
0.5	33	8.0	2.0	40	8.2
1.0	37	8.1	4.0	39	7.9
2.0	38	7.8	6.0	40	8.0
4.0	40	8.0	8.0	40	7.8
8.0	40	7.9	10.0	42	8.1
<b>Raw rice</b>					
—	54	1.6			
<b>Mild steam parboiled<sup>b</sup></b>					
—	41	2.4			

<sup>a</sup>All steamed at 2.5 kg/cm<sup>2</sup> for 15 min after soaking for 1-2 min.

<sup>b</sup>Paddy soaked in water until saturation, drained and steamed at atmospheric pressure for 10 min.

produced by such bisulphite-treated parboiled rice was similar to or better than that of expanded rice produced from the usual dry-heat parboiled rice of commerce. The expansion ratio remained unchanged by bisulphite treatment.

*Effect of salt:* Addition of salt to the parboiled rice, as noted earlier<sup>2</sup>, further increased the expansion ratio (Table 3). The comparative results obtained from rices parboiled by different treatments are shown in Table 3 and Fig 5. Clearly the bisulphite-treated pressure-parboiled rice prepared under optimal conditions gave the highest expansion ratio and was satisfactory with respect to colour and appearance. The expansion ratio by this method went upto as high as 10-10.5, whereas in the trade it is never more than 8-8.5.

*Optimal conditions:* It can be concluded that pressure parboiling will be an excellent method of producing a suitable parboiled rice for making expanded rice. The following conditions would appear to be ideal. Paddy is washed with 2 per cent sodium bisulphite solution and drained. It is then steamed at about 2.5 kg/cm<sup>2</sup> for about 15 min. The treated paddy is dried and milled. The milled rice is adjusted to 10-10.5 per cent moisture (w.b.)

TABLE 3. EFFECT OF DIFFERENT PROCESSING METHODS ON QUALITY OF EXPANDED RICE

Parboiling method	Soaking		Steaming		Salted before expanding	Expansion ratio	
	Medium	Extent	Pressure (kg/cm <sup>2</sup> )	Time (min)			
Raw rice	—	—	—	—	No	2.3	
Normal steam parboiling	Water	Full	0	10	No	2.5	
			0.5	10	No	3.0	
			1.0	10	No	4.8	
			2.0	10	No	5.1	
Dry-heat parboiling <sup>a</sup>	Water	Full	—	—	No	6.3	
					Yes	8.0	
Pressure parboiling <sup>c</sup>	Water	Washing	2.5	15	No	8.0	
		2% sodium bisulphite	Washing	2.5	15	No	8.1
			Yes	10.2			

<sup>a</sup>Soaked paddy not steamed but roasted in hot sand at 250°C for 2.5 min.

<sup>c</sup>Only pressure parboiled milled rice was brown and the corresponding expanded rice was brownish yellow in colour. All others (both milled and expanded rice) were white coloured.

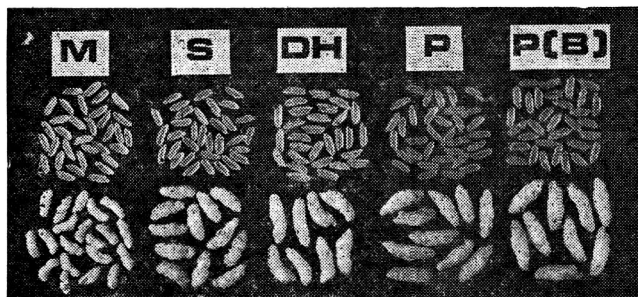


Fig 5. Appearance of expanded rice prepared from various salted parboiled rice. M=mild parboiled (soaked paddy steamed at 0 kg/cm<sup>2</sup> for 10 min), S=severe parboiled (steamed at 1.5 kg/cm<sup>2</sup> for 10 min), DH=dry-heat parboiled, P=pressure parboiled, P(B)=bisulphite-treated pressure parboiled (see conditions in Table 3).

saturated sodium chloride solution is sprinkled on the rice (in the ratio of 1 ml for 40 g) and mixed well. The rice is now expanded by usual HTST treatment.

The above method not only provides better expansion than currently obtained with dry-heat parboiled rice, the product also had excellent colour and other desirable quality. Besides, the method will have an additional advantage of being the first step towards large-scale mechanized production of expanded rice. Expanded rice as now prepared by the cottage industry involves dry-heat parboiling of small quantities of paddy by manual operation in small batches followed by expansion in small batches by HTST treatment. While the second

operation of heat expansion remains to be mechanized and devised into a continuous process, adoption of pressure parboiling for obtaining the parboiled rice will enable the first part of the process to be made into a large-scale operation.

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# Aflatoxin Production in Wheat Flour and Its Effect on Protein and Carbohydrate Content of the Flour

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*Manuscript received 26 November 1984; revised 26 June 1985*

Wheat flour samples collected from various locations of Bangalore contained 0 to 660  $\mu\text{g/kg}$  of aflatoxin. None of the samples contained aflatoxin B<sub>2</sub> and G<sub>2</sub>. Toxin production by *Aspergillus flavus* in inoculated wheat flour increased with increase in moisture content of the flour and incubation temperature. A combination of 30% moisture and incubation temperature of 30°C favoured maximum aflatoxin production. The concentration of water soluble and alkali soluble protein decreased considerably with incubation period in the wheat flour inoculated with *A. flavus*. However, there was an increase in the concentration of acid soluble protein. The alcohol soluble protein showed initial increase up to 9 days followed by a decrease during later stages of incubation. The decrease in reducing and non-reducing sugars in inoculated wheat flour was rapid up to 9 days followed by a gradual decrease. Starch content decreased gradually, the rate being almost uniform throughout the incubation period.

Aflatoxins are hepatotoxic, secondary fungal metabolites produced by *Aspergillus flavus* and its related species. Aflatoxin occurs as a natural contaminant in many stored agricultural products<sup>1,2</sup>. In view of the wide acceptance due to its high carbohydrate (60-65 per cent) and protein (9-15 per cent) content, the question of whether wheat flour can become contaminated with aflatoxin assumes importance. There are several reports on the occurrence of aflatoxin and aflatoxin producing fungi in wheat<sup>1,3-5</sup> and wheat flour<sup>6-8</sup> from developing countries. An attempt was made to determine the natural occurrence of aflatoxins in wheat flour collected from different locations without having any visual contamination or spoilage and the effect of different temperature and moisture levels on aflatoxin production in wheat flour inoculated with a toxigenic strain of *A. flavus* and the effect of mold growth and toxin production on protein and carbohydrate content of wheat flour.

## Materials and Methods

*Natural occurrence of aflatoxin in wheat flour:* Wheat flour samples (1 kg) were collected from eight locations of Bangalore and analysed for aflatoxin content. Aflatoxin was extracted from wheat flour using the Best Food Method<sup>9</sup>. The aflatoxin thus extracted was dissolved in benzene-acetonitrile (98:2, v/v) and subjected to TLC separation in benzene: methanol: acetic acid (90:5:5; v/v) along with authentic standards. Aflatoxins in the samples were identified by exposing the plates to

UV-light. Concentration of aflatoxins in the extract was determined by double beam spectrophotometer UV 200s at 365 nm<sup>10</sup>.

*Effect of temperature and moisture on aflatoxin production in artificially inoculated wheat flour:* Market wheat flour (500 g) taken in a conical flask was autoclaved and adjusted to 30 per cent moisture using sterile distilled water. The wheat flour was inoculated with spore suspension of toxigenic strain of *A. flavus* (courtesy of Dr. V. S. Murthy, CFTRI, Mysore). Such inoculated flasks were incubated at 10, 15, 20, 25 and 30°C and aflatoxin content was analysed at periodical intervals.

In another experiment, autoclaved wheat flour was adjusted to 15, 20, 25 and 30 per cent moisture levels and inoculated with *A. flavus* spore suspension. The flasks were incubated at room temperature ( $24 \pm 2^\circ\text{C}$ ) and aflatoxin content was analyzed at periodical intervals. Uninoculated samples served as controls.

To determine the combined effect of temperature and moisture, autoclaved wheat flour was adjusted to 20, 25 and 30 per cent moisture levels and inoculated. It was incubated at 10, 20 and 30°C along with the uninoculated samples. The aflatoxin content of the flour was analysed at periodical intervals.

*Effect of mold growth and aflatoxin production on protein content of wheat flour:* The autoclaved wheat flour was adjusted to 30 per cent moisture level and inoculated with *A. flavus* and incubated at 30°C. Proteins



were extracted using water, 0.5 N sodium chloride, 0.25 M ascorbic acid, 70 per cent ethanol and 0.01 N sodium hydroxide at regular intervals after incubation and the amount of protein in each fraction was estimated by the method of Lowry *et al.*<sup>11</sup>.

**Extraction of proteins:** Wheat flour suspension (20 per cent) was made using sterile distilled water and extracted for 1 hr under continuous stirring at room temperature. Later, it was filtered through cheese cloth and centrifuged at 5000 rpm for 10 min. The supernatant was dialyzed against distilled water for 24 hr and its protein content was estimated.

Ten grams of wheat flour was extracted with 100 ml of 0.5 N sodium chloride—0.025 M ascorbic acid for 1 hr at room temperature. The supernatant obtained after centrifugation was dialyzed for 24 hr against distilled water and the acid soluble protein content was estimated.

Wheat flour residue obtained after sodium chloride-ascorbic acid extraction was treated with 50 ml of 70 per cent ethanol and the supernatant obtained after centrifugation was dialyzed against distilled water for 24 hr. The residue after alcohol extraction was re-extracted with 0.1 N sodium hydroxide for 1 hr at room temperature and centrifuged. The supernatant was dialyzed against distilled water for 24 hr to obtain alkali soluble protein. The amount of protein extracted in different solvents was determined by the method of Lowry *et al.*<sup>11</sup>

**Effect of mold growth and aflatoxin production on carbohydrate content of wheat flour:** The moisture of autoclaved wheat flour was adjusted to 30 per cent and then inoculated with *A. flavus* and incubated at 30°C. Reducing and nonreducing sugars and starch were estimated at regular intervals, by standard methods of Nelson<sup>13</sup> and Hassed and Newfield<sup>12</sup>.

## Results and Discussion

**Occurrence of aflatoxin in wheat flour:** Aflatoxin B<sub>1</sub> was found in all the samples except in one; G<sub>1</sub> was detected in a sample of the Pilot Bakery of the University. The total aflatoxin content in the samples ranged from 0 to 0.66 µg/g (Table 1). The maximum toxin content was found in the flour from the Pilot Bakery, while the wholesale market sample was cleaner. All other samples contained toxin which exceeded the limit of 30 µg/kg. Thus, wheat flour is susceptible to mold growth under storage and every precaution should be taken to ensure proper storage conditions<sup>5,6,8</sup>.

**Effect of moisture and temperature on aflatoxin production in wheat flour inoculated with *A. flavus*:** The production of aflatoxin in wheat flour at 25 and 30°C was maximum on 9th day (1.25 and 2.4 µg/g respectively; Fig 1), while at 20°C the production was maximum on 15th day (0.75 µg/g of flour). At lower

TABLE 1. OCCURRENCE OF AFLATOXIN IN WHEAT FLOUR

Source of wheat flour samples	Aflatoxin (µg/g)				Total
	B <sub>1</sub>	B <sub>2</sub>	G <sub>1</sub>	G <sub>2</sub>	
G.K.V.K. hostel	0.40	—	—	—	0.40
Agricultural college hostel	0.45	—	—	—	0.45
Veterinary college hostel	0.48	—	—	—	0.48
Pilot bakery project, Hebbal	0.40	—	0.26	—	0.66
G.K.V.K. cafeteria	0.25	—	—	—	0.25
Hebbal cafeteria	0.45	—	0.20	—	0.65
City market (wholesale)	—	—	—	—	—
Retail shop, Hebbal	0.10	—	—	—	0.10

— indicates that no traceable amount of aflatoxin was present.

temperature the toxin production was reduced (Fig 1). At all temperatures toxin production decreased after reaching the peak, probably because of self degradation by sporulating fungus<sup>14</sup>.

In another experiment the effect of different moisture levels on the production of aflatoxin was studied. Aflatoxin production was maximum in wheat flour with 25 and 30 per cent moisture on the 9th day after inoculation (6.70 and 9.20 µg/g, respectively; Fig 2). However,

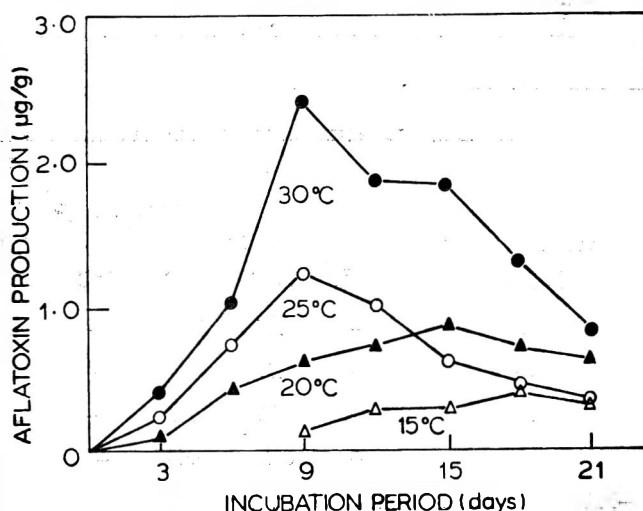


Fig 1. Effect of temperature on aflatoxin production in inoculated wheat flour.

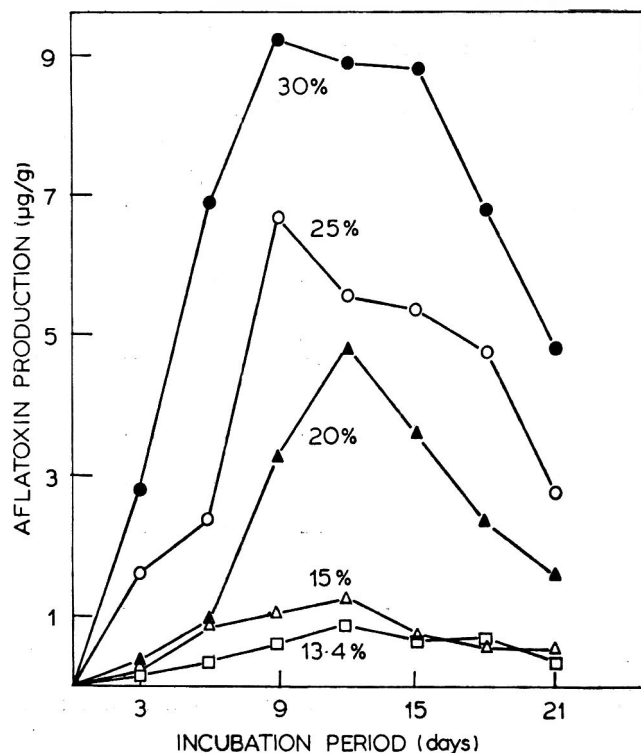


Fig 2. Effect of moisture on aflatoxin production in inoculated wheat flour

in case of the wheat flour with 13.4 (normal moisture per cent), 15 and 20 per cent moisture the aflatoxin production was maximum on 12th day (0.90, 1.30 and 4.80  $\mu\text{g/g}$ ). In all the treatments aflatoxin production was initiated on 3rd day after inoculation.

The combined effect of temperature and moisture on the production of aflatoxin showed that incubation of wheat flour containing 30 per cent moisture at 30°C recorded highest level of aflatoxin on 10th day (17.80  $\mu\text{g/g}$ ; Table 2). At all moisture contents, the toxin

production was maximum in samples incubated at 30°C. The toxin concentration in the samples containing 20 and 13.4 per cent moisture was low at all the temperatures (10, 20 and 30°C) than in samples containing 25 or 30 per cent moisture. Thus, with increase in moisture content of the flour and temperature the production of aflatoxin increased. There was no toxin production in sample containing 13.4 and 20 per cent moisture incubated at 10°C. Thus, moisture content of the flour and incubation temperature have a profound effect on the production of aflatoxin in wheat flour by *A. flavus*. It is widely recognized that most important factor in growth and toxin production by *A. flavus* is the moisture content of the substrate<sup>15</sup>. Temperature controls the enzyme activities in molds and moisture influences the growth<sup>17</sup>. The maximum production of aflatoxin in peanuts<sup>18</sup> and corn and sorghum<sup>19</sup> was obtained in samples having 20-30 per cent moisture and incubated at 25-35°C.

*Effect of mold growth and aflatoxin production on protein content of wheat flour:* Fungal growth reduced the water soluble protein content in wheat flour drastically up to 6th day (Table 3). Later the breakdown was slow up to 15th day and remained constant afterwards. Although, acid soluble protein content decreased initially up to 6th day, it registered a slight increase during later periods of incubation (Table 3), possibly due to conversion of water and alkali soluble proteins to acid soluble proteins. Chetty *et al.*<sup>23</sup> observed that the soluble proteins in peanut inoculated with *A. oryzae* decreased shortly after incubation, but increased during later stages of incubation. The decrease in alcohol soluble proteins during later stages may be due to hydrolytic activity of the fungus<sup>21</sup>. Chetry *et al.*<sup>20</sup> have reported the breakdown of storage proteins into smaller peptide bonds and free amino acids and hydrolysis of ester linkage in peanut seeds infected with *A. oryzae*.

TABLE 2. COMBINED EFFECT OF TEMPERATURE AND MOISTURE ON AFLATOXIN PRODUCTION IN INOCULATED WHEAT FLOUR

Incubation period (days)	Aflatoxin ( $\mu\text{g/g}$ )* at indicated moisture and temp. levels											
	13.4%			20%			25%			30%		
	10°C	20°C	30°C	10°C	20°C	30°C	10°C	20°C	30°C	10°C	20°C	30°C
5	—	0.16	0.25	—	0.30	1.20	0.10	0.80	2.45	0.25	1.20	2.85
10	—	0.65	0.75	—	1.10	3.40	0.50	1.65	13.10	0.75	8.20	17.80
15	—	0.75	0.90	—	1.10	6.35	0.60	3.60	10.60	0.80	4.60	16.70
20	—	0.65	0.65	—	0.90	6.20	0.30	2.90	9.75	0.55	3.80	12.10

—indicates no production of aflatoxin

\*Average of three replications.

TABLE 3. EFFECT OF MOLD GROWTH AND AFLATOXIN PRODUCTION ON SOLUBLE PROTEINS IN WHEAT FLOUR

Incubation period (days)	Proteins (mg/g)*				Total
	Water soluble	Acid soluble	Alcohol soluble	Alkali soluble	
0	11.04	5.84	12.64	54.24	83.76
3	8.88	5.52	13.83	54.24	82.54
6	5.00	4.90	14.68	29.36	53.94
9	4.08	5.04	14.92	27.76	51.80
12	3.68	5.12	12.72	17.76	39.28
15	3.24	5.36	9.48	16.08	34.16
18	3.12	5.43	9.48	11.92	29.95
21	3.12	5.04	8.60	11.92	28.68

\*Average of three replications

TABLE 4. EFFECT OF MOLD GROWTH AND AFLATOXIN PRODUCTION ON CARBOHYDRATES IN WHEAT FLOUR

Incubation period (days)	Sugars		Starch (mg/g)	Total carbohydrates (mg/g)
	Reducing (mg/g)	Non-reducing (mg/g)		
0	5.50	8.04	541.5	555.0
3	3.72	6.12	511.3	521.1
6	2.19	6.02	480.6	488.8
9	1.11	4.56	450.5	456.2
12	0.82	4.46	410.5	415.7
15	0.23	4.08	409.5	413.8
18	0.21	4.02	379.7	383.9
21	0.21	4.03	375.0	379.2

\*Average of three replications.

*Effect of mold growth and aflatoxin production on carbohydrate content of wheat flour:* There was a rapid breakdown of reducing sugars from 3rd to 9th day followed by a very slow breakdown during later stages (Table 4). The breakdown of non-reducing sugars was also rapid till 9th day followed by a gradual decrease. The reduction in non-reducing sugars is probably due to utilization by the fungus for its growth. *A. flavus* utilizes glucose, fructose, ribose, maltose, sorbose, lactose and sorbitol as a source of carbon for growth and production of toxin<sup>22,23</sup>.

The starch content of the wheat flour showed a gradual decrease with incubation and the rate of decrease was

almost uniform throughout. There are reports to show that *A. flavus* utilizes starch for aflatoxin production<sup>23,25</sup>. Thus, the present study suggests that wheat flour is one of the best substrates for fungal growth and toxin production as it contains large amount of carbohydrate which serves as a source of carbon.

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## On Improving the Bread Making Quality of Flour from Field Sprouted Wheat

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*Manuscript received 5 December 1984; revised 25 June 1985*

The flour milled from rain-damaged wheat have shown a significant increase in the alpha amylase activity which lowered the falling number and amylograph peak viscosity, resulting in a sticky dough, and bread with loose and coarse crumb. Stickiness of the dough could be reduced by (i) use of 2-3% salt, (ii) reducing water in the dough by 2-4%, (iii) decreasing fermentation time from 165 to 60-90 min and (iv) reducing initial dough temperature to 17-20°C. Additives such as enzyme-active soy flour, sodium stearoyl-2-lactylate (SSL), glyceryl mono stearate (GMS), lecithin, cysteine hydrochloride, disodium hydrogen phosphate and trisodium phosphate improved dough rheology and handling properties, as well as bread crumb characteristics. Inclusion of 2% salt and 0.5% GMS at an initial dough temperature of 17-19°C, along with any one of the additives such as, SSL, enzyme active soy flour, lecithin, or cysteine hydrochloride further improved the dough and bread characteristics of sprouted wheat.

In the last two years, unseasonal rains during harvest affected wheat quality due to sprouting, giving flour with an excessively high alpha amylase activity<sup>1</sup>. Such flours produce sticky dough and bread having unsatisfactory crumb characteristics.

Many European countries with cold and humid climate face similar problems due to sprout damage. Such wheat is however, generally utilised for feeds whereas in many developing countries, it must be used in the milling and baking industry. Earlier studies covered the effect of germination on the bread making qualities of the flour<sup>1-4</sup>. So far no information is available on the possibilities of improving the bread making quality of such flours. Studies were therefore undertaken to improve the dough characteristics as well as bread quality based on flours from field sprouted wheat; the results are presented in this paper.

### Materials and Methods

A typical flour from field sprouted wheat milled in a commercial roller flour mill was used for bread making studies. Non-fat dry milk (NFDM) from a local market was used. Raw soybean was dehulled and ground in a Kamas mill (Model: Slaggy-200 A) to pass through a 70 mesh sieve.

*Chemical analysis:* Total ash, protein, diastatic activity, alpha amylase activity and colour grade value of flour from rain damaged wheat were evaluated by standard AACC procedures<sup>5</sup>.

*Falling number, amylograph, farinograph and extenso-graph studies:* A Hagberg Falling Number apparatus, a Brabender amylograph, a farinograph and an extenso-graph were used to study effects of additives (sodium chloride, non-fat dry milk, enzyme active soy flour, sodium stearoyl-2-lactylate, glyceryl-mono-stearate,

lecithin, potassium bromate, cysteine-HCl, disodium hydrogen phosphate and trisodium phosphate) on dough characteristics of flour from field sprouted wheat according to standard AACC method<sup>5</sup>. Ice water was used to study the effect of reduced dough temperature on farinograph characteristics. Dough mixing was continued for 5 min after reaching peak consistency and dough temperature was recorded.

*Bread making studies:* To study effects of varying levels of ingredients, additives and processing conditions, the 'Remix' bread making procedure of Irvine and McMullan<sup>6</sup> was followed with the following modification (i) use of 1 per cent hydrogenated vegetable fat, (ii) omission of malt flour, (iii) 1.5 per cent active dry yeast instead of 3.0 per cent compressed yeast.

To examine any possible beneficial effects of reduced dough temperature on dough and bread quality characteristics, ice water (4°C) was used to dissolve ingredients and additives. To disperse dry yeast however, warm (40°C) water was used. Dough was mixed for 3.5 min. During mixing, the outer surface of the mixing bowl was cooled with ice; following mixing dough temperature was recorded.

Bread doughs were graded during mixing, remixing, sheeting and moulding as highly sticky (+++), sticky (++) slightly sticky (+), normal (\*) and better than dough based on flour from sound wheat (\*\*). Similarly, dough machinability was rated excellent (E), good (G), satisfactory (S), fair (F) and poor (P).

One hour after removing bread from the oven, loaf volume was determined by the rape seed displacement method<sup>7</sup>. Cooled loaves were stored overnight in closed tin containers, and evaluated for crust and crumb characteristics according to Pyler<sup>8</sup>.

All the analyses were carried out in triplicate and averages are given.

## Results and Discussion

*Quality characteristics of flour milled from field sprouted wheat:* Flour milled from field sprouted wheat had high alpha amylase activity and diastatic activity, as well as low falling number value and amylograph peak viscosity (Table 1), indicating rain damage.

*Effect of ingredients and additives on the rheological characteristics:* Data on Hagberg falling number, amylograph, farinograph and extensograph characteristics upon addition of different ingredients and additives are presented in Table 2 and Fig 1.

TABLE 1. SOME QUALITY CHARACTERISTICS OF COMMERCIAL FLOUR MILLED FROM FIELD SPROUTED WHEAT

Characteristics	Value <sup>a</sup>
<b>Physico-chemical</b>	
Total ash (%)	0.52
Crude protein (N×5.7), (%)	8.86
Diastatic activity, (mg maltose/10 g flour)	678
Hagberg falling number	160
Kent Jones colour grade value	5.6
Alpha amylase activity, (SKB units)	2.24×10 <sup>-2</sup>
<b>Rheological</b>	
Farinograph water absorption (%)	59.4
Dough development time (min)	1.5
Mixing tolerance index (BU)	100
Amylograph peak viscosity (AU)	60

<sup>a</sup> Expressed on 14% moisture basis

TABLE 2. EFFECT OF INGREDIENTS AND ADDITIVES ON THE FALLING NUMBER, AMYLOGRAPH AND EXTENSOGRAPH CHARACTERISTICS OF FLOUR FROM FIELD SPROUTED WHEAT

Ingredients/additives		Hagberg falling No.	Amylograph peak viscosity A.U.	Resistance to extension (R) B.U.	Extensibility (E) mm	Ratio figure (R/E)
Name	Quantity added (%)					
Control	—	152	40	900	120	7.5
Sodium chloride	2	182	60	900	95	9.5
NFDM	3	152	30	980	110	8.9
SSL	0.5	178	40	920	100	9.2
GMS	0.5	162	40	920	130	7.1
Disodium hydrogen phosphate	0.9	259	120	960	105	9.2
Trisodium phosphate	0.3	208	100	—	—	—
Enzyme active soy flour	0.5	155	30	940	105	9.0
Potassium bromate	0.001	149	30	800	90	8.9
Potassium bromate + cysteine HCl	0.001	148	40	640	105	6.1

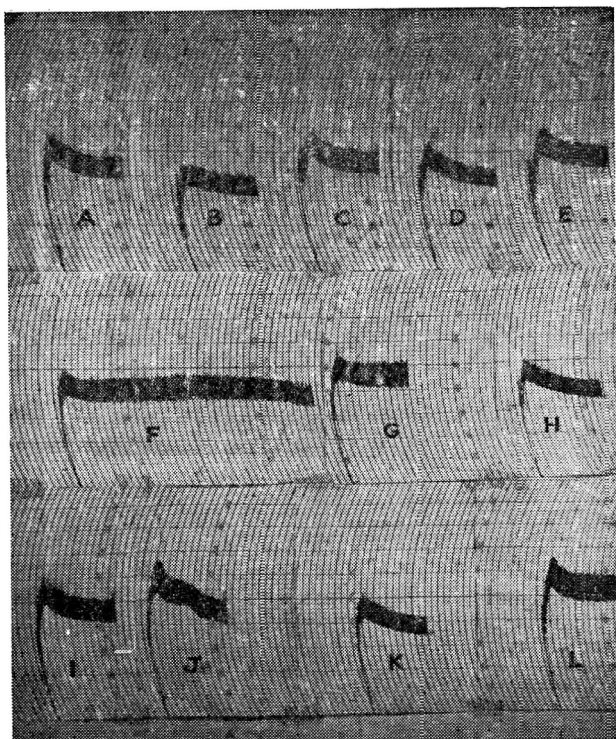


Fig. 1. Effect of ingredients, additives or reduced dough temperature on farinograph characteristics of flour from field sprouted wheat.

A—Control, B—2% Salt, C—3% NFDM, D—0.5% SSL, E—0.5% GMS, F—0.9%  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , G—0.3%  $\text{Na}_3\text{PO}_4$ , H—0.5% Enzyme active soyflour, I—0.001%  $\text{KBrO}_3$ , J—0.001%  $\text{KBrO}_3$ +0.003% cysteine HCl, K—0.003% cysteine HCl, L—Reduced dough temperature: 26.5°C.

Disodium hydrogen phosphate and trisodium phosphate considerably increased falling number values as well as amylograph peak viscosity (Table 2), confirming earlier findings of Westermarck-Rosendahl *et al.*<sup>9</sup>. Salt, GMS and SSL marginally increased falling number.

Potassium bromate alone or in combination with cysteine HCl increased peak consistency of farinograms, while SSL, disodium hydrogen phosphate, and enzyme active soy flour slightly reduced peak consistency. Reduced farinograph dough temperature increased peak consistency as reported earlier<sup>10</sup>, while sodium chloride reduced peak consistency, as observed by Tanaka *et al.*<sup>11</sup>. Trisodium phosphate did not significantly affect peak consistency.

Disodium hydrogen phosphate and trisodium phosphate increased dough stability from 1.5 to 5.0 min and reduced the mixing tolerance index from 70 to 0, confirming findings of Westermarck-Rosendahl *et al.*<sup>9</sup>

Extensograph studies indicated that of the additives tested (Table 2), sodium chloride, SSL, potassium bro-

mate, enzyme active soy flour and disodium hydrogen phosphate considerably increased dough stiffness as indicated by higher R/E values when compared to control confirming earlier results<sup>9-11</sup>. As expected, cysteine-HCl decreased the R/E value.

*Effect of ingredients on bread making quality:* Addition of salt reduces dough stickiness as assessed (Table 3) by handfeel thereby improving its handling properties; however, loaf volume is adversely affected<sup>8</sup>; this effect is more pronounced at 3 per cent (Fig 2) salt and bread tastes salty. Three per cent NFDM improves bread quality profile and dough characteristics, without affecting loaf volume<sup>8,12</sup>.

Reducing water level to 2-4 per cent less than normal decreased dough stickiness but affected final bread yield. Increasing fat or reducing sugar level did not improve machinability (Table 3).

*Effect of processing conditions:* Reducing fermentation from 165 to 60-90 min reduces dough stickiness (Table 4), apparently because there is less time for amylase activity. However, loaf volume decreases due to insufficient ripening of gluten. Considerable improvement in dough machinability and bread quality occurred by reducing initial dough temperature (Table 3 and Fig 3a); this however, necessitated an increase in final proof period to 70 min.

*Effect of additives:* Additives such as enzyme active soy flour, GMS, SSL and lecithin improve dough machinability and bread quality from sound wheat<sup>8,13-15</sup>. The present study showed (Table 4 and Fig 3a and 3b) that enzyme active soy flour, GMS and SSL considerably improve dough properties and also bread from field sprouted wheat. Disodium hydrogen phosphate and trisodium phosphate significantly improve dough properties but not bread quality confirming findings of Westermarck-Rosendahl *et al.*<sup>9</sup> However since these chemicals are not permitted in Indian bread, further studies were not carried out. Lecithin only marginally improved dough and bread quality (Table 4). Cysteine-HCl considerably improved both dough and bread properties probably because it reduces the dough ripening period from 165 to 60 min thus minimising the adverse effect of excessive alpha amylase activity.

*Improved formulation and processing conditions for bread making:* Our studies as discussed above, suggest suitable conditions to overcome the deleterious effect of field sprouted wheat on dough and bread quality (Fig 4 and Table 5). Use of 2 per cent salt and 0.5 per cent GMS considerably improves both dough and bread characteristics; inclusion of SSL, enzyme active soy flour or lecithin, and reducing the dough temperature further improves these characteristics. Cysteine-HCl (30 ppm) along with 2 per cent salt and 0.5 per cent GMS

TABLE 3. EFFECT OF LEVEL OF INGREDIENTS ON THE CHARACTERISTICS OF DOUGH AND BREAD

Name	Ingredients Quantity added (%)	Dough stickiness		Dough machinability	Loaf vol. (cc)	Crust	Crumb
		While mixing	After fermentation <sup>b</sup>				
Sugar	1	+	+++	Poor	500	Fair	Fair
	2.5 <sup>a</sup>	+	+++	Poor	515	Fair	Fair
	4	+	+++	Poor	525	Satisfactory	Satisfactory
Fat	1	+	+++	Poor	520	Fair	Fair
	2 <sup>a</sup>	+	+++	Poor	535	Fair	Fair
	3	*	++	Poor	540	Fair	Good
Salt	1 <sup>a</sup>	+	++	Poor	510	Fair	Fair
	2	*	*	Fair	460	Satisfactory	Good
	3	**	**	Good	430	Fair	Good
NFDM	3	**	**	Good	500	Good	Fair
Water	56	**	**	Good	470	Poor	Poor
	58	**	*	Good	490	Fair	Fair
	60	*	+	Fair	500	Fair	Fair
	62.2 <sup>a</sup> (FWA)	+	+++	Poor	505	Fair	Fair

+ Slightly sticky  
 ++ Sticky  
 +++ Highly sticky

\* Normal compared to dough based on flour from sound wheat

\*\* Better than dough based on flour from sound wheat

FWA Farinograph water absorption

NFDM Non fat dry milk

<sup>a</sup> Levels of different ingredients used in control bread for different trials (Tables 3-5)

<sup>b</sup> Consisting of 165 min primary and 25 min secondary fermentation.

TABLE 4. EFFECT OF PROCESSING CONDITIONS OR ADDITIVES ON THE CHARACTERISTICS OF DOUGH AND BREAD

	Dough stickiness		Dough machinability	Loaf vol. (cc)	Crust	Crumb
	While mixing	After fermentation				
Control <sup>a</sup>	+	+++	Poor	510	Fair	Fair
<b>Processing conditions</b>						
Primary fermentation time (min)						
120	+	++	Poor	505	Fair	Fair
90	+	+	Poor	495	Fair	Fair
60	+	*	Fair	480	Fair	Fair
30	+	*	Fair	470	Fair	Fair
Initial dough temp (°C)						
17 <sup>b</sup>	**	**	Good	560	Excellent	Excellent
<b>Additives</b>						
Enzyme active soy flour 0.5%	*	*	Fair	510	Fair	Fair
GMS 0.5%	**	**	Good	525	Good	Good
SSL 0.5%	**	*	Good	525	Satisfactory	Good
Lecithin 0.5%	*	+	Fair	510	Fair	Good
Cysteine HCl <sup>c</sup> 0.003%	+	*	Good	530	Good	Good
Disodium hydrogen phosphate 0.9%	**	**	Good	510	Satisfactory	Satisfactory
Trisodium phosphate 0.3%	**	**	Good	500	Fair	Fair

+ Slightly sticky

\* Normal compared to dough based on flour from sound wheat

++ Sticky

\*\* Better than the dough based on flour from sound wheat

+++ Highly sticky

<sup>a</sup> Primary fermentation period and initial dough temperature were 165 min and 30°C respectively.

<sup>b</sup> Proof time required to get a constant height of 9.5 cm was 70 min.

<sup>c</sup> Primary fermentation period was 60 min.

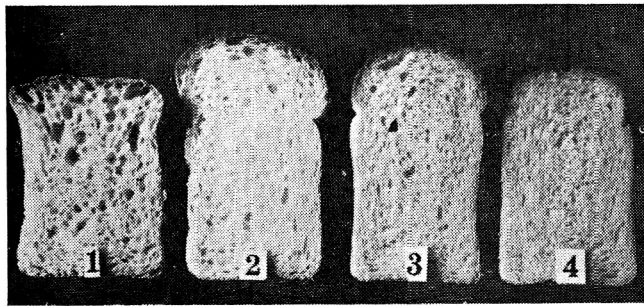


Fig 2. Effect of different levels of salt on bread quality.  
1—0%, 2—1%, 3—2%, 4—3%.

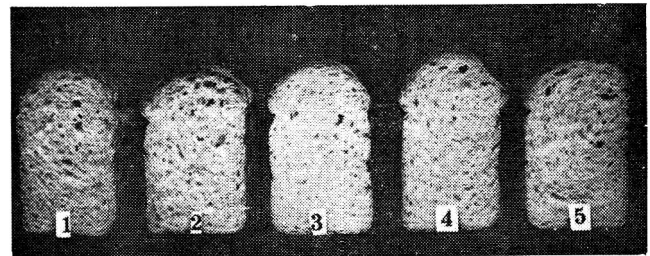


Fig 3b. Effect of additives on bread quality.

1—Control, 2—0.5% Enzyme active soy flour, 3—0.5% GMS, 4—0.5% SSL, 5—0.9%  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ .

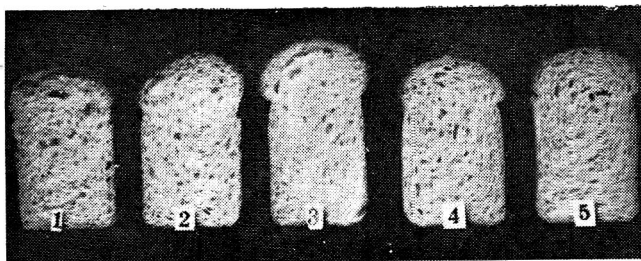


Fig 3a. Effect of various ingredients, cysteine-HCl and processing conditions on bread quality.

1—Control, 2—2% salt, 3—IDT initial dough temperature: 17°C, 4—Primary fermentation period: 60 min, 5—0.003% cysteine HCl.

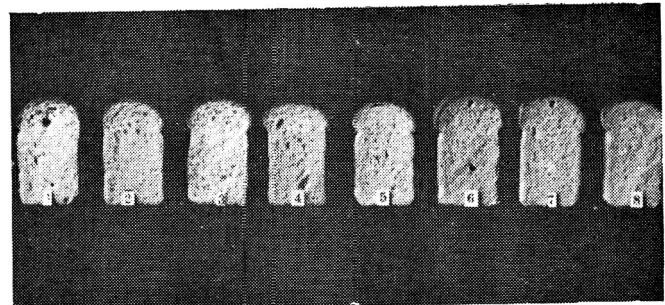


Fig 4. Desired levels of ingredients, additives and processing conditions for improving bread quality from field sprouted wheat.

1—Control; 2—2% salt; 3—IDT 17°C; 4—2% salt; IDT: 17°C; 5—2% Salt+0.5% GMS; IDT: 19°C; 6—2% salt+0.5% GMS+0.5% SSL; IDT: 19°C; 7—2% Salt+0.5% GMS+0.5% lecithin; IDT: 19°C; 8—2% salt+0.5% GMS+0.003% cysteine HCl; IDT: 19°C.

TABLE 5. DESIRED RECIPE AND PROCESSING CONDITIONS FOR IMPROVING THE BREAD MAKING QUALITY OF FIELD SPROUTED WHEAT

Name	Additives Quantity added	Dough temp.	Dough stickiness		Dough machinability	Loaf vol (cc)	Crust	Crumb
			While mixing	After fermentation				
Control	—	Normal	+	+++	Poor	510	Fair	Fair
	—	17°C	**	*	Good	495	Good	Good
GMS	0.5%	Normal	**	**	Good	535	Good	Good
GMS	0.5%	17°C	**	**	Excellent	520	Good	Good
GMS	0.5%+	Normal	**	**	Good	540	Excellent	Excellent
SSL	0.5%							
GMS	0.5%+	19°C	**	**	Excellent	560	Excellent	Excellent
SSL	0.5%							
Soy	0.5%+	19°C	**	*	Good	550	Good	Good
GMS	0.5%							
GMS	0.5%+	19°C	**	**	Good	550	Good	Excellent
Lecithin	0.5%							
GMS	0.5%+	19°C	**	**	Excellent	530	Good	Excellent
Cysteine HCl	0.003%							
GMS	0.5%+	Normal	**	**	Excellent	555	Excellent	Excellent
Cysteine-HCl	0.003%							

+ Slightly sticky; ++ Sticky; +++ Highly sticky; \* Normal compared to dough based on flour from sound wheat; \*\* Better than dough based on flour from sound wheat.

Salt was added at 2% level to all treatments except control.



yielded, even at room temperature, dough having excellent machinability and bread having a high rating.

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## Effect of Heat Developed During Grinding Wheat in a Disc Mill on Some Chemical, Rheological and Chapati Making Characteristics of Flour

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*Manuscript received 13 December 1984; revised 26 June 1985*

The studies on the effect of heat developed during grinding of wheat into whole wheat flour (*atta*) in a disc mill (*chakki*) and subsequent cooling rates, showed a decrease from 0.99 to 0.73% in bound lipids, 4.9 to 4.2% in glutenin fraction and 30.5 to 27.1 mg/100g in protease activity. The standard plate count decreased considerably from 15,300 to 3,900/g. Though the rheological characteristics were not affected significantly the chapati making quality was altered considerably. The heat developed during grinding improved the quality of chapati, particularly its flavour.

In the Indian sub-continent, whole wheat flour (*atta*) used for chapati making is generally milled in disc mills known as *chakki*. Often due to the frictional grinding action, the *atta* attains high temperatures of upto 95°C by the time it comes out of the mill. In households, sometimes, if such hot *atta* is filled into air tight containers without cooling, there is every likelihood that the quality characteristics of *atta* may be affected depending on the temperature as well as time required for cooling of the *atta* to room temperature.

Against this background, the effect of heat generated during grinding on the chemical, rheological and chapati making quality characteristics of *atta* was assessed and the results are presented in this paper.

### Materials and Methods

*Wheat*: Medium hard Punjab wheat procured from the local market was used.

*Whole wheat flour (atta) cooled at different rates*: About 10 kg of wheat sample was ground in a disc mill

(*chakki*). The temperature of *atta* at the delivery end of the mill was recorded. Half of the *atta* taken in an open tray was cooled immediately to room temperature using air circulation. The temperature of *atta* sample was recorded every 10 min. The remaining portion of hot *atta* was transferred immediately into a tin. After closing the lid this *atta* was allowed to cool by itself. Temperature of this *atta* was read by inserting the thermometer in a hole made at the centre along the height of the tin. The control sample was ground in a pestle and mortar with negligible heat development, to almost the same particle size as that of disc milled flour as indicated by sieve test.

**Chemical characteristics:** Moisture, diastatic activity damaged starch and free lipids were determined in flours according to standard AACC methods<sup>1</sup>. Protein fractions like salt soluble proteins, gliadin and glutenin were determined by the methods described by Kent Jones and Amos<sup>2</sup>. Bound lipids were estimated by extracting the sample with chloroform methanol mixture (2:1 V/V) after extracting the free lipids with petroleum ether (40-60°C)<sup>3</sup>. Lipase was determined according to the procedure of Kantharaj Urs *et al*<sup>4</sup>. and proteolytic activity by the method of Lusena and McFarlane<sup>5</sup>. Statistical analysis of the data was done according to Duncans new multiple range test<sup>6</sup>.

**Standard plate count:** The standard plate count of *atta* was determined using ISI method<sup>7</sup>.

**Rheological characteristics:** Farinograph and extensograph characteristics of *atta* were determined at the optimum chapati dough consistency using the method described by Haridas Rao *et al*<sup>8</sup>. The fraingrams and extensograms were evaluated according to standard procedures<sup>1</sup>.

**Chapati making quality:** Chapati was made according to the method standardized earlier<sup>8</sup>. For the preparation of the dough, 200g flour was mixed for 3 min in a Hobart Mixer (N-50), with water equivalent to chapati water absorption measured on Research Water Absorption Meter. About 40 g of dough was sheeted to thickness of 2 mm on a specially designed aluminium platform and cut into a circular shape of 15 cm diameter using a metallic cutter. Chapati was then baked on a hot plate maintained at 400°F for 45 sec on one side and 105 sec on the reverse side and puffed for 25 sec in a gas *tandoor* maintained at 700°F. The puffed chapati was then cooled before evaluation.

**Sensory evaluation of chapati:** Six semitrained panelists evaluated the chapati for its appearance (colour of the spots and their uniformity), hand feel (rough, smooth, pliable) eating quality (sweetish, wheaty, bland) according to the method of Shurpalekar and Prabhavati<sup>9</sup>.

The texture of chapati was evaluated objectively using Warner Bratzler shear press. The chapati was folded,

so as to have 4 layers and placed in the centre of a conical blade provided on the instrument. The maximum force (in pounds), as indicated on the dial for shearing the chapati was recorded.

The pliability of chapati was determined using pliability tester<sup>8</sup>. The puffed height of baked chapati was determined using the scale.

## Results and Discussion

**Cooling rate of whole wheat flour:** The rate of cooling of *atta* in an open tray and in a closed tin is given in Fig 1. The temperature of *atta*, when it came out of the delivery end of disc mill was 92°C. However, during the collection process in a tray, the temperature dropped to 72°C. The *atta* kept in an open tray reached the room temperature of 30°C within an hour while the one kept in a closed tin took a little over 9 hours.

**Changes in the quality characteristics:** Data on the chemical characteristics of different *atta* samples are given in Table 1.

**Moisture:** Moisture content decreased from 10.1 per cent in wheat to 6.8 per cent in *atta* due to frictional heat developed during grinding. Little effect was observed on the moisture, when *atta* was cooled either immediately in trays or slowly in tins. However, no loss of moisture was found in the sample ground in a pestle and mortar, where the heat development was negligible.

**Lipids:** A significant increase in free lipids and decrease in bound lipids was observed as a result of heat development during grinding of wheat. A similar observation of decrease in the bound lipids has been

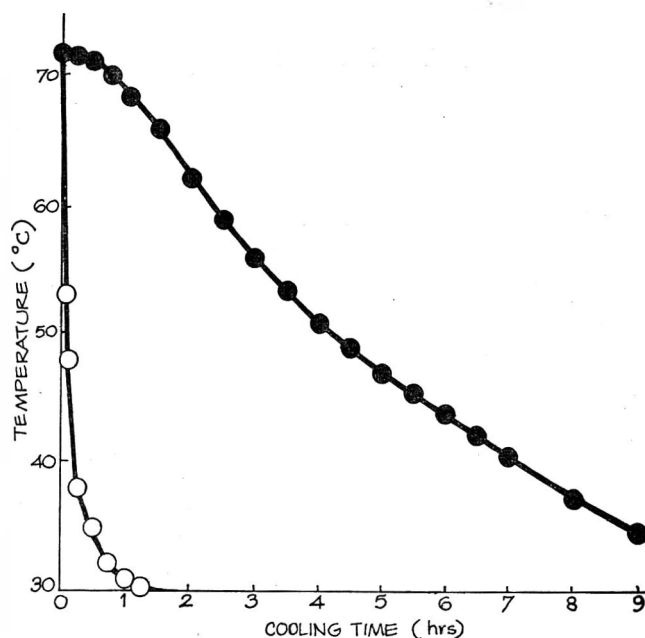


Fig 1. RATE OF COOLING OF FRESHLY GROUND WHOLE WHEAT FLOUR KEPT IN OPEN TRAY (○) AND CLOSED TIN CONTAINER (●).

TABLE 1. EFFECT OF HEAT DEVELOPED DURING GRINDING AND SUBSEQUENT COOLING ON SOME CHARACTERISTICS<sup>1</sup> OF WHOLE WHEAT FLOUR

Whole wheat flour	Moisture (%)	Lipids		Proteins (N×5.7)				Enzyme activity			Damaged starch (%)	Total plate counts (×10 <sup>3</sup> /g)
		Free (%)	Bound (%)	Total (%)	Salt soluble (%)	Gliadin (%)	Glutenin (%)	Diastase (mg/10g)	Lipase (mlNaoH)	Protease (mg/100g)		
Control	10.2	1.40 <sup>a</sup>	0.99 <sup>a</sup>	11.6 <sup>a</sup>	2.5 <sup>a</sup>	4.2 <sup>a</sup>	4.9 <sup>a</sup>	196 <sup>a</sup>	4.62 <sup>a</sup>	30.5 <sup>a</sup>	4.5 <sup>a</sup>	15.3
Cooled immediately	6.8	1.74 <sup>b</sup>	0.73 <sup>b</sup>	11.5 <sup>ab</sup>	2.8 <sup>b</sup>	4.5 <sup>b</sup>	4.2 <sup>b</sup>	373 <sup>b</sup>	4.54 <sup>a</sup>	27.1 <sup>b</sup>	12.1 <sup>b</sup>	3.9
Cooled slowly	7.2	1.74 <sup>b</sup>	0.72	11.4 <sup>a</sup>	2.7 <sup>ab</sup>	4.5 <sup>b</sup>	4.2 <sup>b</sup>	367 <sup>b</sup>	4.65 <sup>a</sup>	27.0 <sup>b</sup>	12.1 <sup>b</sup>	1.5
SE <sub>m</sub> (9 df)	—	±0.08	±0.08	±0.04	±0.08	±0.06	±0.08	±2.7	±0.04	±0.32	±0.06	—

<sup>1</sup> Values expressed on 14% moisture basis

<sup>2</sup> Means of the same column followed by different superscripts differ significantly (P<0.05). Percentages were transferred to arc sine before carrying out the statistical analysis.

reported during roasting of grains<sup>10</sup> and the same was attributed to the breakage of hydrogen bonds between lipids and the other non-lipid materials present in bound lipids. However, no difference in free or bound lipids was observed in *atta* samples cooled differently.

**Protein fractions:** Total proteins in different *atta* samples remained the same while the amount of different fractions varied significantly. Salt soluble proteins as well as gliadin increased slightly by 0.3 per cent as a result of heat development. On the other hand, high molecular weight glutenin fractions decreased from 4.9 to 4.2 per cent as a result of heat generated. This was probably due to partial disaggregation of glutenin due to heat, resulting in an increase in both salt soluble and gliadin protein fractions. Similar observations of disaggregation of high molecular weight glutenin into low molecular weight proteins was reported by Jean

*et al*<sup>11</sup>. Cooling the ground flour at different rates however did not affect the protein fractions.

**Enzyme activity:** Diastatic activity increased considerably as a result of grinding in disc mill. Diastatic activity of control sample ground with minimum heat development was 196 mg/10g flour and that of flour milled in disc mill was 373 mg. The increased diastatic activity may be due to extensive starch damage taking place as a result of the severe grinding action in disc mills<sup>12</sup>. However, the diastatic activity of the *atta* was not affected by different rates of cooling as both *atta* have similar values of about 370 mg.

Lipase enzyme was found to be quite stable to heat produced during grinding. However, proteolytic activity was found to be affected by the heat generated during grinding, as about 10 per cent loss in the activity was observed. Cooling rate, however had no effect on any

TABLE 2. EFFECT OF HEAT DEVELOPED DURING GRINDING AND SUBSEQUENT COOLING ON THE QUALITY OF CHAPATI

Whole wheat flour	Height of puffed chapati (cm)	Pliability (cm)	WB shear value lb (lb)	Appearance	Handfeel	Texture	Flavour	Overall quality
Control	6.0 <sup>a</sup>	1.8 <sup>a</sup>	8.2 <sup>a</sup>	Dull grey spots, non-uniform	Slightly rough less pliable	Slightly tough	Bland	Satisfactory
Cooled immediately	7.1 <sup>b</sup>	2.2 <sup>b</sup>	5.8 <sup>b</sup>	Light brown spots, uniform	Smooth, pliable	Soft	Wholesome wheaty	Good
Cooled slowly	7.2 <sup>b</sup>	2.1 <sup>b</sup>	6.0 <sup>b</sup>	„	Smooth, pliable	Soft	„	Good
SE <sub>m</sub> (9 df)	±0.08	±0.05	±0.10					

<sup>1</sup> Means of the same column followed by different superscripts differ significantly (P<0.05) according to Duncan's New Multiple Range Test.

of the enzymes studied. It can be inferred that though the temperature attained during grinding was as high as 92°C its effect on the enzymes was negligible possibly due to the low moisture content of materials.

**Standard plate count:** Total bacterial load in control *atta* sample was quite high ( $16.3 \times 10^3$  per g) and it decreased by 76.1 per cent, when wheat was ground in the disc mill. The destruction was as high as 90.8 per cent when the *atta* was cooled slowly.

**Rheological characteristics:** Very little difference was observed in both the farinograph and extensograph characteristics of flours, except for the lower farinograph water absorption of the control samples. The water absorption of control sample was found to be 65 per cent as compared to 76 per cent observed for *atta* ground in a disc mill. The higher water absorption of disc milled flour could be attributed to its higher damaged starch content<sup>12</sup>. The cooling rate however did not affect the different farinograph and extensograph characteristics.

**Chapati making quality:** The quality of chapatis made from different *atta* samples is given in Table 2. The *atta* obtained from disc mill gave a chapati with a puffed height of 7.1 cm as compared to 6.0 cm for control sample. The higher puffed height of test samples was due to the higher water absorption, which helps in generating sufficient steam for puffing the chapati<sup>8</sup>. Also, the chapati made from disc milled *atta* was quite pliable, as indicated by the higher pliability value and it was quite soft as shown by the low shear values.

The chapatis made from disc milled flour had the desired light brown spots as compared to the greyish spots observed in chapatis made from the control flour. The dark coloured spots of chapati were due to the higher diastatic activity, which helps in formation of more sugars during mixing and subsequent operations, thus contributing to increased browning reaction. These chapatis were also soft, unlike control which were slightly tough. The chapatis made from disc milled *atta* also had a better and sweetish taste and wholesome flavour, as compared to that of control. The quality of chapati however was similar irrespective of whether the *atta* was cooled immediately or gradually after grinding.

It is concluded that the heat produced during grinding of wheat into *atta* in a discmill: (1) had a significant effect on lipids, protein fractions and diastatic and proteolytic activities, (2) lowered the total microbial load, and (3) improved the quality of chapati particularly with respect to flavour. However rate of cooling of hot *atta* had no effect on any one of the above characteristics.

#### Acknowledgement

The authors thank Mr. C. T. Dwarakanath and his group, Microbiology, Fermentation and Sanitation Discipline for the help in microbiological analysis and Mr. B. S. Ramesh of Industrial Development and Consultancy Services for the help in statistical analysis.

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# Studies on Effect of Evacuation of Paddy on Quality Characteristics of Parboiled Rice\*

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*Manuscript received 21 February 1985; revised 28 June 1985*

Paddy samples were evacuated for removal of intergranular air and then soaked in water at different temperatures and durations. The quality of rice obtained was evaluated and compared with the rice obtained from non evacuated samples soaked at the same temperature and duration. Light coloured parboiled rice with higher head yield and reduced process time (by about 1.5 hr) could be obtained by subjecting paddy to vacuum prior to soaking.

Rice yield from paddy depends not only on the type of processing machines but also on various premilling factors that contribute substantially towards the poor milling yields such as the variety, delayed harvesting and threshing, rapid and nonuniform drying, insect infestation, improper moisture content, etc.

Conventionally parboiling consists of soaking, steaming and drying. However, RPEC method<sup>1</sup> consists only of soaking at 70 to 75°C for 3 to 5 hr and drying eliminating the steaming process completely. It has been reported that parboiled rice produced by this method is of good quality<sup>1</sup>. Deeper colour and relatively harder product are not normally acceptable to most of the consumers. Hence this study was undertaken with a view to determine the effect of evacuation of paddy, prior to hydration at different soaking temperatures and durations, on the quality of parboiled rice produced by RPEC method.

## Materials and Methods

Paddy variety 'Jaya' was soaked directly or after evacuation. In one experiment 150g of paddy (13.27 per cent moisture, dry basis) were mixed with 225 ml hot water ( $t_w$ ), placed in a beaker and soaked in a pre-stabilized water bath at desired temperature ( $t_s$ ), (Equation 1).

$$t_w = t_s + \frac{M_p S_p (t_s - t_p)}{M_w S_w} \quad \dots(1)$$

Where,

$M_p$  = Mass of paddy, kg.

$S_p$  = Specific heat of paddy, Kcal/kg. °C.

$S_w$  = Specific heat of water, Kcal/kg. °C.

$t_p$  = temperature of paddy at the time of soaking, °C.

$t_s$  = temperature of soaking (paddy water mixture), °C.

$t_w$  = temperature of hot water, °C.

In another trial paddy was subjected to absolute vacuum (-76 cm of Hg as indicated by the compound gauge) for 5 min before soaking. Hot water at pre-determined temperature ( $t_w$ ) was released into the flask. After thorough mixing of paddy and water, the vacuum was released and temperature of mixture noted and this was found to be within 1°C of the desired values. The samples were soaked, by keeping in a water bath, at room temperature 30, 40, 50, 60, 70, and 80°C for 15, 30, 45 min and 1, 2, 3, 4, 5 and 6 hr (three replicates). After soaking the moisture content was determined<sup>3</sup> and samples were shade dried to 14 per cent w.b. moisture content and milled (6 per cent polish)<sup>4</sup>.

Colour of the polished rice was measured using different tristimulus filters (amber, blue, green) in a reflection meter<sup>5</sup> (Model 670) and expressed as yellowness index (Y.I.) calculated as follows:

$$Y.I. = \frac{\text{Yellowness of treated rice samples}}{\text{Yellowness of raw rice sample}} \quad \dots(2)$$

Where,

$$\text{Yellowness} = \frac{\text{Amber-Blue}}{\text{Green}} \quad \dots(3)$$

Hardness (breaking strength under compression) of polished rice was measured using Kiya Hardness Tester<sup>2</sup> and chalky kernels were separated using chalky grain

\*Paper (no. 84-0419) presented at XXI, ISAE Convention held at IARI, New Delhi during 5-7 April, 1984.

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detector<sup>1</sup> (wooden box fitted with an electric bulb, a reflector and a glass observation surface 10 cm dia).

### Results and Discussion

**Milling quality:** Even from the varieties that suffer heavy breakage in raw condition, high head yield can be obtained after parboiling<sup>6</sup>. However, parboiled rice requires 3-4 times more abrasive force than raw rice for same percentage of polish<sup>7</sup>. Also, due to the oiliness of the surface of rice, bran sticks to parboiled milled rice and this gives a bad appearance<sup>8</sup>. In spite of this the paddy is parboiled for increasing head yield outturn and nutritive value of rice<sup>8,9</sup> and attempts were made to improve the rice quality.

When paddy was milled as raw, head yield of 'Jaya' rice was 60.8 per cent, it was lower for evacuated samples (Table 1) than for ordinarily soaked samples below the gelatinisation temperature (gelatinisation temperature was reported<sup>10</sup> in the range of 62-69°C for paddy). This may be due to early changes in internal structure, development of cracks, and early loosening of starch binding due to evacuation. At higher soaking temperatures (above 60°C) considerable increase in head yield was observed in both the cases and normally more for evacuated samples (Table 1). Head yield of nearly 71 per cent was achieved on soaking at 70°C for 5 hr directly or for 3.5 to 4 hr in the case of paddy subjected to vacuum before soaking thus indicating the definite improvement in milling quality and saving of nearly 1.5 hr process time. However, this was not true for 80°C where head yield decreased after 2 hr soaking and this may be due to the bursting of paddy grains. This observation is in line with the reported decrease of 2 per cent

in head yield due to bursting<sup>7</sup>. It therefore indicates that a critical level of temperature for parboiling of 'Jaya' paddy by RPEC method ranges between 70 and 80°C and needs further studies with reduced interval(s) of time temperature combinations.

With this in view, all the quality characters are reported, for samples giving head yield equal to or more than raw rice and mainly compared for higher temperatures for raw and treated samples.

**Hardness:** It increased with increase in soaking temperature (Table 2). While analysing the hardness data pertaining to a sample (20 readings) for standard deviation, it ranged from 0.1 to 0.6 kg from mean. Such a high variation in hardness of a sample treated under same condition may be due to crack formation, insect infestation or presence of white belly kernels. Variation in the hardness values under given conditions was not much probably because the quantum of heat treatment received by grains was same in both the cases. The perusal of data (Table 2) for same head yield and temperature level indicates considerable difference in hardness; and low values for evacuated samples clarify the effect of reduced heat treatment.

It is well known that parboiled rice is harder than raw rice, and requires longer time to cook<sup>11</sup>. It has been also established that harder the rice more the cooking time<sup>11</sup>. Thus an attempt viz. evacuation, etc. to get parboiled rice with low hardness would result in saving of energy required in cooking of parboiled rice.

**Rice colour:** Various reasons are ascribed for the deep colour in parboiled rice. viz. pigments of bran<sup>8</sup>, formation of Maillard reactions products<sup>7</sup>, diffusion of husk pigment into endosperm<sup>7</sup>, temperature of soaking<sup>12</sup>,

TABLE 1. VALUES OF HEAD YIELD (%) AT DIFFERENT SOAKING CONDITIONS

Soaking time (hr)	Head yield at indicated soaking temperatures(°C)							
	50		60		70		80	
	Vacuum	Ordinary	Vacuum	Ordinary	Vacuum	Ordinary	Vacuum	Ordinary
¼	38.67	45.67	37.84	48.33	53.50	55.33	46.33	45.00
½	38.50	48.33	39.50	54.00	58.17	57.00	54.50	55.17
¾	36.83	51.67	38.67	54.50	58.83	58.00	60.67	61.67
1	40.00	52.00	54.33	55.67	65.67	60.33	65.00	62.67
2	47.00	55.16	55.17	60.17	68.33	66.17	68.33	68.33
3	57.50	58.34	64.17	65.50	70.17	69.00	62.33	67.00
4	57.67	61.50	64.50	66.33	70.67	68.67	60.83	59.34
5	60.17	65.33	64.83	67.67	69.67	70.84	59.92	50.50
6	61.00	67.67	65.33	64.67	69.34	69.83	51.31	51.50

Total yield: 75%, head yield of raw 'Jaya' paddy: 60.8%  
The figures in block indicate the higher head yield than raw samples.

TABLE 2. HARDNESS VALUES (KG) OF POLISHED RICE OBTAINED AT DIFFERENT CONDITIONS (AVERAGE OF 20 REPLICATES)

Soaking time (hr)	Hardness at indicated soaking temperatures (°C)					
	60		70		80	
	Vacuum	Ordinary	Vacuum	Ordinary	Vacuum	Ordinary
$\frac{3}{4}$	1.376	1.855	1.553	1.960	1.970	2.065
1	2.020	1.285	1.827	2.380	2.800	2.356
2	1.790	1.820	1.980	2.225	3.520	3.700
3	1.856	2.080	2.430	2.595	3.977	4.280
4	2.046	2.000	2.610	2.600	4.790	4.135
5	1.720	2.240	3.710	2.905	3.920	4.990
6	2.023	2.100	3.183	2.985	4.210	5.015

Hardness of raw rice 0.477 kg

The figures in block represent those with higher head yield than raw samples.

TABLE 3. YELLOWNESS INDEX OF POLISHED RICE

Soaking time (hr)	Yellowness at indicated soaking temperatures (°C)					
	60		70		80	
	Vacuum	Ordinary	Vacuum	Ordinary	Vacuum	Ordinary
$\frac{3}{4}$	1.38	1.25	1.40	1.32	1.37	1.40
1	1.40	1.20	1.37	1.47	1.42	1.36
2	1.36	1.30	1.45	1.46	1.53	1.65
3	1.41	1.38	1.50	1.55	1.53	1.71
4	1.45	1.34	1.46	1.50	1.63	1.71
5	1.29	1.23	1.56	1.54	1.78	1.77
6	1.42	1.40	1.46	1.48	1.76	1.80

Yellowness index of raw rice: 1.

The figures in block indicates, those with higher head yield than raw samples

TABLE 4. CHALKY KERNELS IN POLISHED RICE AT DIFFERENT CONDITIONS

Soaking time (hr)	Chalky kernels at indicated soaking temperatures (°C)					
	60		70		80	
	Vacuum	Ordinary	Vacuum	Ordinary	Vacuum	Ordinary
$\frac{3}{4}$	79.0	79.5	77.5	80.0	81.0	80.5
1	76.5	77.5	76.0	79.0	73.5	74.5
2	75.5	75.5	60.5	67.5	47.5	41.5
3	76.5	72.5	56.5	61.0	30.0	37.0
4	76.0	70.5	56.0	60.0	6.0	17.0
5	73.0	69.0	53.5	57.0	0.0	4.5
6	63.5	68.5	33.5	41.0	0.0	0.0

In raw state: 88%

The figures inside block represent those with the higher head yield than raw samples.

pH of soak water<sup>9</sup> and processing conditions during parboiling<sup>3</sup>. In this study, the yellowness index (Y.I.) was less for vacuum applied samples than ordinarily soaked samples and it increased with increase in soaking time and temperature (Table 3). This indicates the desirability of application of vacuum to paddy not only to reduce soaking time<sup>3</sup> but also to get light coloured parboiled rice. However, in either case, low temperature soaking did not affect colour, may be due to opaqueness.

**Chalky kernels:** 'Jaya' rice under raw state contains 88 to 90 per cent chalky kernels and the number decreased with increase in soaking temperature and duration. The rate of decrease was more in samples subjected to vacuum than ordinary soaking (Table 4). Kernels without chalkiness were obtained at 80°C when evacuated samples were soaked for 5 hr whereas 6 hr were needed for samples soaked directly. Comparison of corresponding head yield values (Table 1) indicate very low levels (much less than raw rice) and undesirability. However, in case of high head yield values obtained for parboiling by RPEC method for evacuated and ordinary soaked paddy i.e. about 70 per cent at 70°C the respective chalky kernels were 33.5 and 41.0 per cent (Table 1 and 4) This observation confirms the results reported by Patil *et al.*<sup>11</sup> indicating the chalky kernels present in the rice obtained by pressure parboiling, CFTRI method and double steaming method were 11, 14.33 and 34.33 per cent respectively.

#### Acknowledgement

The help of Shri Anilkumar D. Kulkarni is highly acknowledged.

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## Changes in Phytate Phosphorus and Minerals During Germination and Cooking of Moth Bean (*Phaseolus aconitifolius* Jacq) Seeds

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*Manuscript received 5 July 1984; revised 1 April 1985*

Phytate phosphorus, phosphorus, calcium, magnesium, iron and potassium were determined in moth bean (*Phaseolus aconitifolius* Jacq) seeds. Phytate phosphorus (P) comprised 71.1% of the total P. Forty one per cent of phytate P had disappeared on the 5th day of germination with a simultaneous increase in available inorganic P. After cooking for 45 min, with the beans-to-water ratio of 1:4 whole seeds and cotyledons did not show any breakdown in the phytate P or losses of minerals after initial leaching.

Grains and seeds and processed food products derived from them contain an appreciable amount of phytate.

Phytate is a complex salt of calcium and magnesium with myoinositol and is the principal storage form of phos-



phorus in many seeds. In most legumes, phytate phosphorus accounts for about 80 per cent of the total phosphorus<sup>1</sup> and is primarily present as a complex salt of minerals<sup>2</sup> or complexed with proteins<sup>3</sup>. The phytates form complexes with multivalent cations<sup>4,5</sup> such as  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{Fe}^{++}/\text{Fe}^{+++}$  which decreases their bioavailability. Moreover, phytates interact with proteins and form insoluble complexes<sup>6</sup> many of which are unavailable to the human system under normal physiological conditions.

Moth bean (*Phaseolus aconitifolius* Jacq) seeds were selected for studying the phytate and mineral interactions in view of the scarce data on this legume. Present investigation reports the content of total P, phytate P, nonphytate P, calcium, magnesium, total iron and potassium in moth bean seeds after germination and cooking.

### Materials and Methods

**Germination:** Seeds of moth bean were procured from the local market at Parbhani. Forty grams of seeds of uniform size were weighed and kept in dry glass petri plates. On the first day, about 40 ml distilled water was added and an additional 25 ml after 12 hr interval. The petri plates were incubated at 21°C (25°C ambient temperature). Samples were removed every 24 hr until the 5th day of germination and were sun-dried. The minimum and maximum temperature during sun-drying were 21 and 27°C, respectively. Dry weights of seedlings from each set of germination were noted. Samples were powdered to 60 mesh size and stored at 4°C for analysis.

**Cooking:** Moth bean seeds and cotyledons obtained after removal of testa were used for cooking. The samples were cooked as follows: 100 g beans were placed in beakers in 400 g water and covered with aluminium foil. The samples were autoclaved for 5, 10, 15, 20, 25, 30, 35, 40 and 45 min at a steam pressure of 10 psi and at 116°C. The cooking water was discarded and bean samples were sun-dried immediately as before. The sun-dried samples were powdered to 60 mesh in a waring blender and stored at 4°C for analysis.

**Preparation of samples:** Samples for mineral analysis

were prepared according to Singh and Reddy<sup>7</sup>.

**Dry weight determination:** Samples were dried at 115°C to constant weight in a vacuum oven.

**Determination of minerals:** Ashing procedure for the determination of minerals was based on the AACC<sup>8</sup> method. Calcium, magnesium, total iron and potassium were determined as per AOAC<sup>9</sup> methods.

**Determination of phosphorus:** Total phosphorus was determined by the colorimetric method of Fiske and Subba Row<sup>10</sup>. Inorganic phosphorus was estimated by the method of Pons and Guthrie<sup>11</sup>. Phytate phosphorus was determined as per the method of Widdowson<sup>12</sup> as described by Chauhan<sup>13</sup>.

### Results and Discussion

It is known that during the initial stages of germination seeds utilize stored substances to provide substrates and energy for growth processes. A net loss of dry weight occurs as a result of oxidation and breakdown of stored compounds<sup>14</sup>. The changes in dry weight and ash contents of moth bean during a 120 hr germination period, are shown in Table 1. At the end of this period, the dry weight was reduced to about 87 per cent which is, similar to observations on oats<sup>15</sup>, lettuce<sup>16</sup> and black gram seeds<sup>17</sup>. The total ash content increased gradually throughout the germination period.

TABLE 1. EFFECT OF GERMINATION ON DRY WEIGHT AND ASH CONTENT IN MOTH BEAN<sup>a</sup>

Germination time (hr)	Calculated dry wt. (%)	Ash (%)
0	100.00	3.84
24	98.81	3.94
48	97.00	3.99
72	95.59	4.02
96	93.08	4.18
120	86.79	4.25

<sup>a</sup> Each value is the mean of three determinations and is expressed on dry weight basis.

TABLE 2. CHANGES IN PHOSPHORUS FRACTIONS OF MOTH BEAN SEEDLING DURING GERMINATION<sup>a</sup>

Germination time (hr)	Total P (mg/g)	Phytate P (mg/g)	Phytate P as total P (%)	Nonphytate P (mg/g)	Nonphytate P as total P (%)	Inorganic P (mg/g)
0	4.5	3.2	71.1	1.3	28.8	0.49
24	3.6	2.5	69.4	1.1	30.5	0.42
48	3.1	2.1	67.7	1.0	32.2	0.50
72	2.8	1.8	64.2	1.0	35.7	0.62
96	4.0	1.5	37.5	2.5	62.5	1.10
120	4.1	1.2	29.2	2.9	70.7	1.80

<sup>a</sup> Each value is the mean of three determinations and expressed on dry weight basis.

It is observed from Table 2 that moth bean, is a good source of phosphorus, as it contains 71.1 per cent of total P. It was reported that phytate P represents 53.6 per cent of total P in beans (*Phaseolus vulgaris* L.) and 78.6 per cent in black gram (*Phaseolus mungo*)<sup>17</sup>.

Sobolev<sup>18</sup> has reported that the enzymatic hydrolysis of phytate during germination of seeds is accomplished by two phosphatases. One of these namely phytase, is responsible for the initial breakdown of phytate upto the stage of formation of inositol monophosphate, and the other enzyme completes dephosphorylation of inositol phosphates into inositol and phosphate.

In the present study the phytate P decreased gradually during initial stages of germination and reached 41.0 per cent on 5th day (Table 2), with simultaneous liberation of inorganic phosphorus. At the same time non-phytate P increased rapidly. Inorganic P levels decreased slightly during the first 24 hr germination and reached their highest on the 5th day. This suggests that phytase activity was rather low in moth bean during the first 1 or 2 days of germination.

Data on Ca, Mg, Fe and K are shown in Table 3. No significant changes were observed in their concentrations during germination. Calcium and iron contents were 200 and 14.2 mg/100g, respectively. Gopalan *et al*<sup>19</sup> had reported that moth bean contained 202 mg/100g calcium and 9.5 mg/100g of iron. The low values of potassium reported may be partly due to cultivar differences.

Beans absorb water during cooking. The observations presented in Table 4 were made on cooked beans and on cotyledons after removal of testa. Cooking of beans and cotyledons with water in 1:4 ratio did not show significant effects on phytate breakdown but during short time cooking the losses of phytic acid P and total P were greater. These losses may be attributed to the water soluble nature of bean phytic acid.

TABLE 3. CALCIUM, MAGNESIUM, IRON AND POTASSIUM CONTENT OF MOTH BEAN SEEDLING DURING GERMINATION<sup>a</sup>

Germination time (hr)	Ca (mg/g)	Mg (mg/g)	Fe (mg/g)	K (mg/g)
0	2.00	2.42	0.142	0.71
24	1.88	2.35	0.161	0.64
48	1.76	2.40	0.172	0.61
72	1.87	2.54	0.129	0.61
96	1.95	2.48	0.152	0.59
120	1.95	2.54	0.152	0.63

<sup>a</sup> Each value is the mean of three determinations and is expressed on dry weight basis.

TABLE 4. CHANGES IN TOTAL P AND PHYTATE P IN MOTH BEAN DURING COOKING<sup>a</sup>

Cooking time (min)	Total P (mg/g)		Phytate P (mg/g)	
	Beans	Cotyledons	Beans	Cotyledons
0	4.50	4.55	3.25	4.00
5	4.10	4.00	2.70	3.50
10	3.10	3.80	2.60	3.20
15	3.90	3.60	2.60	3.15
20	3.85	3.55	2.70	3.00
25	3.80	3.45	2.75	3.10
30	3.75	3.45	2.90	3.20
35	3.75	3.55	3.00	3.40
40	3.80	3.60	2.95	3.60
45	3.75	3.75	3.10	3.80

<sup>a</sup> Each value is the mean of three determinations and is expressed on dry weight basis.

As regards minerals, cooking of beans and cotyledons with water in 1:4 ratio for 10 min resulted in a major loss due to leaching, Tables 5 and 6 cooking for 30 min did not cause much further loss after initial leaching. Increase in minerals content in beans and cotyledons when cooked for prolonged time may be by reabsorption of minerals from the cooking water.

It is concluded that in moth bean phosphorus is mainly present as phytate P, which would reduce the availability of minerals. Cooking and germination of moth bean improve the availability of certain minerals through phytate breakdown by phytase enzyme during germination and by breakdown of phytate metal complexes during cooking at relatively high temperatures. Decreased phytate P content in germinated seeds is an indication of its superiority over ungerminated seeds.

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## Effect of Parboiling on Hydration and Sedimentation Characteristics of Cassava (*Manihot esculenta* Crantz) Chips

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*Manuscript received 15 October 1984; revised 29 May 1985*

Studies were carried out with 'Malayan-4' (M-4) variety of cassava. It was found that parboiling of cassava chips affects the hydration behaviour of chips both at room temperature (RT) (28-30°C) and cooking temperature (96-98°C.) At RT equilibrium moisture content by soaking (EMC-S) was attained after 8 hr. Among the three samples studied EMC-S was the highest (61.91% wet basis) for the sample that was parboiled by dipping in boiling water for 10 min. At cooking temperature, water uptake (W) during the initial period of 5 min of soaking was low. After 20 min the W value of parboiled sample attained was higher (1.23) than plain dried sample (0.994). The sediment volume of the flour prepared from parboiled cassava chips was also higher than the corresponding one prepared from plain dried chips. The sediment volume also showed a direct relationship with EMC-S at RT.

Cassava is an important food crop of tropical countries. Unlike yam and potato tubers fresh cassava is susceptible to faster spoilage. Tubers are therefore stored as dry chips. Often fresh slices are parboiled by soaking in boiling water and subsequently dried.

Parboiling is widely employed in rice which has the advantages of modifying texture and cooking quality<sup>1</sup>. No detailed study relating to the modified properties of parboiled cassava chips has been reported. Earlier studies carried out in this laboratory<sup>2</sup> have indicated the scope of improving the pasting characteristics of cassava flour prepared from parboiled chips. In the present paper results of the study on hydration characteristics of plain and parboiled cassava chips and the sedimentation characteristics of the flour prepared therefrom are presented.

### Materials and Methods

Cassava cultivar 'Malayan-4' (M-4) was used in the present study. Fresh tubers of seven to eight months maturity were procured from local farms. These were peeled and processed as follows:

- (i) *Plain dried chips*: Fresh slices of 4.0-5.0 mm thickness were dried at  $58 \pm 2^\circ\text{C}$  for 1 hr in a cross-flow dryer. The dried chips had an average moisture content of 10 per cent.
- (ii) *Parboiled and dried chips*: Two samples (I and II) were prepared by dipping fresh slices in boiling water for 5 and 10 min respectively and the chips were dried for 8 hr as mentioned above. The dried samples had an average moisture content of 8 per cent.

*Studies on hydration characteristics:* Hydration characteristics of plain and parboiled chips were studied at: (1) RT (28-30°C), (2) four different higher temperatures of 60, 80, 92 and 96°C for an identical soaking time of 15 min and (3) cooking temperature of 96-98°C after soaking for different time intervals of 5, 10, 15 and 20 min. Studies beyond 20 min were not conducted as the material got disintegrated.

For the hydration studies at RT, 5.0g of cassava chips of uniform thickness (1-2 mm) were soaked in 50 ml of water taken in a beaker. Samples were removed at definite intervals. Surface water was removed by a filter paper (Whatman No. 41). The moisture content in the samples was determined by drying the samples at  $105 \pm 1^\circ\text{C}$  in an air oven for 24 hr. The EMC-S was determined by prolonging the soaking time till absorption remained constant as reflected from the moisture content values.

For determining the hydration behaviour at higher temperatures, 5.0 g of parboiled chips of 1-2 mm thickness were added to 50 ml distilled water taken in a Pyrex boiling tube and preheated to the desired temperature by immersing in a thermostatically controlled water bath. Samples were stirred using a glass rod to remove trapped air, and the tubes were closed using loose glass stoppers to prevent evaporation of water. After completion of the desired soaking time, samples were removed and the moisture content was determined by air oven method as mentioned earlier. From the moisture content values (g/g dry wt basis) water uptake (W) was calculated as follows:

$$W = \frac{M_f - M_o}{1 + M_o}$$

where  $M_f$  and  $M_o$  are the values for final and original moisture contents in the samples<sup>3</sup>.

*Sediment volume:* Cassava chips were ground in a dry grinder (Sumeet make) and sieved to obtain flour passing through 60 mesh. Five grams of the flour was transferred into a 50 ml graduated measuring cylinder.

Fifty millilitre of 0.05 N HCl was added and the material was mixed well by inversion<sup>4</sup>. The sediment volume was noted after 8 hr when it gave a constant value.

### Results and Discussion

A comparative study of the water absorption by plain and parboiled chips at RT (Table 1) showed that the latter has a higher water absorption capacity. The EMC-S attained by soaking for 8 hr was higher in the case of parboiled samples. Between the two parboiled samples the one which was parboiled by soaking in boiling water for a longer time of 10 min showed higher water absorption. Increase in the water absorption noticed in the case of parboiled samples could be explained as the result of pregelatinisation of starch causing it to swell and expand during the gelatinisation process. A similar observation has been made in the case of rice<sup>3</sup>.

Hydration behaviour of cassava chips, showed a direct correlation with temperature (Table 2). However, when samples were soaked at identical cooking temperature of 96-98°C for different soaking time till they showed a constant W value, it could be noticed that plain dried chips absorbed 70 per cent of their final W value within 5 min of soaking (Table 3); the parboiled chips showed a lower percentage of absorption (61.50 and 54.32 respectively). This time lag observed in the hydration of parboiled samples during the initial period of soaking could be presumably due to the partial resistance offered by the thin layer of gelatinised and reassociated starch formed during processing of parboiled chips. When the soaking time is prolonged at cooking temperature the expanded gelatinised starch molecules, as well as loosened cell structure may be facilitating increased absorption. In the case of plain dried chips, though the initial water uptake was higher the final value reached after 15 min was lower than those of parboiled samples. Cassava chips showed a direct relationship between degree of parboiling and the amount of water absorbed at higher temperatures also.

TABLE 1. HYDRATION OF CASSAVA CHIPS (PER CENT WET BASIS) AT ROOM TEMPERATURE (28-30°C)

Sample	1 hr	2 hr	4 hr	6 hr	8 hr	24 hr
	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.
Plain dried	43.04 $\pm$ 1.38 <sup>a</sup>	46.96 $\pm$ 1.60	51.76 $\pm$ 1.05	52.36 $\pm$ 0.99	53.54 $\pm$ 0.53	54.09 $\pm$ 0.86
Parboiled I	45.62 $\pm$ 1.14	48.92 $\pm$ 1.68	51.32 $\pm$ 0.69	54.33 $\pm$ 0.38	56.69 $\pm$ 1.60	56.69 $\pm$ 0.55
Parboiled II	50.01 $\pm$ 1.36	54.59 $\pm$ 0.39	57.92 $\pm$ 1.26	60.80 $\pm$ 1.28	61.86 $\pm$ 1.28	61.91 $\pm$ 0.63

<sup>a</sup> Each value is the mean of six samples

TABLE 2. WATER UPTAKE (W) BY CASSAVA CHIPS AT DIFFERENT TEMPERATURES FOR IDENTICAL SOAKING TIME (15 min)

Sample	60°C Mean±S.D.	80°C Mean±S.D.	92°C Mean±S.D.	96°C Mean±S.D.
Plain dried	0.534±0.02	0.669±0.05	0.749±0.03	0.994±0.04
Parboiled I	0.634±0.08	0.765±0.03	0.965±0.01	1.175±0.01
Parboiled II	0.783±0.05	0.973±0.05	1.106±0.01	1.193±0.01

<sup>a</sup> Each value is the mean of six samples

TABLE 3. WATER UPTAKE (W) OF CASSAVA CHIPS AT COOKING TEMPERATURE (96-98°C) FOR DIFFERENT SOAKING TIME INTERVALS

Sample	5 min Mean±S.D.	10 min Mean±S.D.	15 min Mean±S.D.	20 min Mean±S.D.
Plain dried	0.704±0.05 (70.0)	0.743±0.04 (74.7)	0.994±0.04 (100.0)	0.994±0.08
Parboiled I	0.757±0.01 (61.5)	1.043±0.09 (84.72)	1.175±0.01 (95.45)	1.231±0.01
Parboiled II	0.672±0.08 (54.32)	1.06±0.05 (85.69)	1.193±0.01 (96.44)	1.237±0.01

Each value is the mean of six samples

Values in parentheses are per cent of 'W' at 20 min.

As indicated in Table 4, parboiled samples showed a higher sediment volume than plain dried flour. A positive correlation between EMC-S and sediment volume was also observed. Further, as sediment volume

TABLE 4. SEDIMENT VOLUME OF CASSAVA FLOUR PREPARED FROM PLAIN AND PARBOILED CHIPS

Flour sample	Initial vol (ml)	Final vol (ml)
Plain dried	15.0	15.5
Parboiled I	7.0	31.0
Parboiled II	7.0	40.0

is a measure of the strength of the flour<sup>6</sup> it is to be inferred that parboiling of fresh cassava slices increases the strength of the flour and is related to the time of soaking in boiling water during processing.

#### Acknowledgement

Authors wish to thank Dr. C. S. Narayanan, of Food Division and Sri S. V. Ramakrishna, Project Leader for their keen interest shown in the present work.

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# Thermal Processing of Whole Tomatoes Under Low pH Conditions

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*Manuscript received 24 July 1984; revised 24 May 1985*

Process has been developed for canning of whole tomatoes under low pH conditions. Canned tomatoes are usually processed in boiling water for 30 min. Under low pH conditions, the processing time has been reduced by half, thus resulting in considerable savings (about 30%) in energy requirements by the tomato canning industry. After a storage period of six months, the canned tomatoes had excellent sensory quality and were well received by the consumers.

Tomato (*Lycopersicon esculentum* Mil.) is valued not only for its culinary properties, but is also considered a good source of vitamins and certain minerals<sup>1</sup>. The tomato canning industry in India is in its infancy and there is considerable scope for canned tomatoes so that this product can reach the areas where fresh tomatoes cannot be made available. The food spoilage micro-organisms have been shown to offer less heat resistance during thermal processing under acidic conditions<sup>2</sup>. Low pH preservation of tomato juice and acidified canned tomatoes has been studied earlier<sup>3,4</sup>, but the energy savings during thermal processing of whole tomatoes under acid conditions have not been reported so far. The present investigation has been directed towards this goal.

## Materials and Methods

'Punjab Chuhara' and 'Punjab Kesri' varieties of tomatoes were procured from the local market of Ludhiana.

**Canning:** Tomatoes were washed thoroughly in water and those with sunburns, cracks and blemishes were discarded. After steam blanching and removing the skin, each variety of tomatoes was divided into three lots and filled into A.1 tall cans. The pH of the two per cent brine was adjusted to 4.2 (control), 2.8 and 1.4 with citric acid. The brine was filled into cans leaving a head-space of 3/8 inches. The cans were exhausted to an internal temperature of 85°C, sealed and then processed in boiling water for 30 min in case of pH 4.2 and for 15 min each in case of pH 2.8 and 1.4 samples. At the end

of processing, the cans were cooled to room temperature under running tap water. Representative samples of the canned tomatoes were incubated at 37 and 55°C for about two weeks to assess microbial spoilage.

**Chemical analysis:** Fresh tomatoes were analysed for total solids, total soluble solids, pH, acidity and ascorbic acid by the methods as reported earlier<sup>4</sup>. The canned tomatoes were also analysed for these constituents at two months intervals, for a total period of six months. Sensory evaluation of canned tomato for colour, appearance, texture and flavour were carried out on a 9-point Hedonic scale by semi-trained panelists. Statistical analysis of the data was carried out for analysis of variance and critical difference<sup>5</sup>.

**Microbiological examination:** Tryptone glucose agar, glucose extract agar and McConkey's medium were used for standard plate count of bacteria, fungi and coliform, respectively, as per procedure mentioned in Difco manual<sup>6</sup>.

**Energy calculations:** In order to calculate the enthalpy differences between the two thermal processes, the following assumptions were made:

- (i) The primary mode of heat transfer within the can is conduction.
- (ii) The heat transfer is only along the radial direction.
- (iii) The product within the can has uniform properties.
- (iv) The surface resistance to heat transfer is negligible.

**Discussion of the assumptions:** Whole tomatoes immersed in the brine solution prevent the setting up of natural convection currents and since the cans are not agitated there is little mixing of the contents at different

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locations. The assumption, then of conduction being the primary mode of heat transfer seems to be reasonable.

The temperature at different locations in the cans are much more affected by the heat moving inwards from the periphery than from the ends as the distance of slowest heating point is almost twice as large from the top and the bottom surface than from the periphery. The heat transfer is further impeded by the head space and moreover the manner in which the filled cans are stacked one a-top the other also impedes heat transfer from the ends. The assumption permits us to represent the finite cylinder as an infinite cylinder for heat transfer calculations.

The third assumption is patently untrue as water and the tomatoes have different properties. But it is a simplifying assumption and allows us to arrive at a reasonable estimate of the energy differences that exist between the two processes.

*Calculations:* For calculating the heat contents of the two process, the following property values are used.

- Density of the products,  $S=612.56 \text{ kg/M}^3$
- Sp. heat of the product,  $C_p = 4090 \text{ J/kg k}$
- Thermal conductivity of product,  $k = 0.580 \text{ W/M k}$
- Heating time,  $t = 15 \text{ min, } 30 \text{ min}$
- Initial temp,  $T_o = 75^\circ\text{C}$
- Surrounding temp,  $T_p = 98^\circ\text{C}$
- Radius of the can,  $R = 0.043656 \text{ m}$
- Height of the can,  $H = 0.11589 \text{ m}$
- Variable radius,  $r = 0.2R, 0.4R, 0.6R, 0.8R, 1.0R$

The temperature at various locations in the can are determined by using the Heisler's charts for infinite cylinder for the transient heat conduction with:

Fourier's number,  $N_{Fo} = \frac{kt}{A\phi R^2}$   
 and Biot's number,  $N_{Bi} = \infty$  (Assumption 4)

The temperatures obtained at various locations are shown in Table 1.

Since the temperature of the product is constantly varying along the radial direction, for heat content calculations, it is assumed that the can the cylinder is composed of a solid cylinder of radius 0.2R and concentric

hollow cylinders of outer radii 0.4R, 0.6R, 0.8R and 1.0R. The amount of material present in each such cylinder is calculated by multiplying the volume of each cylinder by the density of the material. The average temperature of the material present in each cylinder is taken to be the geometric mean of the temperature of the enclosing surfaces. Based on this average temperature, the heat content of each cylinder is calculated and the sum of these gives us the total heat content of the product. The difference in the heat content of the two processes gives us the energy saving. A computer programme written in BASIC was used to calculate the heat content differences.

- Volume of material in each cylinder,  $V = HTc(V_n^{2+1} - V_u^2)$
- Mass of material present in each cylinder,  $= M = sV$
- Average temp,  $T_{av} = (T_{n+1} \times T_n)^{\frac{1}{2}}$
- Heat content of each cylinder,  $q_i = MC_p (T_{av} - T_o)$
- Total heat content,  $Q = q_i$
- Heat content after 15 min processing,  $Q_{15} = 2.6014 \text{ KJ}$
- Heat content after 30 min processing,  $Q_{30} = 3.3033 \text{ 012 KJ}$

*Convection and radiation losses:* The convection and radiation losses were calculated as reported earlier<sup>4</sup>. The total number of cans in the retort was 100 and the calculations are on per can basis. The retort had a diameter of 0.51 m and height of 0.53 m.

$Q \text{ Convection} = h A T (t/n)$   
 where,  
 $h$ -Convection heat transfer coefficient,  $\text{W/m}^2 \text{ C}$   
 $A$ -Area of heat transfer,  $\text{m}^2$   
 $T$ -Temperature difference,  $^\circ\text{C}$   
 $t$ -time, sec.  
 $n$ -number of cans, dimensionless  
 $\gamma$ -Stefan-Boltzmann constant  
 $Q^{215} = 3.118 \text{ KJ/can}$   
 and  $Q^{230} = 6.237 \text{ KJ/can}$   
 $Q \text{ radiation} = F_{12} A(T_1^4 - T_2^4)$   
 where,  
 $F_{12}$  - Shape factor, dimensionless  
 $T_1, T_2$ -Temperature  $^\circ\text{K}$   
 So  $Q^{315} = 6.111 \text{ KJ/can}$   
 and  $Q^{330} = 12.223 \text{ KJ/can}$

*Energy savings:*

Total energy used in first process  $= Q \frac{T}{15}$   
 $= Q^{115} + Q^{215} + Q^{315}$   
 Total energy used in second process  $= Q \frac{T}{30}$   
 $= Q^{130} + Q^{230} + Q^{330}$

TABLE 1. TEMPERATURE AT DIFFERENT LOCATIONS IN THE CAN AT THE END OF 15 AND 30 MIN OF PROCESSING

Processing time (min)	Radius					
	0	0.2	0.4	0.6	0.8	1.0
15	75.0	77.3	79.6	86.0	89.0	98.0
30	90.6	92.0	92.7	93.9	96.0	98.0

$$\text{Total energy saved } Q \frac{T}{30} - Q \frac{T}{15} = 16.229 \text{ KJ/can}$$

$$\text{Per cent energy saved} = \frac{Q \frac{T}{30} - Q \frac{T}{15}}{Q \frac{T}{30}} \times 100 = 31.53\%$$

### Results and Discussion

**Fresh tomatoes:** The physico-chemical composition of the fresh tomatoes of both the varieties is given in Table 2. The fresh tomatoes of 'Punjab Kesri' had lower total solids, total soluble solids, acidity and ascorbic acid, but had a slightly higher pH than 'Punjab Chhuhara'. These differences were, however, statistically insignificant.

**pH and acidity changes during storage:** The physico-chemical changes in control and acidified canned tomatoes of these varieties are given in Table 3. The pH of the brine which was adjusted to 4.2, 2.8 and 1.4

with citric acid equilibrated with the pH of tomatoes during storage. The pH of the control and acidified canned tomatoes after processing changed to 4.06, 3.8, 2.69 in 'Punjab Chhuhara' and 4.05, 3.68, 2.54 in 'Punjab Kesri', respectively. The pH and acidity of the different samples of canned tomatoes varied significantly among themselves as also during storage. The pH and acidity of these samples during storage also varied significantly.

**Ascorbic acid:** No significant difference was found in the ascorbic acid content of canned tomatoes under different pH conditions (Table 3); however, there was a significant reduction in the content of ascorbic acid during storage.

**Cut-out analysis:** The cut-out analysis of canned tomatoes at the end of six months storage is presented in Table 4. The average drained weight of canned tomatoes in all these samples was above the prescribed limit (50 per cent) of FPO. The average vacuum in cans ranged between 16.4 and 17.1 inches. There was no corrosion or blackening on the inside of cans in case of control samples. However, a slight feathering was observed in cans in which acidified tomatoes had been canned. The drained weight loss in these samples ranged between 7.9 and 12.3 per cent.

**Sensory analysis:** At the end of 6 months of storage, the canned tomatoes were evaluated for colour, appearance, texture and flavour (Fig 1 and 2); the panelists could not find significant differences among the samples. However, they observed the colour of 'Punjab Chhuhara'

TABLE 2. PHYSICO-CHEMICAL COMPOSITION OF FRESH TOMATOES\*

Variety	Total solids (%)	Total soluble solids (°Bx)	pH	Acidity (% anhy. citric acid)	Ascorbic acid (mg/100g)
'Punjab Chhuhara'	4.4	4.0	4.0	0.32	11.5
'Punjab Kesari'	4.4	4.0	4.1	0.32	10.5

\*Mean of five samples

TABLE 3. EFFECT OF DIFFERENT STORAGE PERIODS ON THE CHEMICAL COMPOSITION OF CANNED TOMATOES

Brine pH	pH at indicated storage period				Acidity (% anhy. citric acid) at indicated storage period				Ascorbic acid (mg/100g) at indicated storage period			
	0	2	4	6	0	2	4	6	0	2	4	6
<b>'Punjab Chhuhara'</b>												
4.2	4.06	4.01	4.12	4.13	0.32	0.34	0.30	0.29	11.5	7.9	7.6	6.3
2.8	3.80	3.91	4.00	4.09	0.41	0.36	0.34	0.32	11.5	8.5	7.6	7.3
1.8	2.69	2.60	3.01	3.03	1.28	1.31	1.20	1.10	11.5	8.7	8.1	7.6
<b>'Punjab Kesri'</b>												
4.2	4.05	3.99	4.10	4.15	0.32	0.29	0.27	0.25	10.5	8.0	6.2	4.3
2.8	3.69	3.75	3.90	4.14	0.41	0.35	0.34	0.29	10.5	8.3	6.8	4.7
1.8	2.54	2.62	2.82	3.00	1.40	1.34	1.20	1.10	10.5	8.3	7.3	5.4

Parameter	Storage periods	Brine pH
pH	16.62*	221.9**
Acidity	7.09*	237.73**
Ascorbic acid	101.87**	NS

Significance at: \*5% level; \*\*1% level.



TABLE 4. CUT-OUT ANALYSIS OF CANNED TOMATOES (MEAN OF FIVE CANS)\*

Brine pH	Drained wt. (%)	Vacuum (in.)	Internal can condition
‘Punjab Chuhara’			
4.2	53.7	16.4	O.K.
2.8	50.1	16.8	Slight feathering
1.8	55.0	16.4	Slight feathering
‘Punjab Kesri’			
4.2	54.5	17.0	O.K.
2.8	55.0	17.1	Slight feathering
1.8	53.0	17.0	Slight feathering

\*At the end of 6 months storage period

to be significantly better than ‘Punjab Kesri’. The ‘F’ values (at 5 per cent level) for the average score of these samples for colour, appearance, texture and flavour were 9.58, 1.47, 2.25 and 3.35 respectively.

**Energy savings:** The short time, low pH thermal processing of whole canned tomatoes resulted in considerable energy savings (about 30 per cent) as seen from the calculations. In an industrial situation, saving in energy would probably be slightly less, because in our calculations, all the losses were not considered. Besides energy saving, this low pH thermal processing would result in increased production without any additional expenditure on processing equipments and personnel. A comparison of the temperature histories of the two processes shows that heat damage to the product under low pH thermal processing is much less because the degradations associated with high temperature are minimised.

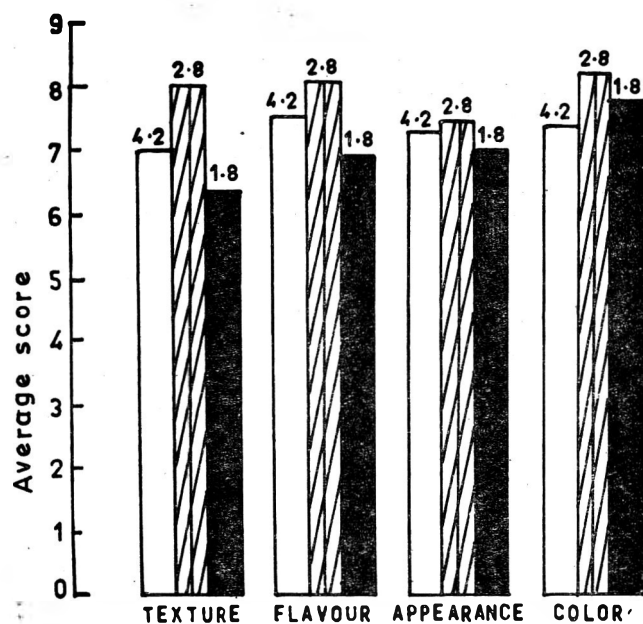


Fig. 1. Comparison of sensory qualities of canned tomatoes (‘Punjab Chuhara’) under different pH conditions

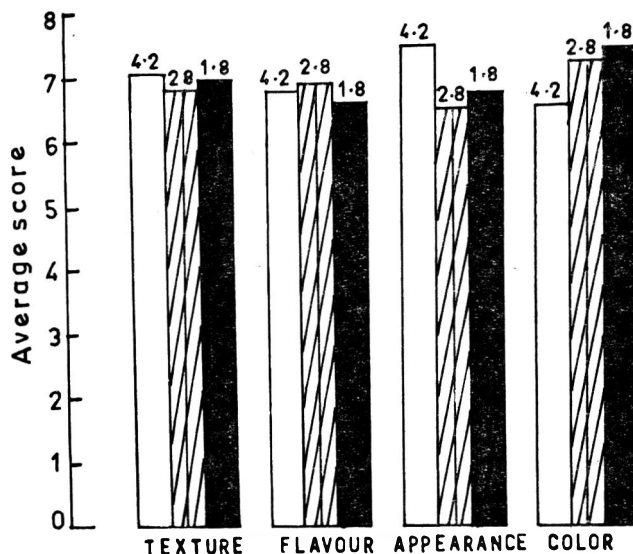


Fig. 2. Comparison of sensory qualities of canned tomatoes (‘Punjab Kesri’) under different pH conditions

**Microbiology:** Microbiological examination for standard plate count and coliforms for the canned tomatoes were carried out. The standard plate count in cans of each of ‘Punjab Chuhara’ and ‘Punjab Kesri’ under pH conditions of 4.2 and 1.8 were found to be nil; however canned tomatoes of ‘Punjab Chuhara’ and ‘Punjab Kesri’ with a pH of 2.8 had a standard plate count of 400 and 100/ml of brine, respectively. According to microbiological standards, these counts can very safely be considered negligible. The *E. coli* was, however, absent in all these samples.

#### Acknowledgement

The authors thank Dr. Rajinder Prasad Gupta, Microbiologist, Department of Microbiology, PAU, Ludhiana, for his help in carrying out microbiological examination of canned tomato samples.

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# Evaluation of Dye Reduction as a Quality Index for Raw Meat Under Tropical Conditions

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*Manuscript received 20 August 1984; revised 26 April 1985*

The reduction time of methylene blue and resazurin dyes was used to monitor total viable counts in beef and pork packaged in polythene bags or kept loose and stored on bench-top under ambient conditions as for meat under sale in Nigerian open-air markets or under storage in homes without refrigeration. Results show a nearly linear inverse relationship between total viable counts and dye reduction times. The highly significant ( $p < 0.01$ ) negative correlation ( $r \geq 0.93$ ) obtained suggests dye reduction test to be a useful rapid indicator of the microbiological quality of raw meat, packaged or unpackaged, under ambient tropical (Nigerian) conditions. Both dye reduction time and the viable count were better for packaged than unpackaged meat showing the relative efficiency of simple polythene packaging in enhancing microbiological quality. Each of the dyes studied appeared as good as the other.

Routine monitoring of meat quality and sanitation necessarily includes evaluation of the microbial load. For this purpose simple, rapid methods requiring expertise and facilities are ideal. Dye reduction by micro organisms is one such simple and easy-to-perform test<sup>1</sup>. The principle underlying the procedure is that samples will reduce and cause discolouration of an oxidation-reduction sensitive dye such as methylene blue or resazurin at rates proportional to the number and virility of micro organisms present in the sample<sup>2,3</sup>. The remarkable success of dye reduction test in the milk industry has attracted interest for its possible application in the meat industry<sup>2,4,5</sup>.

Researchers in the Danish Meat Research Institute and Meat Products Laboratory (Ministry of Agriculture) have intensively investigated the possible use of dye reduction test for meat microbiological quality and for sanitary appraisal of meat industries and process operations<sup>4</sup>. The present study seeks to explore the applicability of dye reduction test under hot, humid, relatively insanitary meat production and marketing/storage conditions as in Nigeria.

## Materials and Methods

Beef and pork lion steaks (*Longissimus dorsi*) were obtained pre-rigor immediately after slaughter from a local (Nsukka) open-air market and the university farm respectively. Each meat was trimmed of visible fat and connective tissue, and split into longitudinal halves for storage either as such or after packing in a transparent air-permeable polythene bag. All four samples were

left on laboratory bench-top simulating the normal holding of meat during sale in local markets or during storage in homes without refrigeration. Test samples were taken from each meat at 12 hr intervals with sterile cork borer (steel pipe sharpened at one end) of 2 cm internal diameter, cutting off with sterile forceps and scissors at a depth of about 3 mm. These test samples were taken, at every sampling time, from twelve different locations on each meat for viable count, methylene blue reduction and resazurin reduction tests in quadruplicate.

Methylene blue (0.0025 per cent) and resazurin (0.005 per cent) solutions were prepared<sup>2</sup> by dissolving 0.025g methylene blue and 0.05g resazurin in 200 ml sterile distilled water and making up to litre with sterile distilled water. For dye reduction test, 1g of the top transverse portion of each bored-out meat piece was put into a sterile test tube containing 9 ml of 0.1 per cent peptone water. One milliliter of the dye was then added and the tube stoppered and mixed by inverting several times. The tubes were incubated in a water-bath at 37°C and the time taken for the dye to turn colourless was noted.

For total viable count, 1g of the top transverse portion of each bored-out meat piece was homogenized aseptically in 99 ml of sterile 0.1 per cent peptone water and further decimal dilutions were made for microbial enumeration in Plate Count (Nutrient) Agar by the pour plate method<sup>6</sup>. Resulting plates were incubated at 37°C alongside a control un-inoculated agar plate (for sterility check) for 24 hr, after which colonies formed were counted.

TABLE 1. MICROBIAL COUNTS AND DYE REDUCTION TIMES FOR BEEF UNDER AMBIENT CONDITIONS

Storage period (hr)	Unpackaged			Packaged in polythene bag		
	Total viable count (log)	Dye reduction time, (min)		Total viable count (log)	Dye reduction time, (min)	
		Methylene blue	Resazurin		Methylene blue	Resazurin
12	7.075	119.0	115.0	6.975	119.0	113.5
24	7.575	110.5	78.0	7.30	109.0	97.5
36	8.275	84.0	52.0	7.850	98.0	89.0
48	9.225	62.0	41.0	8.250	84.5	78.5
60	9.50	41.5	38.5	8.625	75.0	67.0
72	—	—	—	8.925	67.5	52.5
84	—	—	—	9.225	51.5	43.5

Each value is the mean of four replicates.

Unpackaged meat spoiled after 72 hr.

TABLE 2. MICROBIAL COUNTS AND DYE REDUCTION TIMES FOR PORK UNDER AMBIENT CONDITIONS

Storage period (hr)	Unpackaged			Packaged in polythene bag		
	Total viable count (log)	Dye reduction time, min		Total viable count (log)	Dye reduction time, min	
		Methylene blue	Resazurin		Methylene blue	Resazurin
12	6.950	109.5	99.5	7.0	109.5	100.0
24	7.250	90.0	83.0	7.075	104.0	90.0
36	8.050	69.5	61.0	7.250	103.0	84.0
48	8.925	45.0	40.0	7.825	89.5	77.0
60	9.50	34.0	21.0	8.325	84.0	60.0
72	—	—	—	8.775	75.0	51.5
84	—	—	—	8.975	63.0	43.5

Each value is the mean of four replicates.

Unpackaged meat was spoiled after 72 hr.

## Results and Discussion

Tables 1 and 2 show the total microbial counts and the corresponding dye reduction times for beef and pork, unpackaged or in transparent polythene bags, during storage under ambient conditions. In both meat types total viable counts and dye reduction times were better for packaged than for unpackaged samples showing the relative efficiency of simple polythene packaging in enhancing the microbiological quality of meat through reduced contamination.

In all cases as storage progressed and microbial counts increased, the time required to decolorize the methylene blue and resazurin dyes decreased. Fig 1 and 2 show this inverse relationship between microbial count and dye reduction time to be almost linear for both methylene blue and resazurin. As Table 3 shows the dye reduction time correlated significantly ( $P < 0.01$ ) with the total count of viable micro organisms in the meat, regardless of the meat type and whether packaged or not. The correlation coefficients are  $\geq 0.93$  showing the high

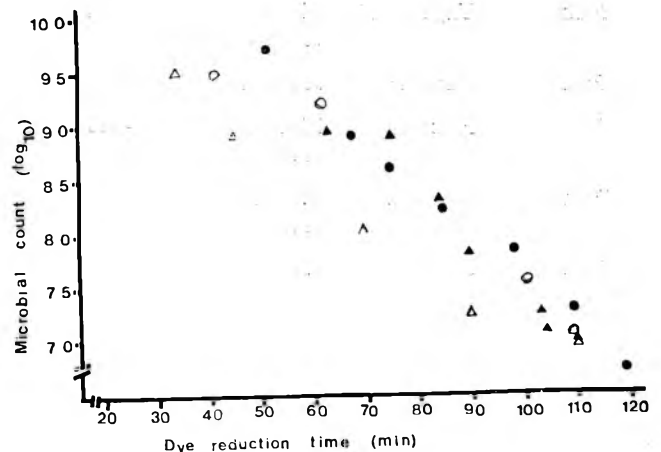


Fig 1. Relationship between microbial count and methylene blue reduction time for meat stored under ambient (Nigerian) conditions.

○ - Beef, unpackaged; ● - Beef, packaged;  
 △ - Pork, unpackaged; ▲ - Pork, packaged.  
 Each value is the mean of 4 replicates.

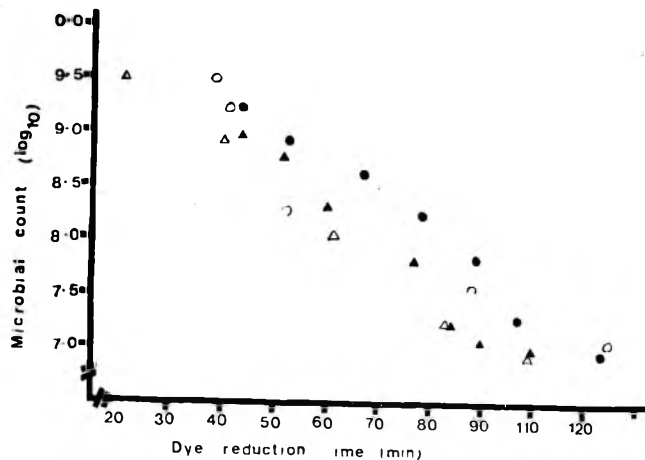


Fig 2. Relationship between microbial count and resazurin reduction time for meat stored under ambient (Nigerian) conditions.

○ - Beef, unpackaged; ● - Beef, packaged;  
 △ - Pork, unpackaged; ▲ - Pork, packaged.

Each value is the mean of 4 replicates.

TABLE 3. CORRELATION OF DYE REDUCTION TIME (DRT) WITH TOTAL VIABLE COUNT

Meat type	Condition	Dye used	Corr. coef. DRT Vs. Microbial count
Beef	Unpackaged	Methylene blue	-0.99**
Beef	Unpackaged	Resazurin	-0.93**
Pork	Unpackaged	Methylene blue	-0.98**
Pork	Unpackaged	Resazurin	-0.99**
Beef	Packaged	Methylene blue	-0.99**
Pork	Packaged	Methylene blue	-0.98**
Pork	Packaged	Resazurin	-0.98**

\*\*Highly significant

sensitivity of both dyes to microbial numbers/activity. In this regard each dye appeared as good as the other; however, reduction times were generally lower for resazurin than for methylene blue at the same microbial count (Table 1 and 2), even though these dyes are known to be non-inhibitory to microbial activity in the concentrations normally used<sup>1,5,7</sup>.

The results indicate the need for further studies that could lead to establishing equations for predicting from dye reduction times the microbial load of meat under tropical conditions. However, the dye reduction times obtained in this study appeared too good for the organoleptic quality (especially odour) of the meats as storage progressed. Thus while the unpackaged meat samples had gone sensorily objectionable after 36 hr of storage the dye reduction times were still indicating, by Danish standards<sup>4</sup>, "acceptable" or even "good quality" meat. There is, therefore, need to correlate dye reduction time not only with microbial count but also with organoleptic quality and acceptability under tropical conditions.

#### Acknowledgement

The author is grateful to the University of Nigeria, Nsukka, for financial support through Senate Research Grant No. 0175/76.

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# Textural Classification of Foods Based on Warner—Bratzler Shear

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*Manuscript received 6 July 1984; revised 26 April 1985*

Several foods have been classified into three groups based on the ratio of Warner-Bratzler shear stresses obtained by using horizontal (WBH, kg. cm<sup>-2</sup>) and traditional conical (WBC, kg. cm<sup>-2</sup>) blades, respectively. The first group of foods represent fairly cohesive foods, where the ratio, WBH/WBC, is greater than 1.1. The second group of foods exhibit snappy nature in general and the ratio of shear stresses lies between 0.9 and 1.1. The third group of foods are crisp and the shear stresses ratio is less than 0.9. Cereal foods, *roti* and biscuits; confectionery; fruits, vegetables; candies; cheese; and mutton are classified into these three groups. Differences between firmness and hardness are made clear and either new or modified definitions have been proposed for cohesiveness, snappiness and crispness. Conceptual relationships between cohesiveness, firmness, stickiness, toughness and chewiness have been indicated.

Texture is important in food preferences and selection, and hence its variations are considered in menu planning<sup>1</sup>. It is defined as "the attribute of a substance resulting from a combination of physical properties and perceived by the senses of touch (including kinaesthesia and mouthfeel), sight and hearing. Physical properties may include size, shape, number, nature and conformation of constituent structural elements", and hence active manipulation and mastication are considered essential in expressing and sensing the texture of foods<sup>2</sup>.

Detailed work carried out by Szczesniak and co-workers<sup>3</sup> and by the Japanese workers<sup>4</sup> on the words used by consumers to describe texture of a number of food materials indicates that the words, thus collected and appropriately analysed, denoted, mostly, the different degrees of hardness (soft to hard), cohesiveness (fracturable to cohesive) and moisture content (dry to wet). Later on, sensory texture profiling was developed to record and quantify the sequentially perceived textural experiences from first bite through chewing to the swallowing and aftertaste<sup>5,6</sup>. Instrumental texture profiling techniques were reported<sup>7-9</sup> to measure the corresponding parameters in sensory texture profile. The whole attempt of all these was to finally classify food products according to their textural characteristics.

Warner-Bratzler (WB) shear device has been used to measure the tenderness of meat in particular and various food products in general. A number of workers<sup>10-12</sup> attempted to describe the mechanism of the type of shearing occurring during cutting a sample in WB shear apparatus. Recently, the dependence of force

required to shear on cone angle of the blade employed has been established<sup>13</sup>. Present communication describes the classification of foods based on a measure of cohesiveness, determined through the use of WB shear apparatus, along with the toughness measurements through the traditional parameter from the WB-shear-force deformation curves, i. e., the peak force per unit area of cross section.

## Materials and Methods

Some of the food materials were purchased from the market and others were prepared in the laboratory. In all the cases, sample dimensions were carefully measured and finally shear stresses (peak-force from the force deformation curves) were expressed in kilogram per unit initial-cross-section area, i. e., kg. cm<sup>-2</sup>. Cooked mutton was cut into strips of 1 × 1 cm<sup>2</sup> cross section with fibres running parallel to the length and sheared across the fibres. *Roti* (Indian unleavened bread) was cut into 2.5 cm wide strips and sheared across the length. Chocolates, vegetables, *Sohan-papri* (an Indian sweet meat of layered texture), cheese and *Sohan-halwa* (Indian sweet meat), were cut into rectangular pieces, in nearly cubical forms or bars of square cross section. Other types of food materials were used as such, as sizing meant tampering with texture, and their relevant dimensions were measured.

## Results and Discussion

Fig 1 shows the blades with conical (slanted cutting edges) and rectangular (single horizontal cutting edge)

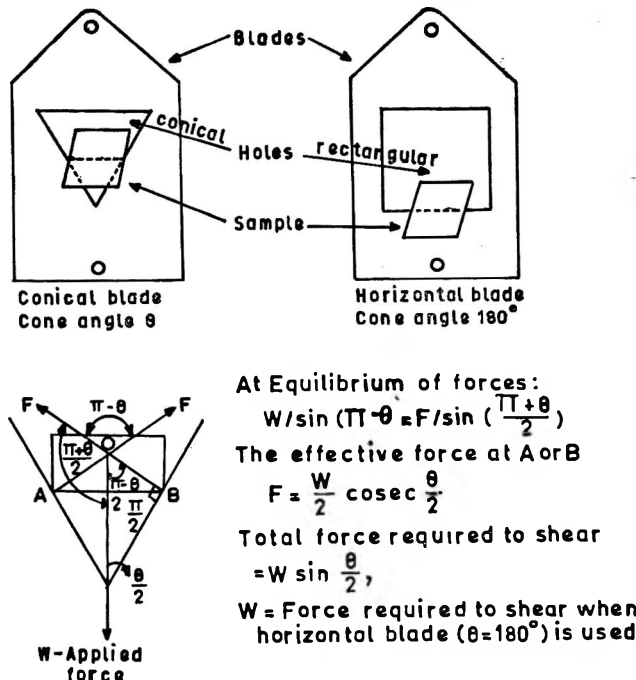


Fig 1. Conical and horizontal blades employed to shear samples, and the equilibrium of forces acting on a specimen, in a conical blade, at any moment. (Adapted from Ref. 13).

holes and the equilibrium of forces acting on a specimen placed in the conical blade at any moment. For a cohesive food material, maintaining its shape throughout, the force required to shear is equal to  $W \sin \theta/2$ , where  $W$  is the force required to shear when a horizontal blade is used, and  $\theta$  is the cone angle. Hence, the theoretical value of the ratio<sup>13</sup> of shear stresses required to shear samples using horizontal (WBH) and traditional conical (WBC,  $\theta=60^\circ$ ) blades is 2, i.e.,  $WBH/WBC=2$ . (The coefficient of variation<sup>13</sup> for WBC was 7 to 11 per cent and for WBH it was 4 to 10 per cent). It made the basis of present work since in practice different food products exhibit different textural properties and their geometry and shapes are also affected differently during shearing. The component of  $F$  (Fig 1) acting perpendicular to  $W$ , i.e., compressive forces acting on the specimen at both ends are equal to  $F \cos \theta$  and tend to cancel each other; but depending on mechanical properties of a food material, the sample might bend, get folded, or remain unaffected. For  $\theta=60^\circ$ , the force  $F \cos \theta$  is equal to  $W/2$ .

Food products offer resistance to biting. Firmer foods possess greater resistance to penetration by teeth (or by a blade or a probe). As teeth penetrate into the food continually, increasing stresses are required to maintain the rate of penetration (or strain) until rupture. So, essentially two factors assume importance (i) the fundamental resistance of a food material to deformation (upon penetration, or compression, etc.) and (ii) the

extent of deformation at which a food ruptures. The initial slope of a force-deformation curve is termed as firmness (compression-firmness, tensile-firmness, shear-firmness, etc.). The extent of deformation at rupture is a function of cohesiveness; therefore, greater the cohesiveness, greater would be the deformation to and the stress required at rupture i.e., more work would be required to reputeure the food product. A sample which ruptures at a relatively higher deformation is termed as cohesive and may also be described, additionally, as soft or chewy whereas a food product rupturing at considerably lower deformation would be termed as poorly cohesive (snappy, brittle, or crisp) and may also be described as firm or hard. It is typical of poorly cohesive foods that they would be described as firm or hard, and never as soft in spite of the fact that they in general require relatively smaller stresses to cause rupture as would be shortly seen. Hence cohesive foods could be tough but may not be as firm or hard as the poorly cohesive foods are.

Based on the general textural properties exhibited by foods, as judged by sensory testing and the magnitudes and ratios of shear stresses, WBH and WBC, the food products could be classified into three major groups.

#### Group 1. (The ratio $WBH/WBC > 1.1$ )

This is the group of fairly cohesive foods. Cohesiveness can be defined as the textural property manifested by the substantial deformation (with respect to sample thickness) before a product ruptures. It represents the strength with which the structural components of a food material are bonded to each other; more cohesive a food is, more work would be necessary to rupture it. In other words, for two equally firm foods, rupture would take place at a greater deformation for the more cohesive food. Depending upon the degree of cohesiveness, stickiness, and the changes in geometry and shape during shearing, the ratio ( $WBH/WBC$ ) varies from 1.1 to greater than 3 (Table 1). *Roti*, and raw bitter melon are the products which fold into the conical cavity of the blade thereby registering very low WBC and hence the ratio exceeds 2 (even 3) because, with the use of horizontal blade (WBH) no such folding occurs, on the contrary all the products remain unfolded. Mutton, toffees and grapes (2nd peak, 2 peaks are obtained with grapes) are the products which exhibit the ratio around 2. For other food products, listed in Table 1, the ratio varies between 1.1 and 2. There are exceptions, like fruit-rine-type candies which exhibit sticky properties (verified by sensory testing) in addition to high cohesiveness, hence resulting in the ratio greater than 2. The 'sticky' has been defined as "possessing the textural property manifested by a tendency to adhere to contacting surfaces, especially the palate, teeth and tongue

TABLE 1. SHEAR STRESSES OF FOODS FOR WHICH THE RATIO  $WBH/WBC > 1.1$ 

Food	Shear stress $\text{kg. cm}^{-2}$		WBH/WBC ratio
	WBC	WBH	
1. Fruitrine	7.07	20.35	2.88
2. <i>Roti</i> *	4.67	15.34	3.28
3. Toffees, A	8.30	15.26	1.84
4. Toffees, B	6.73	12.49	1.85
5. Mutton*	3.96	7.22	1.82
6. Confectionery, A	5.21	5.86	1.12
7. Bitter chocolate	4.04	5.10	1.26
8. Biscuits <sup>1</sup> D	1.49	2.88	1.94
9. „ C	1.42	2.46	1.73
10. „ B	0.82	1.56	1.90
11. „ A	0.95	1.34	1.41
12. Bitter gourd	0.41	1.56	3.80
13. Snake gourd	1.35	1.80	1.33
14. <i>Sohan papri</i>	0.60	0.84	1.40
15. Grapes, fresh II peak A	0.28	0.81	2.89
16. Grapes, fresh, B	0.38	0.75	1.97
17. Grapes, fresh, C	0.33	0.73	2.21
18. Chocolate, special	0.32	0.77	2.41
19. Grapes, 3-days at RT, II Peak, C	0.47	0.65	1.38
20. „ „ „ „ A	0.22	0.55	2.50
21. „ „ „ „ B	0.33	0.51	1.54
22. Processed cheese, A	0.13	0.15	1.15

The shear stress are average of triplicates.

\*Average of 8-9 replicates.

A,B,C,D indicate different brands or varieties. RT—Room temperature.

<sup>1</sup> Exposed to atmosphere for one week.

during mastication; perceived by the sense of touch”<sup>2</sup>, whereas in cohesiveness a tendency to adhere to their own particles is observed. In all 12 products are shown under this group. Such products are described as tender, chewy or tough by the consumers<sup>14</sup>.

#### Group 2. ( $0.9 \leq \text{the ratio } WBH/WBC \leq 1.1$ )

Foods exhibiting poor cohesive properties belong to this group. They are snappy, brittle and fracturable in nature. Snappiness could be defined as the textural property manifested by considerably smaller deformations at which such foods break into a few pieces with

TABLE 2. SHEAR STRESSES OF FOODS FOR WHICH  $0.9 \leq \text{THE RATIO } WBH/WBC \leq 1.1$ 

Food	Shear stress $\text{kg. cm}^{-2}$		WBH/WBC ratio
	WBC	WBH	
23. Confectionery, B	4.27	4.27	1.00
24. Milk chocolates, A	4.67	4.30	0.97
25. Milk chocolates, B	3.72	3.47	0.93
26. Milk chocolates, C	2.67	2.63	0.98
27. Potato	2.37	2.23	0.94
28. Biscuit	2.08	2.12	1.02
29. Carrot	1.64	1.70	1.04
30. Grapes, fresh, I peak, A	0.86	0.90	1.04
31. „ „ „ B	0.66	0.69	1.04
32. „ „ „ C	0.56	0.56	1.00
33. Grapes, 3-days RT stored I peak, A	0.65	0.61	0.93
34. „ „ B	0.85	0.80	0.94
35. „ „ C	0.52	0.52	1.00
36. Processed cheese, B	0.28	0.30	1.07

The shear stresses are average of triplicates.

A,B,C indicate different brands or varieties.

RT—Room temperature.

snap sounds and the pieces run away from the point of rupture/fracture. It is perceived by the senses of touch, sight and hearing. Such products are snapped into a few pieces, no sooner the necessary magnitude of force is reached so as to cause major failure (injury), irrespective of the cone angle of the blade. Therefore, the ratio of shear stresses for snappy foods should be equal to 1. But, with due consideration to the sources of error, an arbitrary 10 per cent interval is reported here representing the class of foods. Hence, foods for which the ratio lies between 0.9 and 1.1 would be snappy in nature. The definition of brittle<sup>2</sup> is “possessing the textural property manifested by a tendency to crack, fracture or shatter without substantial prior deformation on the application of force; perceived by the senses of touch, hearing and sight”. Table 2 indicates such products. Hard boiled sugar candies, biscuits, milk chocolates, potato, carrots and grapes (1st peak) are some of the food products constituting this group.

#### Group 3. (*The ratio $WBH/WBC < 0.9$* )

This is the group of food products which are characteristically crisp in nature. Crispness could be defined as possessing the textural property manifested by a

TABLE 3. SHEAR STRESSES OF FOODS FOR WHICH THE RATIO WBH/WBC < 0.9

Food	Shear stress, kg. cm <sup>-2</sup>		WBH/WBC ratio
	WBC	WBH	
37. Chocolate, special, A	4.64	3.34	0.72
38. Chocolate, special, C	3.69	2.98	0.81
39. Chocolate, special, B	4.50	2.24	0.50
40. <i>Sohan halwa</i>	2.16	1.84	0.80
41. Little gourd ( <i>Coccinia indica</i> )	2.24	1.61	0.72
42. Biscuits, A	2.27	1.47	0.65
43. Biscuits, B	1.33	0.94	0.71

The shear stresses are average of triplicates.  
A,B,C, indicate different brands or varieties.

tendency when subjected to an applied force to yield suddenly at considerably lower deformations at a fast rate into small fragments with a characteristic high frequency low sounds and perceived by the senses of touch, hearing, and sight; whereas Jowitt<sup>2</sup> has defined it as "the textural property manifested by a tendency when subjected to an applied force to yield suddenly with characteristic sound, perceived by the senses of touch and hearing" and for porous, dry foods especially biscuits (crackers), potato crisps (chips), the use of the term brittle was suggested by him. Such products break into several pieces upon application of relatively small forces without much deformation, with characteristic high frequency low sounds. All the crisp foods, viz., some biscuits and chocolate belong to this group and the relevant data are presented in Table 3.

Initial slopes of the shear-force-deformation curves (and more so for WBH) indicate firmness which cannot be measured without considerable errors (especially due to errors in deformation measurements), whereas the peak force has been reported to indicate tenderness/toughness and hence was composed of two components—firmness and cohesiveness. The simplest nature of the function appears to be multiplicative in nature, therefore, toughness = firmness (1 + cohesiveness). Hence, products could also be classified based on toughness (shear stress). In each group of foods, the foods are almost ranked according to toughness and a general list could be compiled. It is easy to observe that Group 3 foods are short chewy, of course salivating and quite pleasant to eat, Group 2 foods take relatively more chewy and the group 1 foods are the most chewy.

'Sticky' seems to add to toughness to make a product more chewy. Fruitrine represents such a food here

(Table 1). Chewiness could be written as: chewiness = toughness (1 + stickiness), or chewiness = firmness (1 + cohesiveness) (1 + stickiness). Therefore, food products as represented in the 3 groups (Table 1, 2 and 3) are logically arranged for toughness and cohesiveness.

Fig 2 shows a two dimensional classification of food products along with typical force-deformation curves for each group of foods. It is apparent that in the present set of food products, no product, which is snappy or crisp in nature, required even stresses equal to 5 kg cm<sup>-2</sup> to shear, whereas cohesive foods required very high shear stresses (upto more than 20 kg cm<sup>-2</sup>) to rupture. It is also clear that at a given shear stress, food products could be available with very wide range of cohesiveness—from very cohesive to poorly cohesive (snappy, crisp). The converse is also true but with not so much of variation in shear stress, especially for foods belonging to Groups 2 and 3. These observations are in tune with the examination of 'biting through foods' mentioned earlier. From the description of 'biting through foods' and the data presented in this paper in Tables 1, 2 and 3, it would be apparent that the term 'firm' could be used to describe textural property (as described by Jowitt<sup>2</sup>) of foods belonging to Group 1 foods, whereas terms 'firm' and 'hard' could be used with similar meanings to describe foods belonging to Groups 2 and 3. It would be evident that apart from the 'resistance to deformation', the 'extent of deformation' is important in differentiating between 'firm' and 'hard'. As the 'deformation at rupture' goes on decreasing, the concept changes from firmness to hardness. The latter is applicable to low-moisture foods like biscuits and the former to the high moisture foods like fruits and vegetables (Groups 2 and 3) in addition to Group 1 foods. The notion of 'hurting' (the palate) is associated with hardness.

Another interesting observation is that some of the biscuits which belonged to Groups 2 and 3, crossed over to Group 1 when exposed to atmosphere for 7 days. With absorption of moisture, the biscuits were rendered cohesive and, therefore, snappy and crisp properties were lost. It is in agreement with the findings of Brennan and coworkers<sup>15</sup>. Therefore, the existence of a critical moisture level for each product of these three groups cannot be ruled out. Since, foods are also classified (described) based on their moisture contents<sup>1,3,4</sup>, if a 3-dimensional picture is drawn of various food products, perhaps a better insight could be gained. Therefore, a spatial arrangement of the foods in a space of cohesiveness (WBH/WBC ratio), toughness (WBH kg cm<sup>-2</sup>), and moisture content would give information on the further sub-grouping of foods. Such an exercise (only 2-dimensional, a plot between WBH/WBC ratio and moisture content) revealed that foods varying over the



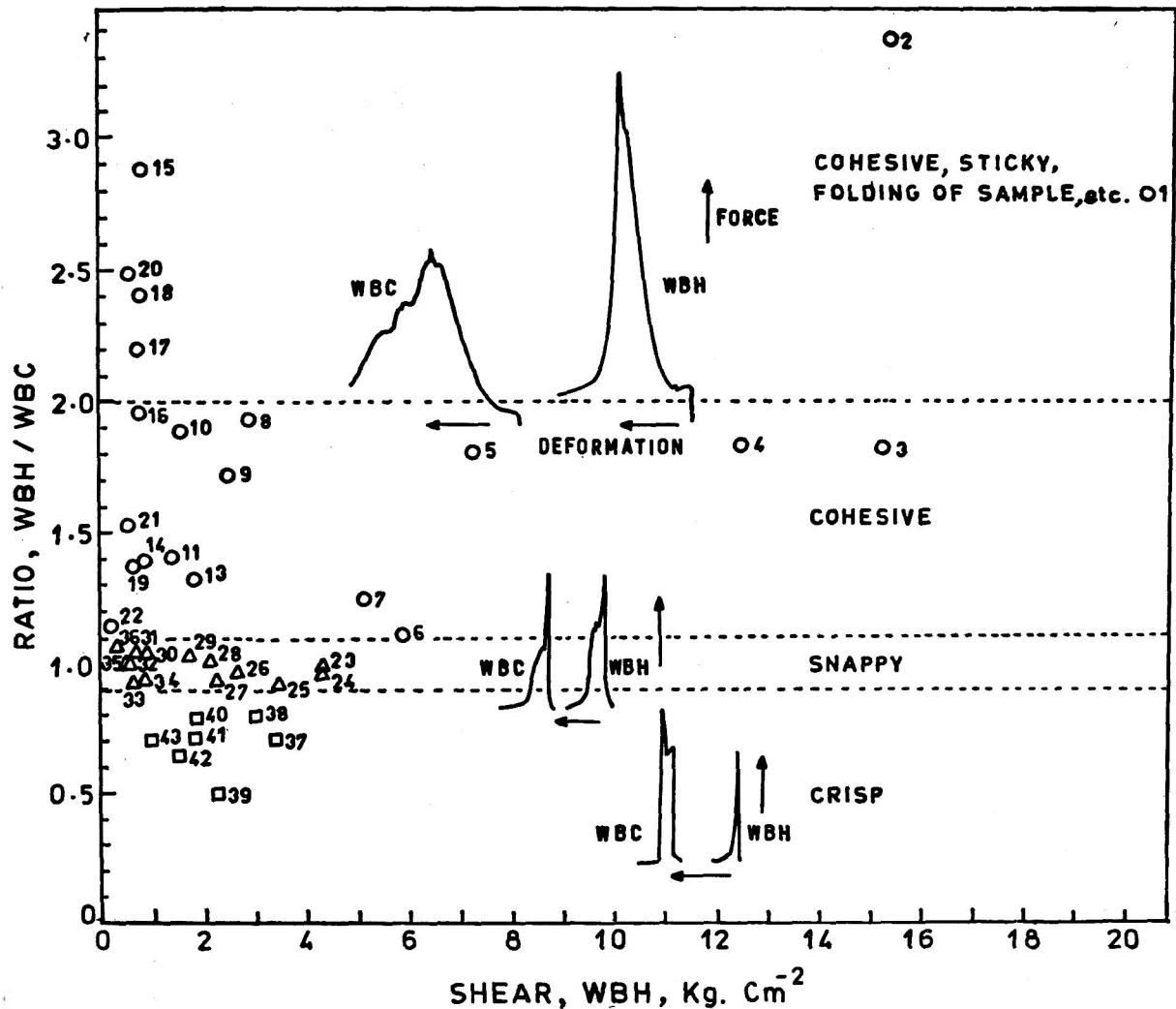


Fig 2. Two-dimensional classification of various food products based on shear stress (toughness) and the ratio of the two shear stresses (WBH/WBC); cohesiveness). Typical force-deformation curves for each group of foods are also shown. The numbers refer to the entries in Tables 1 to 3.

whole range of moisture constitute the Group 1, whereas foods under Groups 2 and 3 polarized into two subgroups, one of very low moisture foods (like biscuits) and the other of very high moisture foods, viz., fruits and vegetables. The snappy or crisp nature of Groups 2 and 3 foods also becomes an index of freshness. In case of dry foods, loss of snappy/crisp behaviour is indicative of exposure to surroundings and poor storage, whereas for fruits and vegetables it indicates staleness, over-storage or over-ripening.

#### Acknowledgement

The author is grateful to Shri P. Veerajju, Shri V. S. Govindarajan, and Miss D. Rajalakshmi for their constructive criticism and suggestions.

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## Kinetics of Phosphine Residue Dissipation from Wheat and Its Milled Products in Storage

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*Manuscript received 28 December 1984; revised 23 May 1985*

A detailed and comparative study of the kinetics of the phosphine (PH<sub>3</sub>) residue in two wheat types, hard brown Punjab wheat and soft white wheat and the milled products of wheat (whole wheat flour, white flour, and semolina) has been made for the first time. Using the linear relationship between log of computed PH<sub>3</sub> residues and days in storage, coefficient of desorption of PH<sub>3</sub> for each of the commodities has been calculated. The change in the magnitude of initial PH<sub>3</sub> residue has been followed over 7 days of airing period. Hard brown Punjab wheat holds the highest, and semolina the least amount of initial residue, and dissipation of this residue is the slowest in whole wheat flour and the fastest in semolina. Due to stratification of desorbed PH<sub>3</sub>, waxing and waning in the desorbed amount of PH<sub>3</sub> occurred. This study has provided an answer not only to the problem of prolonged desorption of PH<sub>3</sub> which was not understood till now, but also evidences for the presence of bound residues of PH<sub>3</sub> in fumigated commodities.

Fumigation of food stocks with pH<sub>3</sub> liberated from aluminium phosphide has gained popularity due to ease of fumigation, low cost and significant insect kill. Persistence of PH<sub>3</sub> residue in fumigated stocks is now a non-controversial matter<sup>1-8</sup>. All over the world food stocks fumigated with PH<sub>3</sub> on commercial scale are not critically aired and gas proof fumigation covers are removed only to dissipate excess of free PH<sub>3</sub>. In our model studies it has been conclusively established that the PH<sub>3</sub> residue is not lost completely from food stocks even when aired by spreading in the open in a thin layer<sup>6-8</sup>. The effect of long contact of persisting PH<sub>3</sub> residues with grain constituents may be the formation of PH<sub>3</sub> reversible chemisorbed residue due to its weak nucleophilic properties as established by the absorption spectra of the chromophores from the fumigated wheat and its milled products<sup>7,8</sup>. These reversible chemically bound residues form the source for prolonged desorption of PH<sub>3</sub> over many months from fumigated

cereals<sup>6,7</sup> and the milled products of wheat<sup>8</sup> with a large excess of fumigant that results in significant PH<sub>3</sub> residues in the commodities. The present study reported here has been done employing a dose of 0.1 g/kg followed by exhaustive airing upto 7 days. The purpose of this study is to give an account of desorption kinetics of free PH<sub>3</sub> formed due to decomposition of reversibly bound PH<sub>3</sub> residues in two types of wheat and milled products of wheat.

### Materials and Methods

Hard brown Punjab wheat and soft white wheat were obtained from the local market. Whole wheat flour (*atta*), white flour (*maida*) and semolina (*soji* or *rawa*) were obtained from International School of Milling Technology of this Institute. Experimental fumigation was done at 2 kg levels in 2.5 l B<sub>40</sub> stoppered flasks placing 0.2g phosfume<sup>R</sup> pellet (aluminium phosphide + pelleting and filling agents) in a filter paper pack

underneath the commodity. The commodity was exposed to  $\text{PH}_3$  for 2 weeks in duplicate, by closing the flask with gas-tight, greased  $\text{B}_{40}$  stopper.

The 10g sample of *atta*, *maida* and *soji* were extracted in 48, 30 and 21 ml  $\text{AgNO}_3$  solution respectively immediately after fumigation for determination of initial  $\text{PH}_3$  residue<sup>5</sup>. The remaining commodities were aired in a thin layer in the open. At the end of 2, 4 and 7 days of airing 10g samples of each commodity were drawn for determination of  $\text{PH}_3$ . After 2, 4 and 7 days of airing four replicates of 200g of each fumigated commodity was sealed in 250 ml conical flasks with a side spout and used to study the desorption of phosphine as reported earlier<sup>6-9</sup>. The flasks were stored at room temperature. The cumulative residues at the end of 2, 4 and 7 days of airing were computed by calculating the total amount of  $\text{PH}_3$  desorbed till the 60th day. Both determined and

computed residue levels of  $\text{PH}_3$  are given in Table 1. Regular study of desorption of  $\text{PH}_3$  from these commodities (Table 2) was continued for 60 days. In all these determinations an equivalent quantity of the untreated sample was used as blank.

### Results and Discussion

Fall of the log (residues) versus days in storage in samples of whole wheat flour, white flour and semolina, aired for different periods is shown in Fig 1. It is clear that this fall is linear upto 10 days in sample of *atta* aired for 2 days, while it is 6 days in samples aired for 4 and 7 days. From thereon the two curves representing 2 and 4 day aired samples run almost close to each other and finally merge with one another on the 33rd day. The curve representing the 7 day aired sample runs all through at a lower level. The shapes of these curves

TABLE 1.  $\text{PH}_3$  RESIDUE (PPM) AND COEFFICIENT OF ITS DESORPTION

	0 days	2 days		4 days		7 days	
		After airing	After 60 days storage	After airing	After 60 days storage	After airing	After 60 days storage
<b>Hard brown Punjab wheat</b>							
Determined <sup>5</sup>	$0.89 \pm 0.27$	$0.13 \pm 0.05$	B	$0.15 \pm 0.09$	B	$0.06 \pm 0.03$	B
Computed	—	$0.49 \pm 0.02$	$0.02 \pm 0.0$	$0.46 \pm 0.02$	$0.02 \pm 0.0$	$0.38 \pm 0.01$	$0.03 \pm 0.0$
Coeff. of desorption (-K)	—	0.64	—	0.82	—	1.50	—
<b>Soft white wheat</b>							
Determined <sup>5</sup>	$0.50 \pm 0.02$	$0.11 \pm 0.05$	B	$0.02 \pm 0.001$	B	$0.03 \pm 0.01$	B
Computed	—	$0.70 \pm 0.19$	$0.03 \pm 0.004$	$0.60 \pm 0.08$	$0.03 \pm 0.003$	$0.46 \pm 0.09$	$0.04 \pm 0.01$
Coeff. of desorption (-K)	—	1.17	—	1.07	—	1.80	—
<b>Whole wheat flour (Atta)</b>							
Determined <sup>5</sup>	$0.47 \pm 0.05$	$0.25 \pm 0.13$	B	$0.24 \pm 0.04$	B	$0.07 \pm 0.01$	B
Computed	—	$0.39 \pm 0.08$	$0.03 \pm 0.01$	$0.36 \pm 0.07$	$0.03 \pm 0.01$	$0.31 \pm 0.06$	$0.03 \pm 0.02$
Coeff. of desorption (-K)	—	0.57	—	0.39	—	0.45	—
<b>Wheat flour (Maida)</b>							
Determined <sup>5</sup>	$0.20 \pm 0.01$	$0.06 \pm 0.01$	B	$0.09 \pm 0.04$	B	$0.03 \pm 0.01$	B
Computed	—	$0.17 \pm 0.01$	0.0	$0.19 \pm 0.03$	0.0	$0.13 \pm 0.02$	0.0
Coeff. of desorption (-K)	—	0.66	—	0.43	—	0.20	—
<b>Semolina (Soji)</b>							
Determined <sup>5</sup>	$0.13 \pm 0.06$	B	B	B	B	B	B
Computed	—	$0.28 \pm 0.09$	$0.20 \pm 0.01$	$0.26 \pm 0.08$	$0.04 \pm 0.0$	$0.22 \pm 0.07$	$0.02 \pm 0.0$
Coeff. of desorption (-K)	—	0.60	—	0.49	—	0.22	—

Mean  $\pm$  SD of 8 replicates.

B=Below estimatable limit of the method

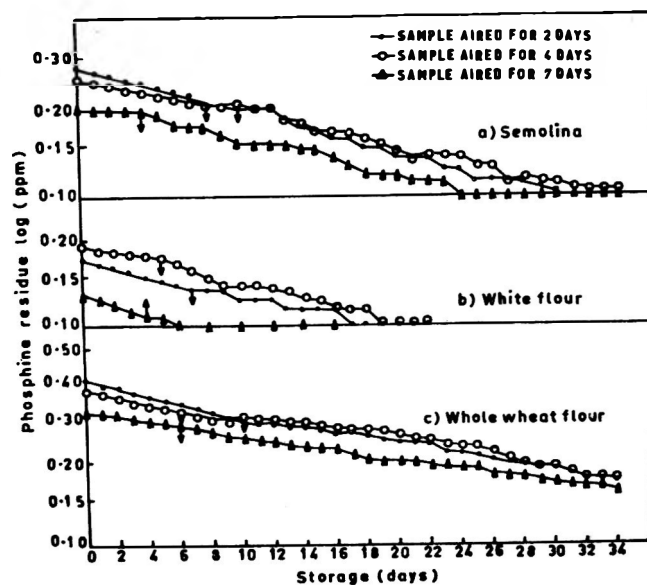


Fig 1. Fall in residues of phosphine in milled products of wheat

(Fig 1) indicate that the rate of fall of  $\text{PH}_3$  residue in all the 3 samples of *atta* is slow.

In case of *maida* the fall of log (residue) versus days is linear for 7, 5 and 4 days in samples aired for 2, 4 and 7 days respectively. The curve representing 4 day aired sample (Fig 1) has started with higher initial residue than the 2-day aired, runs almost parallel to it upto 11 days finally merging with it on 20th day onwards. The corresponding curve for 7-day aired sample runs below the other two upto 6 days and then runs along the days-axis as the residue is levelled off to an almost constant value. During the initial period of storage, the rate of loss in residue is more rapid than in whole wheat flour.

Similarly, the duration of linearity is for 10, 8 and 4 days in samples of 2, 4 and 7 days aired semolina (Fig 1). The curves representing 2 and 4 day aired samples (Fig 1) run almost parallel to one another between 15 and 33 days finally overlapping on the 34th day. The corresponding curve for 7-day aired sample runs below and parallel to the other two upto 24 days and thereafter runs along the days-axis. Irrespective of the period of airing the residue on the 34th day is the same in all the three samples. The rate of fall of residue in 7-day aired sample is slower than in the other two samples of semolina.

Fall of log (residue) during storage in samples of hard brown Punjab wheat (Fig 2) is linear over 15, 13 and 4 days in samples aired for 2, 4 and 7-days respectively while the same in soft white wheat (Fig 2) is linear upto 11, 9 and 5 days respectively in samples aired for corresponding periods. In 2-day aired samples of both types of wheat the fall of residue is linear over certain days is noticed at intervals; as for example 17 to 24 days in

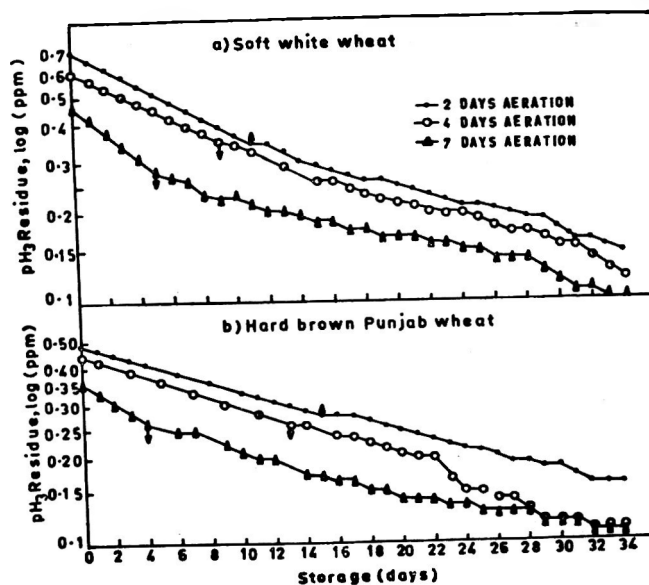


Fig 2. Fall in the residue of phosphine in two types of wheat

hard brown Punjab wheat; 14 to 18, 19 to 24, 25 to 28, 29 to 31 and 32 to 34 days in soft white wheat, which is due to uniform desorption of stratified  $\text{PH}_3$ . On 28th day the residue levels in 4 and 7-day aired samples of hard Punjab wheat have been equalised, while the 4-day aired sample of soft white wheat retains a higher residue than the corresponding 7-day aired sample.

Compared to the milled products, the whole grains of both wheat types retain higher  $\text{PH}_3$  residue on the 34th day in samples aired for shorter durations.

As indicated by the linear fall of  $\text{PH}_3$  residue over a certain number of days, the dissipation of  $\text{PH}_3$  is uniform from these commodities and depends on the initial level of  $\text{PH}_3$  residue in them. The duration of uniform dissipation of  $\text{PH}_3$  is longer in 2 and 4 days aired samples of hard Punjab wheat (Fig 2) than in samples of soft white wheat, and milled products. Two day aired samples of soft white wheat, whole wheat flour and *soji* come next in the order. All the three aired samples of white flour exhibit the least duration of uniform dissipation of  $\text{PH}_3$  residue. Generally 7-day aired samples of wheat types and milled products are the least in this respect due to non-uniformity in the rate of dissipation, although their computed residue levels are nearer to the corresponding 2-day aired samples (Table 1). As seen in Fig 1 and 2, after the linearity period, the rate of dissipation of  $\text{PH}_3$  residue from these commodities is non-uniform probably due to stratification<sup>5-8</sup> and to some extent to oxidation both in the commodity and in the side tube.

As shown earlier<sup>7,8</sup> the rate of desorption during the linearity period of the curve obeys first order kinetics represented by the equation:

$$\log(a - x) = -\frac{K}{2.303} t + \log a$$

where  $(a - x)$  is the residue in ppm at 24 hr,  $t$  is time, viz. 24 hr at the end of which the residue is computed.  $a$  is the initial residue in ppm, so  $\log a$  is constant,  $K$  is the constant of desorption, negative sign for  $K$  appears in the equation because of desorption.

The coefficient of desorption of  $\text{PH}_3$ ,  $-K$ , has been calculated for each sample, using the relation  $\tan \theta =$

$\frac{K}{2.303}$  derived from the equation shown above; where

$\tan \theta$  is the slope of the linear portion of any desorption curve, and the values are shown in Table 1. As demonstrated earlier<sup>7,8</sup> soft white wheat aired for 7 days (Table 1) having the highest value for  $K$  (1.80) is having the fastest rate of desorption of  $\text{PH}_3$  while that of 7 days aired sample of white flour having the least value for  $K$  (0.20), is having the slowest rate of desorption. Next in the order of decrease in the rate of desorption based on  $K$  value, are hard brown Punjab wheat aired for 2 days, ( $K=1.17$ ), soft white wheat aired for 4 days ( $K=1.07$ ), hard brown Punjab wheat aired for 4 days ( $K=0.82$ ), white flour aired for 2 days ( $K=0.66$ ) hard brown Punjab wheat aired for 2 days ( $K=0.64$ ), semolina aired for 2 days ( $K=0.60$ ), whole wheat flour aired for 2 days ( $K=0.57$ ), semolina aired for 4 days ( $K=0.49$ ) whole wheat flour aired for 7 days ( $K=0.45$ ), white flour aired for 4 days ( $K=0.43$ ), whole wheat flour aired for 4 days ( $K=0.39$ ), and semolina aired for 7 days ( $K=0.22$ ). So it may be said that the coefficient of desorption not only indicates the rate of desorption of  $\text{PH}_3$  but also the  $\text{PH}_3$  retaining capacity of the individual samples. Although the differential capacity of these samples of five commodity types examined for desorbing their  $\text{PH}_3$  residues by different rates has not been studied yet, the bondage of  $\text{PH}_3$  with receptive sites of low electron density of the constituents of these commodities may in part be responsible. The evidence in part for such a proposition are available from the absorption spectra of  $\text{AgNO}_3\text{-PH}_3$  residue chromophore from these commodity types.

Under identical conditions of fumigation and handling hard brown Punjab wheat holds the highest initial residue of 0.89 ppm, next in the order are soft white wheat, whole wheat flour, white flour and semolina (Table 1). Of the two types of wheat examined, hard brown Punjab wheat holds nearly twice the initial residue than soft white wheat. Although the computed residue levels in these five commodities decrease as the airing period is prolonged (Table 1), the determinable residues show variance (Table 1). Such anomaly is perhaps, due to that portion of the commodity nearer to the aluminium phosphide packet which would have absorbed more of

TABLE 2. DISSIPATION OF  $\text{PH}_3$  RESIDUE (PPM) FROM FUMIGATED AND AIRED COMMODITIES IN STORAGE

Storage period (days)	2 days aired	4 days aired	7 days aired
<b>Hard brown Punjab wheat</b>			
35	0.007 ± 0.001	0.005 ± 0.000	0.0
39	0.019 ± 0.001	0.014 ± 0.001	0.012 ± 0.001
43	0.020 ± 0.001	0.020 ± 0.001	0.016 ± 0.001
47	0.019 ± 0.001	0.018 ± 0.001	0.022 ± 0.001
51	0.026 ± 0.001	0.016 ± 0.001	0.017 ± 0.001
55	0.025 ± 0.001	0.023 ± 0.001	0.014 ± 0.000
59	0.031 ± 0.002	0.008 ± 0.000	0.009 ± 0.000
60	0.005 ± 0.001	0.0	0.007*
<b>Soft white wheat</b>			
35	0.009 ± 0.003	0.007 ± 0.001	0.002 ± 0.000
39	0.022 ± 0.006	0.018 ± 0.003	0.009 ± 0.003
43	0.028 ± 0.004	0.023 ± 0.004	0.014 ± 0.003
47	0.021 ± 0.004	0.016 ± 0.002	0.005 ± 0.003
51	0.021 ± 0.004	0.019 ± 0.001	0.019 ± 0.005
55	0.021 ± 0.003	0.021 ± 0.005	0.014 ± 0.006
59	0.018 ± 0.003	0.012 ± 0.002	0.027 ± 0.008
60	0.006 ± 0.001	0.006 ± 0.001	0.010 ± 0.001
<b>Whole wheat flour (Atta)</b>			
35	0.007 ± 0.002	0.004 ± 0.001	0.005 ± 0.002
39	0.022 ± 0.007	0.016 ± 0.002	0.020 ± 0.005
43	0.025 ± 0.007	0.029 ± 0.008	0.025 ± 0.004
47	0.022 ± 0.006	0.023 ± 0.010	0.013 ± 0.005
51	0.029 ± 0.007	0.016 ± 0.002	0.014 ± 0.001
55	0.014 ± 0.004	0.015 ± 0.003	0.029 ± 0.006
59	0.022 ± 0.004	0.017 ± 0.003	0.023 ± 0.002
60	0.005 ± 0.003	0.008 ± 0.003	0.005 ± 0.001
<b>White flour (Maida)</b>			
35	0.0	0.0	0.006 ± 0.002
39	0.011 ± 0.001	0.018 ± 0.003	0.013 ± 0.000
43	0.022 ± 0.001	0.020 ± 0.007	0.005 ± 0.001
47	0.010 ± 0.001	0.005*	0.0
51	0.007 ± 0.001	0.0	0.007*
55	0.009*	0.003*	0.0
59	0.0	0.005*	0.0
60	0.0	0.0	0.0
<b>Semolina (Soji)</b>			
35	0.004 ± 0.000	0.004 ± 0.002	0.003 ± 0.002
39	0.009 ± 0.006	0.013 ± 0.004	0.013 ± 0.004
43	0.015 ± 0.007	0.014 ± 0.007	0.007 ± 0.003
47	0.012 ± 0.009	0.016 ± 0.009	0.019 ± 0.002
51	0.018 ± 0.010	0.011 ± 0.004	0.012 ± 0.006
55	0.015 ± 0.004	0.013 ± 0.009	0.008 ± 0.002
59	0.015 ± 0.006	0.010 ± 0.006	0.019 ± 0.004
60	0.008 ± 0.003	0.008 ± 0.000	0.003 ± 0.000

Means ± SD of replicates

\*One sample was analysed.

PH<sub>3</sub> getting to the samples when random replicate samples were drawn for analysis, while such anomaly has not been observed with samples fumigated with gaseous phosphine. Semolina is unique in not showing the determinable PH<sub>3</sub> residue even at the end of the 2-day airing. As indicated by their K values, this absence of determinable residue is not due to fast and complete dissipation of free PH<sub>3</sub> but suggests the rapid formation of interaction complexes of PH<sub>3</sub> which in turn forms the source for such prolonged desorption. White flour is unique in not having the computed residues at the end of 60 days of storage, this may be due to decomposition of unstable PH<sub>3</sub> interaction complexes resulting in complete dissipation of resultant PH<sub>3</sub> or due to formation of firmly bound irreversible complexes.

Examination of the desorbed amounts of PH<sub>3</sub> in Table 2 reveal the following points: On 35 day all the commodities show either less or no desorption, which gradually increases due to decomposition of PH<sub>3</sub> interaction complexes. Generally, 2-day aired samples of hard brown Punjab wheat, soft white wheat and whole wheat flour desorb larger amounts of PH<sub>3</sub> than their 4 and 7 days aired counterparts. Except for 2-day aired samples of hard brown Punjab wheat, which shows a continuous increase in the amount of PH<sub>3</sub> desorbed over 35 to 59 days, all other samples of five commodities show waxing and waning in the amount of PH<sub>3</sub> desorbed, because of establishment of stratification in the bulk of commodity due to the top layer resorbing the PH<sub>3</sub> desorbed by the bottom layers<sup>6-8</sup>. So on such days free PH<sub>3</sub> available for determination is less. On the other hand, when the PH<sub>3</sub> holding capacity of the commodity is exceeded, large amounts of PH<sub>3</sub> is desorbed and on such days large amounts of free PH<sub>3</sub> have been found.

Finally, it may be mentioned that for the first time during the last 50 years of PH<sub>3</sub> fumigation, a detailed

and comparative study about the kinetics and the fate of PH<sub>3</sub> residue in wheat types and milled products have been made. This study has not only provided an answer to the problem of prolonged desorption of PH<sub>3</sub> from fumigated commodities, which has not been understood till now, but also has provided circumstantial evidences for the presence of bound residues of PH<sub>3</sub> in commodities.

#### Acknowledgement

The authors thank Mr. M. Muthu for his critical comments, Mr. S. K. Majumder, Deputy Director for his interest and Dr. B. L. Amla, Director for his interest and encouragement.

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## RESEARCH NOTES

### EFFECT OF POPPING AND BOILING ON PROTEIN QUALITY OF MAIZE (*ZEA MAYS* L.) KERNELS

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*Manuscript received 23 April 1984; revised 13 May 1985*

The effect of popping and boiling of maize kernels on the *in vitro* digestibility of protein and on lysine and tryptophan was evaluated. Dye binding capacity and lysine content were slightly increased by boiling and decreased by popping. *In vitro* protein digestibility improved in pop corn and sweet corn kernels after popping and boiling respectively whereas it decreased slightly in normal and 'Opaque-2' maize following these treatments.

Maize is consumed in different forms. Sweet corn is consumed after boiling in water to soft consistency while pop corn is eaten after popping. Flint kernels may be used after boiling or roasting. However, information on the effect of boiling and popping on lysine, tryptophan and *in vitro* protein digestibility is not available. The present study was aimed at evaluation of the above

mentioned parameters of fully ripe maize kernels after popping and boiling.

Four maize varieties namely 'Shakti' ('Opaque-2' composite), 'Vijay' (with semiflint kernels), 'TV Time' (pop corn) and 'Hawaiian Sugar' (sweet corn) grown at the Indian Agricultural Research Institute, New Delhi during the monsoon season were used in the study.

Clean 20 mesh sand heated on a gas stove was used for popping of kernels having  $11 \pm 1$  per cent moisture. Preliminary investigations indicated that 5 min of heating was sufficient to adequately heat the sand to the temperature ( $230 \pm 5^\circ\text{C}$ ) required for popping of the kernels. For cooking, kernels of 'Vijay', 'Shakti' and sweet corn were kept in boiling water for 40-45 min (1:3 wt/vol.) and then lyophilized to dryness. One hundred grams of sample in triplicate of each variety were popped and boiled separately and were later ground to 60 mesh in a hammer mill. Crude protein ( $\text{N} \times 6.25$ ) was estimated by micro-Kjeldahl<sup>1</sup> method, while lysine and tryptophan were determined colorimetrically<sup>2,3</sup>. Dye-binding capacity (DBC) was determined by the procedure as described earlier<sup>4</sup>. *In vitro* protein digestibility (IVPD) was determined by the method of Saunders and Kohler<sup>5</sup> with slight modification using pronase and trypsin, according to Gupta *et al.*<sup>6</sup>

Protein content was highest in 'Hawaiian Sugar',

TABLE 1. PROTEIN, LYSINE, TRYPTOPHAN AND DBC VALUES IN DIFFERENT MAIZE VARIETIES FOLLOWING TWO TREATMENTS (ON DRY WEIGHT BASIS)

	'Vijay' composite			'Shakti' composite			'TV Time' pop corn		'Hawaiian Sugar'	
	Control	Popped	Boiled	Control	Popped	Boiled	Control	Popped	Control	Boiled
Protein (%)	9.71 $\pm 0.50$	9.67 $\pm 0.19$	9.68 $\pm 0.13$	12.23 $\pm 0.11$	12.16 $\pm 0.03$	12.11 $\pm 0.19$	14.93 $\pm 0.76$	14.82 $\pm 0.39$	15.14 $\pm 0.78$	15.10 $\pm 0.43$
Lysine (g/16g N)	2.52 $\pm 0.09$	2.44 $\pm 0.02$	2.56 $\pm 0.11$	4.69 $\pm 0.13$	4.37 $\pm 0.17$	4.80 $\pm 0.06$	1.92 $\pm 0.03$	1.90 $\pm 0.03$	3.75 $\pm 0.01$	3.85 $\pm 0.07$
Tryptophan (g/16g N)	0.49 $\pm 0.03$	0.48 $\pm 0.01$	0.48 $\pm 0.02$	0.94 $\pm 0.02$	0.80 $\pm 0.04$	0.96 $\pm 0.03$	0.29 $\pm 0.02$	0.25 $\pm 0.02$	0.50 $\pm 0.02$	0.56 $\pm 0.01$
Chemical score	46.00 $\pm 1.69$	44.36 $\pm 0.37$	46.55 $\pm 1.91$	85.27 $\pm 2.44$	79.45 $\pm 3.09$	87.27 $\pm 1.04$	34.91 $\pm 0.60$	34.55 $\pm 0.49$	68.18 $\pm 0.17$	70.00 $\pm 0.91$
DBC value (optical density)	0.209 $\pm 0.004$	0.175 $\pm 0.001$	0.231 $\pm 0.003$	0.283 $\pm 0.004$	0.246 $\pm 0.002$	0.291 $\pm 0.003$	0.239 $\pm 0.002$	0.263 $\pm 0.007$	0.320 $\pm 0.004$	0.331 $\pm 0.007$

Paper presented at the Poster Session in Symposium on Third Indian Convention of Food Scientists and Technologists, India, held at CFTRI, Mysore (India) on 2-4 June, 1983.

TABLE 2. *IN VITRO* PROTEIN DIGESTIBILITY (PER CENT) OF DIFFERENT MAIZE VARIETIES FOLLOWING POPPING AND BOILING\*

	'Vijay' composite	'Shakti' composite	'TV Time' Popcorn	'Hawaiian Sugar'
Control	85.20 ± 1.35	80.13 ± 1.11	68.23 ± 1.68	73.91 ± 3.09
Popped	81.90 ± 3.56	72.47 ± 3.52	84.48 ± 2.16	—
Boiled	83.77 ± 1.95	72.08 ± 2.82	—	82.49 ± 1.17

\* on dry wt basis.

followed by 'TV Time'. Lysine content was highest in 'Shakti' followed by 'Hawaiian Sugar' whereas 'TV Time' popcorn had the lowest (Table 1). The chemical score, based on FAO/WHO<sup>7</sup> requirement of lysine (5.5 g/16g N) for pre-school children was particularly low for the untreated kernels of 'Vijay' and 'TV Time' popcorn. Popping led to reduction whereas boiling caused increase in lysine, tryptophan and improvement in chemical score. However, these changes were not significant among varieties evaluated.

Among control samples, 'Hawaiian Sugar' had the highest DBC value, followed by 'Shakti', 'TV Time' popcorn and 'Vijay'. Upon boiling, the DBC value increased in 'Vijay' (10.5 per cent); however, following popping, a reduction in DBC by 13.1 to 16.3 per cent was observed among the different varieties.

*In vitro* protein digestibility (Table 2) varied considerably among the varieties; it was highest for 'Vijay' followed by 'Shakti,' Hawaiian Sugar' and lowest in 'TV Time' popcorn. After popping and boiling the digestibility decreased in 'Vijay' and 'Shakti' while for 'Hawaiian Sugar' and 'TV Time' popcorn, it increased by 11.9 and 22.5 per cent respectively.

Data collected in the present study indicated that boiling of maize varieties of various endosperm types increased the DBC values. This may probably be due to release of certain free amino acids following hydrolysis of peptide bonds during boiling. *In vitro* protein digestibility was increased in 'TV Time' popcorn after popping and in 'Hawaiian Sugar' after boiling but decreased in 'Shakti' through either treatment. This decrease is similar to what was reported for dent corn by Eggum<sup>8</sup>. Increase in digestibility may be due to the increase in volume after popping (about five fold) and boiling which would lead to more efficient enzymatic digestion<sup>9,10</sup>.

Lyophilizer facility provided by Dr. S. L. Mehta, Nuclear Research Laboratory is gratefully acknowledged.

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## SHELF LIFE OF ANHYDROUS (DRY) BUTTER FAT MADE UNDER DIFFERENT CONDITIONS OF THERMAL CLARIFICATION OF CENTRIFUGALLY SEPARATED MELTED BUTTER

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*Manuscript received 17 September 1984; revised 14 June 1985*

Studies on the shelf life of anhydrous (dry) butter fat prepared by thermal clarification of centrifugally separated melted butter in a ghee pan at three heat treatments, 85°C for 45 min (Product A), 90°C for 30 min (Product B) and 90°C for 45 min (Product C) have shown that during storage over a period of 3 months, Product B was superior to Product A and Product C. It has registered the lowest deterioration as indicated by TBA values and organoleptic quality. This was further confirmed by organoleptic evaluation of the recombined milk.

Anhydrous (dry) butter fat similar to butter oil made by heat clarification of centrifugally separated melted

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butter in the conventional *ghee* making equipment can be used more purposefully than *ghee*, either for production of recombined milk or other products. However, if the product is to be of commercial importance, it has to have an acceptable shelf life. The changes in chemical and organoleptic quality attributes of the anhydrous butter fat during storage are reported in this paper.

Anhydrous butter fat was made as per the procedures of Prasad and Bhanumurthi<sup>1</sup> by thermal clarification of centrifugally separated melted butter with an approximate moisture content of 1.05 per cent in a *ghee* pan at time temperature combinations of 85°C/45 min (Product A) 90°C/30 min (Product B) and 90°C/45 min (Product C). After cooling, the anhydrous butter fat was filtered through a double folded muslin cloth and was packed in sanitised lacquered tins of 250 ml capacity. The trials were carried out in duplicate and the average values reported. The tins were stored separately at 30±1°C and 37±1°C. The samples were analysed in duplicate initially and at monthly intervals during a storage period of 3 months. Recombined milk samples were made to have 3.1 per cent fat with the anhydrous butter fat and reconstituted skim milk and given to the judging panel for sensory evaluation. The anhydrous butter fat was analysed for moisture<sup>2</sup>, total acidity<sup>2</sup> and free fatty acid<sup>1</sup> (FFA)<sup>2</sup> content and thiobarbituric acid (TBA) value<sup>3</sup>. The anhydrous butter fat was evaluated on a 9-point Hedonic scale by a panel of judges and the recombined milk, by the score card method, with 10 points for colour, 20 for odour, 40 for taste and 30 for body. The rating given to the recombined milk was, excellent for a score of 90 points and above, 80-89 good, 60 to 79 fair and 50 and below poor.

**Moisture content:** At the storage temperatures of both 30±1 and 37±1°C, no appreciable change in the moisture content was observed during the 3-month storage period.

**Free fatty acid (FFA) content:** A small increase in the FFA content was observed (Table 1) during storage in Products A, B and C. The U.S. Military Specifications<sup>4</sup> permit a maximum of 0.5 per cent for FFA in butter oil and hence the products in the present study remained acceptable at the end of 3 months storage period.

**Thiobarbituric acid (TBA) value:** The TBA values increased (Table 1) during storage in all the three products at both the temperatures of storage, the final values being higher at the higher temperature. Gaba and Jain<sup>5</sup> reported that TBA values showed a more consistent relationship to sensory evaluation data as compared to FFA and peroxide values in *ghee* during storage. Bhatia<sup>6</sup> reported a higher initial TBA value and a four fold increase during storage of *ghee* made by heating centrifugally separated melted butter with cultured butter milk at 120°C. The anhydrous butter fat in the present study has undergone much less deterioration as compared to *ghee* similarly made excepting for higher temperature of clarification employed.

**Organoleptic evaluation of anhydrous butter fat:** The organoleptic evaluation of the anhydrous butter fat stored showed that the product which had an average score of 8 (liked very much) initially lost score slightly during the 3 month storage and had values of 7.6 and 7.4 at the end of 3 months at 30±1 and 37±1°C respectively. Product C which had a slight cooked (*ghee* like) flavour scored the minimum of 6.9 points. Product A with a less intensive heat treatment at manufacture had a higher score than Product C but was rated inferior to Product B. Vyas and Vyas<sup>7</sup> studying the shelf life of butter oil, reported a more rapid decrease in the flavour score during storage at room temperature for 4 months.

**Organoleptic evaluation of recombined milk:** Recombined milk made with product B got the maximum average score of 88.79 initially (Table 2) corresponding to a rating of 'good' and lost only less than 2 points

TABLE 1. EFFECT OF STORAGE ON THE QUALITY OF ANHYDROUS BUTTER FAT

Quality attributes	Storage temp. (°C)	Values at indicated temp. of clarification and period of storage (months)					
		85°C/45 min		90°C/30 min		90°C/45 min	
		0	3	0	3	0	3
FFA (as oleic acid %)	30	0.23	0.24	0.23	0.25	0.23	0.25
	37	0.23	0.25	0.23	0.28	0.23	0.26
TBA value (Klett reading)	30	16	20	15	17	16	19
	37	16	22	15	20	16	21
*Flavour	30	7.5	7.0	8.0	7.6	6.9	6.9
	37	7.5	6.8	8.0	7.4	6.9	6.7

\*Average score got by organoleptic evaluation

TABLE 2. ORGANOLEPTIC EVALUATION OF RECOMBINED MILK MADE FROM ANHYDROUS BUTTER FAT STORED AT  $30 \pm 1^\circ\text{C}$  AND  $37 \pm 1^\circ\text{C}$

Quality attributes	Storage temp. ( $^\circ\text{C}$ )	Max. score	Scores at indicated temp. of clarification and periods of storage (months)					
			85 $^\circ\text{C}/45$ min		90 $^\circ\text{C}/30$ min		90 $^\circ\text{C}/45$ min	
			0	3	0	3	0	3
Colour	30	10	7.9	7.8	8.9	9.0	7.7	7.9
	37		7.9	7.4	8.9	8.5	7.7	7.7
Odour	30	20	13.1	11.5	16.8	16.5	14.3	13.8
	37		13.1	11.3	16.8	16.3	14.3	13.5
Taste	30	40	25.1	23.3	36.0	33.5	28.8	27.5
	37		25.1	28.5	36.0	33.3	28.8	27.0
Body	30	30	25.1	23.9	27.1	28.5	24.1	23.5
	37		25.1	24.0	27.1	29.0	24.1	22.5
Total	30	100	71.3	66.4	88.8	87.5	74.8	72.6
	37		71.3	66.1	88.8	87.0	74.8	70.7

when the recombined milk was made with 3 months old product stored at  $30 \pm 1$  or  $37 \pm 1^\circ\text{C}$ . These results further confirmed the observations made on the TBA values and organoleptic quality of the anhydrous butterfat: to establish the superiority of Product B. Product C which got a lower score than Product A as anhydrous butter fat gave a higher score for recombined milk made therefrom than from Product A, indicating that the slight cooked flavour observed was probably masked when converted into recombined milk.

Hence it is concluded that anhydrous butter fat made by thermal clarification at  $90^\circ\text{C}$  for 30 min of centrifugally separated butter was superior both initially and after storage for a period of 3 months at  $30 \pm 1$  or  $37 \pm 1^\circ\text{C}$ .

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#### NUTRITIVE EVALUATION OF HIGH PROTEIN FOODS

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*Manuscript received 5 July 1984; revised 7 June 1985*

Evaluation of five different high protein foods for protein efficiency ratio values indicated the superiority of the preparation based on casein over others which are based on predigested milk protein, or those subjected to heat processing or those which are based on vegetable proteins like soy or groundnut. The preparation based on groundnut protein hydrolysate did not sustain growth of weaning rats when fed as the sole source of protein.

Indian standard specification for protein rich concentrated nutrient supplementary foods requires<sup>1</sup> among others that they should have a protein efficiency ratio (PER) of 2.0. With this in view some of the high protein food formulations available in the Indian market were evaluated for protein quality by determining their PER.

TABLE 1. CHARACTERISTICS OF HIGH PROTEIN FOODS

Preparation	Protein content (%)	Ingredients		Physical form	Form while consuming
		Protein	Others		
A	54	Casein	Hydrogenated fat, sugars	Tablets	Like a biscuit
B	51	Casein hydrolysate	Sugar, vitamins	Granules	To be reconstituted with milk
C <sub>1</sub>	42 )	Skim milk powder and solvent extracted soy meal	Hydrogenated fat, iron salt, sorbitol, vitamins	Fine powder	,,
C <sub>2</sub>	42 )				
C <sub>3</sub>	42 )				
C <sub>4</sub>	42	Skim milk powder heated soy meal	,,	,,	,,
D	51	Vegetable protein isolate, skim milk powder	Malt extract powder, vitamins, minerals	Powder	,,
E	51	Groundnut protein hydrolysate	Malt extract, vitamins, minerals	Light granules	,,

The characteristics of the foods tested are shown in Table 1. PER was determined according to the method specified by I.S.<sup>2</sup> and the experimental values were corrected.

Data on PER values of different protein rich foods are presented in Table 2. Preparation A based on casein

TABLE 2. PER VALUES OF HIGH PROTEIN FOODS

Preparations	Wt gain (g)	Protein intake (g)	PER	
			Observed	Corrected
Casein	81.7±5.9	28.1±2.3	2.9±0.3	2.5
A	81.8±5.4	25.6±1.8	3.2±0.2	2.7±0.1
Casein	76.8±6.0	29.7±0.9	2.5±0.4	2.5
B	47.5±7.5	25.5±0.6	1.8±0.2	1.8±0.2
Casein	88.2±8.0	34.4±3.9	2.6±0.2	2.5
C <sub>1</sub>	65.1±3.2	32.2±2.8	2.0±0.1	1.9±0.1
Casein	81.7±5.9	28.1±2.3	2.9±0.3	2.5
C <sub>2</sub>	49.9±6.7	26.3±2.5	1.9±0.2	1.6±0.4
Casein	93.5±5.3	39.3±3.1	2.4±0.2	2.5
C <sub>3</sub>	58.1±4.9	30.6±3.8	1.9±0.1	2.0±0.1
Casein	88.2±8.0	34.4±3.9	2.6±0.2	2.5
C <sub>4</sub>	60.7±8.6	26.4±2.1	2.3±0.2	2.2±0.2
Casein	76.8±6.0	29.7±0.9	2.5±0.4	2.5
D	—	—	—	—
Casein	76.8±6.0	29.7±0.9	2.5±0.4	2.5
Unheated D	18.2±6.1	19.4±1.3	0.9±0.2	0.9±0.2
Casein	88.2±8.0	34.4±3.9	2.6±0.2	2.5
E*	—	—	—	—

Results expressed as mean±S.D. of three individual expts.

\*Rats discarded after three weeks as there was no weight gain.

had a PER slightly higher than that of reference protein, casein whereas Preparation B based on predigested milk protein had a PER of about 75 per cent of that of casein. As this product is prepared by drying the hydrolysed protein in the presence of other ingredients including sugar, the lower nutritive quality is presumably the result of this operation. Preparation C, selected from different processing batches and designated as C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> had PER values ranging between 1.9 and 2.3. The highest PER of 2.3 was shown by the preparation C<sub>4</sub>, which contained precooked soy flour as against the other three preparations, in which, solvent extracted soymeal was incorporated. Soybeans contain several antinutritional factors, which are mostly thermolabile<sup>3</sup>. It may therefore be inferred that the PER of soy protein based preparation will depend upon the extent of heat denaturation of the antinutritional factors. The variation observed in the PER values of the Preparation C manufactured in different batches emphasises the need for consistent quality control of the major raw material soy flour, particularly with respect to adequate inactivation of the antinutritional factors.

A very interesting observation was that Preparation D and Preparation E based on groundnut protein isolate plus skim milk powder and groundnut protein hydrolysate respectively, failed to sustain any growth of weanling rats. The food intake of animals fed these preparations was very poor and the experiments had to be discontinued after three weeks. The low intake of food in these cases, reflects deficiencies of essential nutrients, principally of essential amino acids. The PER of the mix containing a blend of unheated constituents of Prepa-

ration D was found to be 0.9, which suggested that the lack of growth sustaining ability of Preparation D is probably due to compositional imbalance, improper conditions during preparation of isolate itself, as well as thermal damage during the drying of the mix. For Preparation E, the poor performance could be the result of cross linking of sugars and amino acids during the thermal drying process<sup>4-6</sup>. In fact, these studies indicate that a product based on protein hydrolysate was inferior to that based on the intact protein. One reason for this may be poor utilisation of the hydrolytic products due to their rapid absorption resulting in partial wastage<sup>7</sup>.

Thus the present study emphasizes the need for (1) optimising thermal treatment in the preparation of high protein food formulations so as to inactivate any thermolabile antinutritional principles and minimising protein interactions with other ingredients, mainly sugars, and (2) the need for evolving controlled procedure in the preparation of products based on protein hydrolysates or those containing such indirectly hydrolysed ingredients as malt extract so that the nutrients remain in a form 'available' to the consumer.

It must, however, be pointed out that since most of the market products are recommended as nutritive

supplements, the PER values determined by experiments using these as the sole source of protein, may not reflect correctly the efficacy of these products.

The authors gratefully acknowledge M/s. Raptakos Brett and Co. Pvt. Ltd., Bombay for sponsoring the above project.

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## BOOK REVIEWS

*Trends in Modern Meat Technology*: Compiled by B. Krol, P. S. Van Roon and J. H. Houben. Proceedings of the International Symposium on Trends in Modern Meat Technology, Centre for Agricultural Publishing and Documentation, P.O. Box 4,700, AA, Wageningen, Netherlands, 1985; pp. 125; Price: f 50.00/US \$ 20.00. (outside USA and Canada).

Papers from Netherlands, Holland, United Kingdom, Denmark, France, Germany and Finland were discussed in the Symposium.

The Symposium was organised in eight sessions and the summary and conclusions of each session have also been reported.

In the first session on perspectives, three papers highlighted the need of utilizing the results of research in other areas, emphasis on eating habits, factors of socio-ecological and socio-historical origin, local and regional preferences, consumer's concern for the quality of meat produced at the centres far away from the consuming centres and governmental role in assessing and control of quality.

The second session on computer aiding comprised two papers dealing with recent advances in computerisation and robotics in meat industry. A few applications like automatic analyses in an abattoir, optimization of fat content in comminuted products, video image analysis and its application to grading of beef carcasses, robotic butchering and linking of sausages, deboning of bacon and portion cutting of meat chunks were described. P. A. technology is expected to make a major dent in 5-10 years. Computer calculations for completion of chilling have been reported.

Improvement of meat quality and reduction of labour costs by improved gadgetary, and by modern techniques of hot-boning and automated electrical stimulation formed the subject matter of the two papers presented in the third session.

Four papers were presented in the fourth session on product technology; bacon, fermented sausages, new technology of bacon production, sausage extrusion process, meat (bio) technology of fermented sausages and sensory properties were discussed.

In the fifth session on process technology—'emulsions', three papers focussed the attention on physio-chemical principles involved in the formation and stability of emulsions, continuous emulsification process and mathematical models for cooked sausages.

Hot processing, parameters of binding strength,

cohesiveness of cooked ham and pasteurised meat products in coextruded bags were discussed in the three papers presented in the sixth session. Influence of hot boning on curing, chilling, concentration of salt in relation to mechanical treatment, 'cook-in-bag' container method were discussed.

In the seventh session on product technology—'liver products', two papers dealt with the incorporation of liver in comminuted meats (sausages), sliceable and spreadable products. More fundamental research on liver products was recommended.

The eighth session on speciality foods was devoted to processing of low sodium products, production and processing of reindeer meat, a vital industry for nomadic people of polar region of Finland. The last paper by Dr. D. B. Macdougall, admirably summarises the deliberations of the Symposium. Interrelationship between consumer, quality control, use of computer, high technology, product development, materials (quality), processes and product (yield and quality) and profit has been clearly brought out.

The authors of the papers have reported some new findings and applications, pointing out the trends in meat processing.

The book is worthy of possession by students and practicing meat technologists.

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

*Environmental Health Criteria 49—Acrylamide*: World Health Organisation (WHO), Geneva, 1985; pp: 121; Price: Sw. fr. 12.

Acrylamide also known as propenamide, propenoic acid amide is a white crystalline compound, highly soluble in water. This arises from residual acrylonitrile present in technical grades of polyacrylamides in the range 1 to 100 ppm. Commercial polyacrylamides contain 0.05 to 5.0 per cent acrylamide, and these polymers are used as flocculates in the treatment of waste and drinking water, soil, grouting agent in foundations of dams and tunnels. Thus acrylamide monomer gets into atmosphere as a pollutant.

A WHO task force has prepared a good account of this pollutant covering all aspects including an exhaustive

bibliography which runs into 20 pages. Although no method has been described for determination of acrylamide bound to blood and tissue proteins and its metabolites in urine, GC and HPLC have been cited for its determination in air and water. It is unlikely to enter and be transported in the atmosphere to any significant extent because of low vapour pressure, as air and soil also close to acrylamide producing factories fail to show any residue. Biodegradation is likely to occur as a variety of microbes possess ability to degrade it. In water it remains unchanged. In most countries its residue in potable water is limited to 0.25  $\mu\text{g/L}$ .

Occupational exposure occurs mainly through skin and inhalation in manufacturing plants. Absorbed compound is distributed in body water compartments and passes through the placental barrier. Acrylamide and its metabolite accumulate in nerve tissues and blood as protein-bound and haemoglobin-bound respectively in addition to liver, kidney and male reproductive system. The compound is neither mutagenic nor carcinogenic.

The monograph ends with some good suggestions for further work, on methods for analysis in blood, metabolism, and any other effects not studied yet. It is priced at Sw. fr. 12. Indeed a useful bulletin to possess.

J. R. RANGASWAMY  
C.F.T.R.I., MYSORE

*Food Analysis—Principles and Techniques, Volume 3; Biological Techniques:* Edited by Dieter W. Gruenwedel and John R. Whitaker, Marcel Dekker Inc., New York and Basel, 1985; pp: 395; Price: bound \$ 75 (US and Canada).

The book is one of the eight volumes on the subject of food analysis that describes and discusses in detail a number of biological techniques, involving a range of systems starting from the whole animal to pure enzymes, that are of vital importance to the quality and safety assessment of foods, particularly in the areas of nutrition, food science and food toxicology. The six chapters in the book may be considered broadly under two methodological approaches: (1) employing live systems as analytical tools (Chapters 1,2 and 4) and (2) using 'extracellular biochemical techniques' (Chapters 5 and 6). Chapter 3 may be considered to contain materials from both. Each chapter ends with a summary and has been written by an expert(s).

The first chapter reviews the biological techniques using whole animal as an analytical tool, which is of particular importance in the nutritional as well as toxicological evaluation of foods. Though the method is expensive and time consuming, it has been shown to

provide combined assessment of digestibility, absorption, nutritional quality and metabolism of foods. Chapter 2 covers the use of microorganisms such as bacteria, fungi, protozoa and yeasts in the nutritional and toxicological evaluation of foods and chemicals. Of recent vintage is the use of cell and tissue culture methodology in food research. In this direction Chapter 4 gives all the principles, methodology and application of cell and tissue culture in the assessment of food safety, particularly regarding the contamination of foods with mutagens, oncogens and environmental toxicants. It also indicates that the methodology will soon find more and more application in the nutritional evaluation of foods. One important point that is brought out of employing of cultured cells and tissues, or microorganisms is that the techniques are less expensive, less time consuming and statistically more valid than whole animal experiments.

In Chapters 5 and 6, the advantages of the immunochemical and enzymatic methods in terms of sensitivity, speed, high specificity, amenability to automation and inexpensive character have been presented by the authors very systematically along with the principles involved in all such techniques. Both these assay techniques have been found to permit assays to be completed in a few minutes. With regard to the enzymatic methods to become strong and versatile competitive tool, the author has brought out the future needs such as identification and purification of new enzymes for many of the myriad biological compounds and developments in sophisticated instrumentation for automation.

Numerous nonculture methods employed to determine microorganisms or their products in food products have been listed in tabular form and the current status of individual methods have been discussed giving relevant examples in Chapter 3. About 12 non-culture techniques, involving modern instrumentation, enzymes, immunological responses, cell diffusion, haemagglutination, bioassays etc. have received attention at length. The merits and demerits of these methods in terms of sensitivity and rapidity have been brought out very clearly by the author.

In general, the book eliminates the problem of searching through widely scattered sources to achieve thorough understanding of the principles and methodology of the techniques used in the area of biological techniques for analysis. It gives the broad-ranging recent developments in the field and serves as a source book for all food analysis in industry, government, and academic, including food scientists, nutritionists, biochemists, microbiologists, toxicologists, biologists, and environmental chemists. Additionally, graduate students in food science and nutrition will find this volume and perhaps other volumes as well, indispensable in their studies.

S. C. BASAPPA  
C.F.T.R.I., MYSORE



## AFST(I) News

### Ludhiana Chapter

The Annual General Body Meeting of the Chapter was held on 18th January 1986 and the following office bearers were elected:

*President:* Dr. M. S. Kalra  
*Vice-President:* Dr. S. K. Berry  
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*Jt. Secretary:* Dr. K. S. Minhas  
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**ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA)**

**CFTRI Campus, Mysore-570 013**

Dear member,

Date: 1-10-1985

It is proposed to bring out a National Directory of Technical Personnel in food and allied areas. The proposed Directory shall contain as much information about a person as possible. The Directory would be useful to find out the persons with technical backgrounds in various fields. Therefore, I request you to fill up the following proforma and send it immediately. Those who have already responded may please ignore this.

Expecting your whole-hearted cooperation,

**Dr. D. NARASIMHA RAO**  
*Hony. Exec. Secretary*

*To,*

**NATIONAL DIRECTORY OF TECHNICAL PERSONNEL IN FOOD AND ALLIED AREAS**

**Hon. Exec. Secretary, AFST(I), CFTRI Campus, Mysore-570 013**

1. Name :
2. Date of birth and age :
3. Postal Address  
Present :  
Permanent :
4. Educational qualification :
5. Field of specialisation :
6. Posts held with necessary details :
7. Organisation where working at present with designation and duties :

8. Major area of contribution :
9. Experience (Including visits abroad) :
10. Honours/Awards/Deputations, etc. :
11. Membership of Professional bodies :
12. Any other information relevent to the food science and technology, not covered so far :

*Signature*

**(Note: Attach extra sheets to provide full and complete information)**

# INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (in triplicate) should be typewritten in double space on one side of bond paper. They should be complete and in final form, since only minor corrections are allowed at the proof stage. The submitted paper should not have been published or data communicated for publication anywhere else. Only invited review papers will be published.
2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.
3. Names of chemical compounds and not their formulae should be used in the text. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Superscripts and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.
4. **Abstract:** The abstract should indicate the principal findings of the paper. It should be about 200 words and in such a form that abstracting periodicals can readily use it.
5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. They should be typed on separate sheets. Nil results should be indicated and distinguished clearly from absence of data, which is indicated by '—' sign. Tables should not have more than nine columns.
6. **Illustrations:** Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper not bigger than 20 cm (oy axis) × 16 cm (ox axis). The lettering should be such that they are legible after reduction to column width. Photographs must be on glossy paper and must have good contrast; *three copies* should be sent.
7. Abbreviations of the titles of all scientific periodicals should strictly conform to those cited in the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.
8. **References:** Names of all the authors along with title of the paper should be cited completely in each reference. Abbreviations such as *et al.*, *ibid*, *idem* should be avoided.

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

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

- (a) *Research Paper:* Jadhav, S. S. and Kulkarni, P. R., Presser amines in foods. *J. Fd Sci-Techmol.*, 1981, **18**, 156.
  - (b) *Book:* Venkataraman, K., *The Chemistry of Synthetic Dyes*, Academic Press, Inc., New York, 1952, Vol. II, 966.
  - (c) *References to article in a book:* Joshi, S. V., in *The Chemistry of Synthetic Dyes*, by Venkataraman, K., Academic Press, Inc., New York, 1952, Vol. II, 966.
  - (d) *Proceedings, Conferences and Symposia Papers:* Nambudiri, E. S. and Lewis, Y. S., Cocoa in confectionery, *Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India*, Mysore, May 1979, 27.
  - (e) *Thesis:* Sathyanarayan, Y., *Phytosociological Studies on the Calcicolous Plants of Bombay*, 1953, Ph.D. Thesis, Bombay University.
  - (f) *Unpublished Work:* Rao, G., unpublished, Central Food Technological Research Institute, Mysore, India.
9. Consult the latest issue of the *Journal* for guidance.

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