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(INDIA)

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# Effect of Washing/Steeping on the Acceptability of Karnal Bunt Infected Wheat for Bread, Cookie and Chapati Making

K. S. SEKHON, S. K. RANDHAWA, A. K. SAXENA AND K. S. GILL

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*Manuscript received 18 December 1979; revised 4 July 1980*

The effect of washing/steeping on the acceptability of bread, cookie and chapati at 1.0, 3.0, 5.0, 10.0 and 14.0 per cent of Karnal Bunt infection are reported. The disease did not adversely affect the loaf volume and specific volume, but affected the crumb texture, crumb colour and taste. The colour, taste and flavour of chapatis and cookies were also adversely affected. Washing/steeping improved the loaf volume and specific volume, and brought about a marked improvement in the colour, flavour and taste of bread, cookie and chapati. The washed/steeped samples having 5 per cent infection produced bread, cookie and chapati comparable to those produced from disease-free sample. These products became acceptable even at 10 per cent infection after washing/steeping treatment.

Karnal Bunt is a serious disease of wheat caused by *Neovossia indica* (Mitra) Mundker. It affects the quality of the grains severely and makes them unsuitable for human consumption. Mehdi *et al*<sup>1</sup>. reported that even the presence of 1 per cent diseased grains made the chapatis unacceptable for human consumption. Our earlier studies<sup>2</sup> indicated that the disease had no adverse effect on the protein content and amino acid make-up. The chapatis made from 3 per cent Bunt-infected wheat were acceptable for human consumption.

## Materials and Methods

A Karnal Bunt affected sample of wheat variety 'WL-711' having 14 per cent disease infection and a disease free sample of 'WL-711' were obtained from

the crop grown in 1977-78 under similar conditions at the Punjab Agricultural University Farm. These samples were mixed in different proportions to obtain duplicate samples having 1, 3, 5, 10 and 14 per cent of disease infection. These were further divided into three equal proportions of 2 kg each. One lot was left as such. The second lot was washed with water at room temperature till the water coming out was free of black colour. The third lot was washed and then steeped for half an hour (grains were left in water and dipped for half an hour at room temperature) and the water drained off.

Grain moisture was reduced to 14 per cent by drying/conditioning and maintained for 24 hr prior to milling. Half kilogram of each sample was ground in a small stone 'chakki' in the laboratory for making chapatis

TABLE 1. BREAD MAKING PROPERTIES OF BUNTED WHEAT FLOUR

Character	Control	Control (diseased)					Washed					Steeped				
		Per cent infection					Per cent infection					Per cent infection				
		1	3	5	10	14	1	3	5	10	14	1	3	5	10	14
Loaf vol. (cc)	500	500	525	520	515	530	510	545	560	510	532	530	478	510	525	545
Loaf wt. (g)	135	135	135	135	140	140	140	140	140	137	138	135	137	138	137	140
Specific vol. (cc/g)	3.7	3.7	3.89	3.86	3.69	3.78	3.64	3.89	4.00	3.72	3.85	3.93	3.48	3.69	3.83	3.89
Crumb colour	Yellow Cream	Yellow Cream	Dull White	Black -ish	Black -ish	Black -ish	Yellow Cream	Cream -ish	Cream -ish	Dull Cream	Black -ish	Yellow Cream	yellow Cream	Cream -ish	Cream -ish	Dull Cream- ish
Crumb texture	A	A	A	B	B	C	A	A	A	A	B	A	A	A	A	B
Taste score	5	5	5	3	0	0	5	5	5	3	2	5	5	5	3	2

A = very good; B = good; C = poor.

and 1 kg of each sample was milled through Quadratic Junior Mill for preparing bread and cookie. Chapati, bread, and cookie were tested as described earlier.<sup>2,3</sup>

### Results and Discussion

The disease had no adverse effect on the loaf volume and specific volume of bread but affected the crumb colour, crumb texture and taste (Table 1). The colour and taste of the bread were markedly affected at 5 per cent level of infection. Washing markedly improved crumb colour, crumb texture and taste of bread. The loaves produced from washed grains of 5 per cent infection were comparable to those produced from disease-free samples in all respects. Even at 10 per cent level of infection the loaves produced from washed samples looked normal except for some differences in colour and taste. Steeping the grains further improved the loaves with respect to these characteristics.

Spread factor (W/T) was higher for cookies prepared from diseased samples as compared to control. However, this increase did not show a uniform trend with the increase of the disease intensity. The taste of the cookies was affected even at 3 per cent level of infection (Table 2). Washing/steeping had a decreasing effect

on the width but increased the thickness of the cookies. Consequently the spread factors of cookies prepared from washed/steeped samples were low. No difference was observed in taste of cookies after washing/steeping in samples infected upto 5 per cent and the cookies prepared from 10 per cent infected grain were acceptable.

Colour, taste and flavour of chapatias prepared from upto 3 per cent infected grain were little affected. But at 5 per cent level of infection these characteristics were severely affected (Table 3). Our previous studies<sup>1</sup> showed that chapatias remain acceptable upto 3 per cent infection. Mehdi *et al*<sup>1</sup>, however reported that chapatias were unacceptable even at 1 per cent level. Washing/steeping brought about considerable improvement in the colour, taste and flavour of chapatias. The chapatias were quite normal at 5 per cent level of disease and were acceptable even at 10 per cent level of infection after washing/steeping.

### References

1. Mehdi, V., Joshi, M. and Abrol, Y. P., *Bull. Grain Technol.*, 1973, 11, 195.
2. Sekhon, K. S., Gupta, S. K., Bakhshi, A. K. and Gill, K. S., *Crop Improv.* (accepted for publication).
3. Sekhon, K. S., Saxena, A. K., Randhawa, S. K. and Gill, J. *Fd Sci. Technol.*, 1980, 17, 233.

TABLE 2. COOKIE MAKING PROPERTIES OF BUNTED WHEAT FLOUR

Character	Control	Control (diseased)					Washed					Steeped				
		Per cent infection					Per cent infection					Per cent infection				
		1	3	5	10	14	1	3	5	10	14	1	3	5	10	14
Width(W) (cm)	5.9	6.35	6.50	6.10	6.00	6.25	6.15	5.80	5.75	5.60	5.65	5.70	6.00	6.10	6.10	6.10
Thickness(T) (cm)	1.0	0.83	0.83	0.93	0.85	0.87	0.85	1.00	0.92	1.15	1.10	1.25	1.00	0.875	0.80	0.80
W/T	5.9	7.6	7.8	6.6	7.0	7.2	7.2	5.8	6.2	4.8	5.1	4.6	6.0	6.96	7.63	7.63
Appearance	A	A	B	B	B	C	A	A	B	B	B	B	B	B	B	B
Taste score	5	5	4	3	0	0	5	5	5	3	1	5	5	5	3	1

A = very good; B = good; C = fair.

TABLE 3. CHARACTERISTICS OF CHAPATIES MADE FROM BUNTED WHEAT "ATTA"

Character	Control	Control					Washed					Steeped				
		Per cent infection					Per cent infection					Per cent infection				
		1	3	5	10	14	1	3	5	10	14	1	3	5	10	14
Colour	5	5	5	3	1	0	5	5	5	3	0	5	5	5	4	0
Taste	5	5	5	2	1	0	5	5	5	3	0	5	5	5	3	0
Flavour	5	5	5	2	1	0	5	5	5	3	0	5	5	5	3	0
Rating	A	A	A	B	C	C	A	A	A	B	C	A	A	A	B	C

A = very good; B = acceptable; C = unacceptable

# Use of Starch Phosphate in Bread and Biscuits

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*Manuscript received 12 February 1980; revised 2 May 1980*

Studies on the effect of starch phosphate, on the rheological characteristics, and bread and biscuit making quality of wheat flour (*maida*) showed that Farinograph characteristics were affected adversely at 0.5 - 5.0% levels, while the extensograph characteristics improved at levels higher than 2.0%. The loaf volume, crumb grain, texture and eating quality of bread deteriorated with higher levels of starch phosphate. The spread, hardness and breaking strength, of biscuits increased at higher levels (0.1-1.0%) of starch phosphate.

Starch phosphate is used as a substitute for gum, as a binding agent in bakery products, particularly in gluten-free breads<sup>1</sup>. The effect of gum on the rheological characteristics as well as quality of bread based on soft wheat flour or composite flour has been well studied<sup>2,3</sup>. The high cost and limited availability of gum has led to the use of starch phosphates. However, the information on the effect of starch phosphates on the quality of dough and bakery products is lacking. This is more important in case of Indian wheats, which in general are not suited for bread making due to their gluten quality. The effect of incorporating starch phosphate on the rheological and amylograph characteristics as well as bread and biscuit making quality are reported in this article.

## Materials and Methods

**Materials:** Wheat flour (*maida*) for bread was obtained by milling a commercial wheat variety from Punjab - 'WG-357' in a Buhler Laboratory Flour Mill (Model : MLU 202) after conditioning the wheat overnight to 15% moisture level. For biscuits, 'Pissi Local' - a soft wheat variety from Madhya Pradesh - was milled similarly after conditioning to 13.5 per cent moisture. Commercial *Maida* procured from the local market was used separately for comparison. Commercial sample of starch phosphate was used. Homogeneous blend of *maida* with starch phosphate was prepared by mixing in a Hobart Mixer (Model N-100), followed by sieving and remixing.

**Flour analysis:** Moisture, total ash, dry gluten and sedimentation value of different wheat flours (*maida*) were determined according to AACC methods<sup>4</sup>. Crude protein ( $N \times 5.7$ ) was estimated by micro-kjeldahl method.

**Rheological characteristics:** Using Brabender Farinograph and Extensograph, standard AACC procedures<sup>4</sup>

were adopted to study the dough characteristics of *maida*.

**Amylograph characteristics:** Effect of starch phosphate on the hot paste viscosity of *maida* was studied using Brabender amylograph under standard conditions<sup>5</sup>.

**Bread making:** Starch phosphate was incorporated at 0.25, 0.5, 1.0, 2.0, 3.0 and 5.0 per cent levels for bread making and evaluated according to standard remix procedure<sup>6</sup>, with a fermentation time of 120 min. The breads were evaluated by loaf-volume (rape seed displacement method), shape, crust colour, crumb grain and texture and eating quality by a panel of six judges.

**Biscuit making:** Starch phosphate was added at 0.1, 0.5 and 1.0 per cent levels in the preparation of biscuits which were prepared according to the recipe and method described earlier<sup>7</sup>. After cooling, the thickness (T) and diameter (W) of five biscuits were measured to determine W/T ratio and the spread factor. The colour, crispness and eating quality of biscuits were assessed by a panel of six judges. Hardness of individual biscuit was measured in a General Foods Texturometer (Model GTX- 2) with the following operating conditions: plunger: lucite 18 mm; platform: flat aluminium cup; clearance: 1 mm; voltage: 0.5V; and speed: low. Hardness of biscuits was measured from the height of the curve expressed as kg/v. The force required to develop a crack (breaking strength) in the biscuit was measured using Grain Hardness Tester (Kiyu Seksakusho Ltd., Tokyo).

## Results and Discussion

**Physico-chemical characteristics of wheat flour:** The data in Table 1 show the quality of the flours (*maida*) used for bread and biscuit making. Commercial *maida* contained significantly lower gluten than the 'WG-357' wheat flour. The low gluten as well as



TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF WHEAT FLOURS (*Maida*)

Flour used	Total ash (%)	Crude protein (Nx5.7) (%)	Dry gluten (%)	Sedimentation value (ml)
Bread ( <i>WG - 357</i> ) flour	0.52	9.9	9.6	40.1
Biscuit ( <i>Pissi Local</i> ) flour	0.44	8.7	8.3	15.5
Commercial	0.64	7.6	7.3	21.4

\*Expressed on 14% moisture basis

sedimentation value of commercial *maida* may be due to the use of medium hard to soft wheat and it was not of desired quality for bread making. The higher ash content of commercial *maida* as compared to the laboratory milled *maida* samples was indicative of the high extraction rate and higher bran content of commercial *maida*.

**Farinograph characteristics:** The farinograph characteristics are given in Table 1. Though the water absorption increased as the level of starch phosphate increased, the dough development time, the dough stability and the mixing tolerance index remained almost unchanged upto 1 per cent. Higher levels of starch phosphate resulted in significant increase in dough development time and decreased stability. Similar observation of increased dough development time with

TABLE 2. EFFECT OF ADDITION OF STARCH PHOSPHATE ON THE FARINOGRAPH CHARACTERISTICS OF FLOUR

Starch phosphate (%)	Water absorption (%)	Dough development time (min)	Stability (min)	Mixing tolerance index (BU)
0.0	69.6	4.5	6.8	40
0.5	70.2	4.5	6.8	40
1.0	71.0	4.5	6.8	40
2.0	72.0	4.8	5.8	40
3.0	72.0	5.0	5.0	50
4.0	72.1	6.0	4.3	60
5.0	72.5	6.5	3.8	60

the addition of gums reported by Christianson<sup>8</sup> was attributed to slower water absorption of gums. Heubner and Wall<sup>2</sup> have reported increased peak time and decreased stability when polysaccharides were incorporated into the flour.

It is interesting to note that shape of the farinograms have gradually changed as the levels of starch phosphate increased. (Fig. 1). However, 2 peaks are observed for the dough containing 3 per cent or more of starch phosphate. Such unusual peaks were also observed by Heubner and Wall<sup>2</sup> when carrageenan was added to flour.

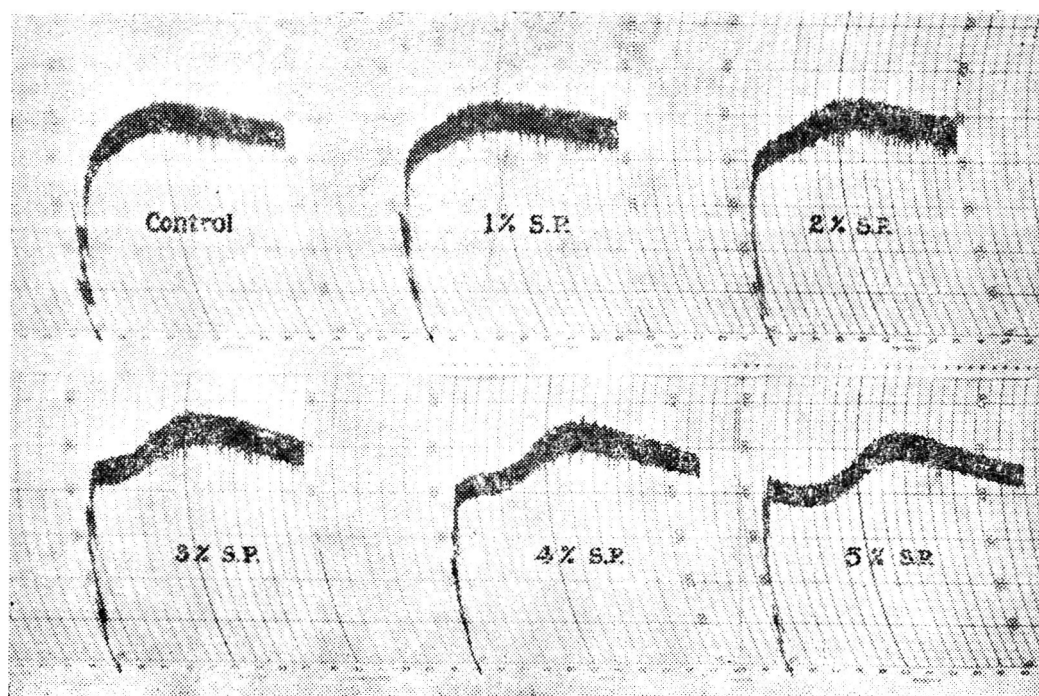


Fig. 1. Farinograms of wheat flour dough containing different levels of starch phosphate.

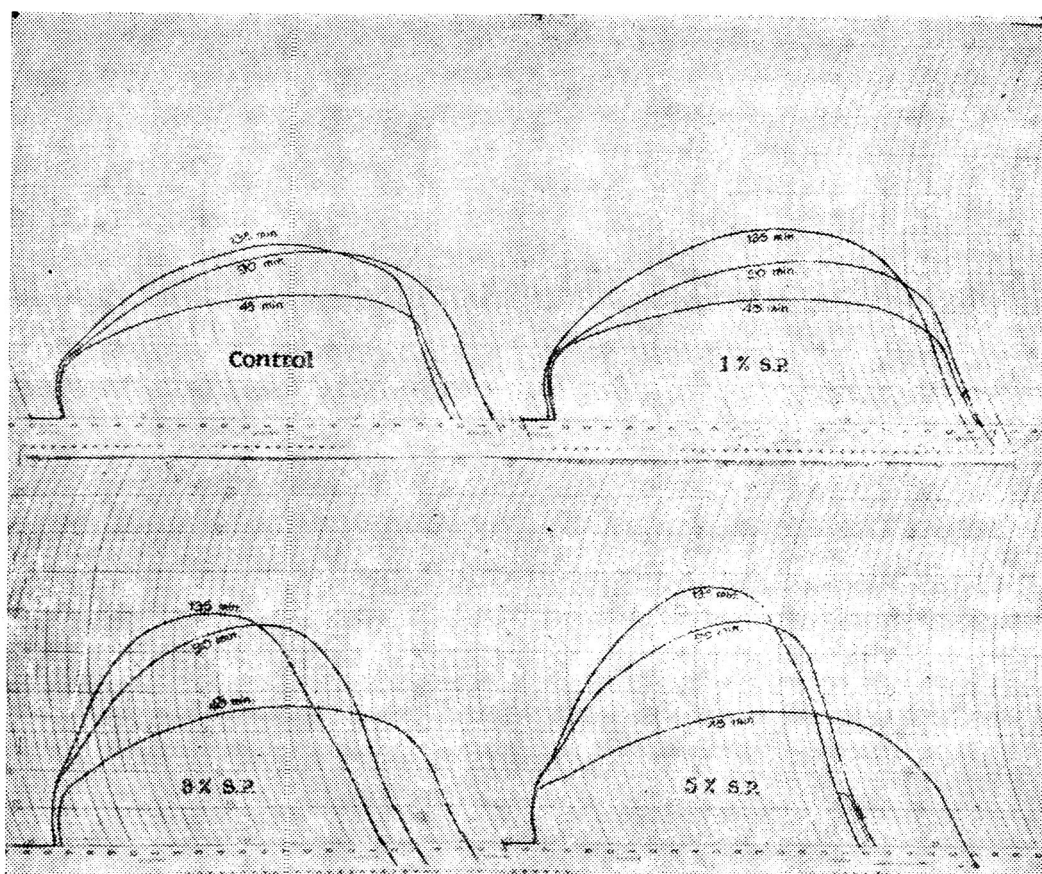


Fig. 2. Extensograms of wheat flour dough containing different levels of starch phosphate.

**Extensograph characteristics:** Data in Table 3 show that resistance to extension increased significantly only at 3 per cent or higher levels of starch phosphate. The successive increases in the resistance to extension in the doughs rested for periods of 45, 90 and 135 min (Fig 2) were much higher in doughs containing starch phosphate, as compared to doughs based only on *maida*. Such increase became more significant as the level of starch phosphate increased.

The strengthening effect of dough due to starch phosphate was similar to that of oxidising agents like potassium bromate<sup>9</sup>. The increase in the ratio of resistance

to extension to extensibility (Table 3) showed that the dough stiffened with the incorporation of starch phosphate. The strength of the flour as indicated by the total area also increased.

The above data on the rheological characteristics of flour show that starch phosphate at 2 per cent or more levels was beneficial on the extensograms, but farinograph characteristics were adversely affected.

**Amylograph characteristics:** Significant increases were observed in peak viscosity at 3-5 per cent levels of starch phosphate (Table 4). This may be attributed to the ability of starch phosphate to thicken the wheat

TABLE 3. EFFECT OF ADDITION OF STARCH PHOSPHATE ON THE EXTENSOGRAPH CHARACTERISTICS OF FLOUR

Starch phosphate (%)	Resistance to extension (R) (BU)	Extensibility (E) (mm)	Ratio (R/E)	Energy (Cm <sup>2</sup> )
0	480	179	2.5	105
1	500	177	2.6	107
3	585	154	3.5	153
5	660	153	3.9	153

TABLE 4. EFFECT OF ADDITION OF STARCH PHOSPHATE ON THE AMYLOGRAPH CHARACTERISTICS OF FLOUR

Starch phosphate (%)	Gelatinisation temp. (°C)	Peak viscosity (BU)	Temp. at peak viscosity (°C)
0	58.0	360	88.0
1	58.0	360	89.0
3	56.5	370	89.0
5	56.5	440	89.0

TABLE 5. EFFECT OF INCORPORATION OF STARCH PHOSPHATE ON THE QUALITY\* OF BREAD

Level used (%)	Local volume (ml)	Specific volume (g/ml)	Crumb grain	Crumb texture	Eating quality	Overall quality
<i>Flour of WG-357</i>						
0	535	3.87	Fine and uniform	Soft	Highly acceptable	Excellent
0.25	535	3.84	"	"	"	"
0.5	525	3.83	Slightly coarse and uniform	Soft	"	"
1.0	525	3.80	"	"	"	"
2.0	515	3.66	"	Slightly hard	Acceptable	Good
3.0	505	3.60	Slightly coarse and non-uniform	"	"	"
4.0	480	3.44	"	"	"	"
5.0	475	3.38	Coarse and non-uniform	Hard	Slight off-taste	Satisfactory
<i>Commercial flour</i>						
0	495	3.50	Coarse and non-uniform	Slightly hard	Just acceptable	Satisfactory
2.0	475	3.37	"	Hard	"	Fair

\*The crust shape and colour were normal in all the breads.

flour slurry. No significant changes were observed in temperature for gelatinisation or peak viscosities.

*Bread making quality:* The data on the quality of bread as influenced by starch phosphate are given in Table 5. The loaf volume decreased gradually with increase in the level of starch phosphate. Other characteristics like crumb grain and texture were affected adversely by starch phosphate at 2 per cent or more. Handling of the dough containing 4 per cent or more of starch phosphate was difficult, as it tended to be sticky. However, improvement in the overall quality of bread containing 5-7 per cent starch phosphate has

been reported by Bergthaler and Stephan<sup>10</sup>. Although some improvement was observed in the dough characteristics by the addition of starch phosphate the same effects were not found in the quality of bread.

*Biscuit making quality:* Addition of starch phosphate did not improve the colour and taste of biscuits. They were slightly hard and less crisp to bite when starch phosphate was added.

The results in Table 6 show that starch phosphate increased the spread slightly and decreased the raise of biscuits. This is also evident by the higher width to thickness ratios for biscuits with higher starch phosphate.

TABLE 6. EFFECT OF INCORPORATING STARCH PHOSPHATE ON THE QUALITY OF BISCUIT

Level used (%)	Width (cm)	Thickness (cm)	W / T ratio	Spread factor (%)	Hardness* (kg/volt)	Breaking** strength (kg/biscuit)
<i>Flour of Pissi Local</i>						
0.0	5.07	0.50	10.14	—	31.0	3.5
0.1	5.11	0.50	10.22	100.8	32.5	3.8
0.5	5.12	0.49	10.45	101.2	37.0	4.0
1.0	5.14	0.48	10.70	102.4	39.4	4.9
<i>Commercial flour</i>						
0.0	4.85	0.45	10.78	—	36.0	6.0
0.5	5.11	0.39	13.10	121.52	42.5	6.7

\*Average values for 5 biscuits determined using General Foods Texturometer.

\*\*Average values for 10 biscuits determined using Grain Hardness Tester.

Both the hardness of biscuits as measured in General Foods Texturometer and the breaking strength (indicating force required to develop a crack in the biscuits) measured in the Grain Hardness Tester increased with higher starch phosphate levels. The observation on the breaking strength showed that addition of starch phosphate reduced the checking of biscuits to some extent. These suggest that appropriate level of starch phosphate may overcome higher breakages, especially in biscuits based on hard doughs.

*Bread and biscuit making quality of commercial maida:*

Bread and biscuits were prepared from commercial *maida* using 2.0 and 0.5 per cent of starch phosphate, respectively, as these levels were found somewhat critical. The bread was rated as satisfactory, as compared to excellent rating for bread from wheat of 'WG-357'. This may be due to the lower quantity of protein of inferior quality as indicated by sedimentation value. Adverse effects on bread quality similar to the bread from 'WG-357' were observed in bread based on commercial *maida* containing 2 per cent starch phosphate.

The biscuits made from commercial *maida* had lesser spread and raise, but higher hardness and breaking strength as compared to biscuits made from 'Pissi Local'. Higher strength of commercial flour used may be responsible for this. More pronounced changes in spread, W/T ratio, hardness and breaking strength were observed in biscuits based on commercial *maida* with or without 0.5 per cent starch phosphate as com-

pared to biscuits based on *maida* from 'Pissi Local' wheat.

*Conclusions:* Addition of starch phosphate improved the dough characteristics as indicated by extensograms, while the farinograms indicated adverse effect. The overall bread and biscuit quality was adversely affected with higher levels of starch phosphate. Starch phosphate at 0.5 per cent level tended to increase the hardness of biscuits.

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## Hardness and Colour in Parboiled Rices Produced at Low and High Temperatures

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'IR 20' paddy soaked by cold, warm and hot soaking methods was parboiled at 70, 80, 90, 100, 110 and 120° C for 5 and 10 min duration. For parboiling soaked paddy at temperatures below 100°C, a new system has been developed. The degree of milling and the milling breakage were not influenced by the parboiling conditions adopted. The parboiled rice obtained from cold soaked paddy was harder than from warm and hot soaked paddy. The parboiling temperatures and duration had significant influence on the hardness. Rice colour depended on the temperature of soaking and parboiling. The possibility of producing light coloured parboiled rice at low temperatures with the least milling breakage has been indicated.

As the hardness and colour are the prime factors that decide the marketability and economics of parboiled rice, a new method of parboiling has been developed

to study the influence of temperatures and duration of parboiling on these factors and the results are reported here.

## Materials and Methods

One month old 'IR 20' paddy procured locally was used. To get paddy of uniform size and shape, clean paddy was graded by dipping in salt (NaCl) solution of 1.1 sp. gr. The paddy was thoroughly washed in water until free of chloride. It was immediately soaked in cold<sup>1</sup> (CS), warm<sup>2</sup> (WS) and hot<sup>3</sup> (HS) water and parboiled.

**Parboiling in a closed box immersed in oil bath:** A rectangular box (30×22×1.2 cm) made of 30-gauge tin having 5 cm diameter opening for filling and discharging the paddy and provided with handles was used for parboiling by closed heating (CH). A thermometer with rubber cork fitting and a rubber cushion at the bottom was fitted inside the box. The box was immersed in a rice bran oil bath and resting on a perforated stand; the oil was heated 10°C above the required temperature. The oil bath was fitted with stirrers and a thermometer.

Soaked paddy after removing the adhering moisture was filled to full in the box and immersed in the oil bath. The box was kept open till the paddy temperature rose to 60°C to expel the entrapped air and then tightly closed. The box was held at the temperature for the prescribed period (5 or 10 min), taken out, cooled in running water and the paddy was shade dried after spreading in a thin layer.

The soaked paddy was also parboiled by open steaming (100°C) at 0 psig (OS), and by autoclaving at 110° and 120°C for 5 and 10 min duration. Two lots of raw paddy were merely soaked at 70° and 80°C for 5 and 3 hr respectively (designated as 70-m and 80-m). All samples were shade dried<sup>2</sup> to uniform moisture content before milling.

**Milling:** Paddy was dehulled in a Satake grain testing mill and polished in a McGill miller No. 3 for 30 sec with constant load (6 lb) and the breakage was assessed. The degree of milling was also determined by weighing the bran. Another sample of brown rice was milled to 6% degree of milling by varying the time.

Moisture content was determined by drying at 105°C for 24 hr and the results are expressed on wet basis.

**Hardness:** Hardness was determined with a Kiyahardness tester<sup>4</sup> and by grinding 20 g brown rice in an automatic Moulinex coffret No. 4 grinder for 15 sec and noting the fraction passing through 30-mesh screen.

**Colour:** After removing the adhering bran, the reflectance was measured in a Photovolt reflection meter using a green tristimulus filter<sup>5</sup>.

## Results and Discussion

Except in 70°C closed heating (CH) (6 to 9% kernels with white-core endosperm), kernels parboiled in different conditions exhibited uniform translucency

indicating completion of parboiling. During parboiling, husk splitting was noticed in all cases except in 70-CH, 80-CH and 70-m; the degree of husk splitting being 80-m>120>110>OS>100-CH>90-CH. The parboiled paddy produced by closed heating had 0.5 to 2.0 per cent less moisture than the corresponding soaked paddy; in other cases an increase of 1.5 to 3.0 per cent grain moisture after parboiling was noticed. Reduction in grain moisture during parboiling of soaked paddy by closed heating has also been reported<sup>6</sup>.

The moisture content during milling was 10.0 to 10.4 per cent in paddy and 10.2 to 10.6 per cent in brown rice. The 30-sec degree of milling for raw rice was 9.0 per cent; whereas after parboiling it ranged from 4.0 to 5.2 per cent. Broken were negligible (below 0.25 per cent) in all cases except in 70-CH and 70-m, where it ranged from 1.0 to 3.8 per cent. No correlation was found between the parboiling conditions and the degree of milling or milling breakage. Bhattacharya<sup>7</sup> noticed that method of drying rather than the degree of parboiling influenced the milling breakage.

The breakage hardness value (BHV) for raw rice was 4.69 and it ranged from 5.34 to 9.30 for parboiled rices. Increase in BHV for parboiled rice was also noticed earlier<sup>8</sup>. Close positive correlations ( $r=0.998^{***}$ ;  $r=0.989^{***}$ ) existed between parboiling temperature and BHV (Fig. 1). The duration of parboiling also significantly influenced the hardness of rice (Table 1). Parboiled grains obtained from cold water soaking were significantly harder than that of warm and hot water soaked paddy. The fraction that passed through 30-mesh screen was

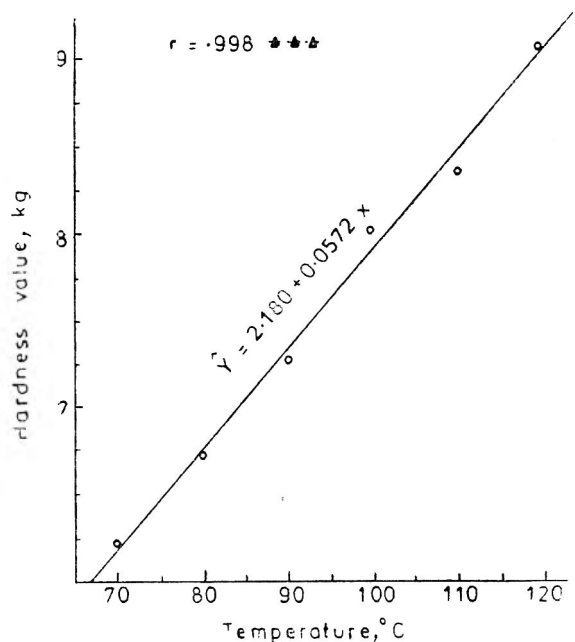


Fig. 1. Effect of parboiling temperature on hardness of rice.

TABLE 1. INFLUENCE OF PARBOILING TEMPERATURES, DURATION AND SOAKING METHODS ON THE HARDNESS AND REFLECTANCE VALUES

Characteristics	Breaking Hardness	Powdering	Reflectance
	C.D. (P = 0.05)	C.D. (P = 0.05)	C.D. (P = 0.05)
Parboiling temp (T)	0.14**	0.33**	1.91**
Parboiling period (D)	0.08*	0.19	1.10
Soaking method (S)	0.10**	0.23**	1.35*
T×D	0.20*	0.47**	2.70
T×S	0.24**	0.57**	3.30
D×S	0.14**	0.33**	1.91
T×D×S	0.34**	0.81**	4.67

\*at 0.10 level; \*\*at 0.01 level; figures without asterisk are not significant.

58.20 per cent for raw rice, whereas on parboiling under different conditions this fraction progressively reduced to 53.80 to 36.90 per cent; it correlated negatively ( $r = -0.995^{***}$ ;  $r = -0.996^{***}$ ) with the temperature of parboiling (Fig. 2). The soaking method also influenced the extent of fraction (Table 1).

The parboiling temperatures correlated negatively with reflectance readings ( $r = -0.980^{***}$ ;  $r = -0.913^{***}$ ); but the temperature influenced the colour only between 100 and 120°C. This is in agreement with the results of Jayanarayanan<sup>9</sup> but not with that of Roberts *et al.*,<sup>10</sup> who observed progressive increase in colour above 123.5°C. Among the three soaking methods, cold soaking had the least colour inducing effect on rice and the hot soaking the most. Roberts *et al.*,<sup>10</sup> indicated that the steeping temperature and time of soaking have lesser effect on colour development but the steaming temperature had more effect. But others found that temperature and duration of steeping<sup>5,9</sup> and steam-

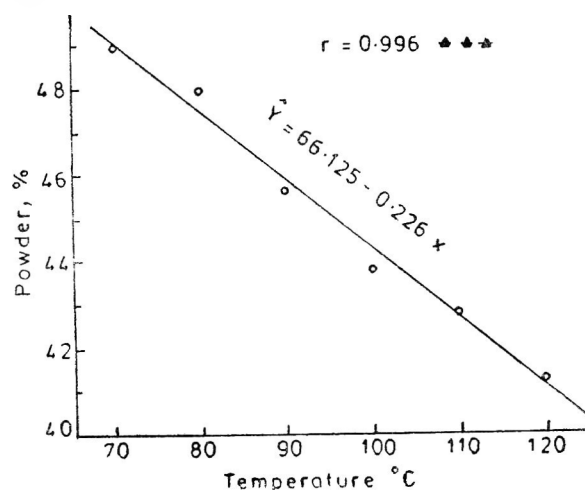


Fig. 2. Effect of parboiling temperature on smaller fraction passing through 30-mesh screen.

ing<sup>11,12</sup> had their own colour inducing effects, but in the present study, the duration of parboiling showed no significant influence on the colour (Table 1) probably due to shorter duration employed.

Higher the temperature and duration of parboiling, harder were the rices produced (Table 1) and the cooking qualities of such rices were comparatively poor<sup>13</sup>. The hardness of rices of cold soaked samples was superior to warm and hot soaked samples and the reason for the same is not known; the colour of the former is lighter. Considering the preferences for light coloured translucent rice<sup>14</sup>, parboiling at below 100°C temperature seems to be proper. The eating quality of such rices as determined by taste-panel and extrusion test was better than those produced at higher temperatures<sup>15</sup>. This may be due to a lesser degree of starch solubilisation until a steaming temperature of 100°C<sup>10</sup> and may also partly be due to a low gelatinization during steaming at 10 psig<sup>16</sup> particularly for 5 and 10 min duration.

#### Acknowledgement

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# Detection and Estimation of Total Catechin from *Katha* by Thin Layer Chromatography

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A new solvent for extraction and a simple and accurate TLC method has been developed for determining total catechin in *katha*, the extractives of heart wood of *Acacia catechu*. Catechin is extracted by methanol: acetone: ether: carbon-disulphide (1 : 1 : 1 : 1) and identified on TLC plate. It is scraped and eluted by acetone and determined spectrophotometrically at 515 nm by developing colour with the chromogenic reagent Fast blue salt B.

The purity of *Katha* (dried aqueous extract of heart-wood of *Acacia catechu*) is determined by its catechin content. There is no suitable method for the determination of catechin in *Katha*. However, Indian Standards Institution<sup>1</sup> has developed a gravimetric method but the results are not reproducible<sup>2</sup>. The method of Bokuchava *et al.*<sup>3</sup> was also found to be unsuitable. Eastmond and Garduer<sup>4</sup> developed a GLC method for detection of catechin in beer. The method is complicated and will not be suitable for routine analysis. This communication suggests a new solvent for extraction and simultaneous estimation of the extracted catechin by TLC.

## Materials and Methods

**Spraying reagent:** Fast blue salt B (0.1g) dissolved in 100 ml distilled water; solution should be prepared freshly and used within 10 min.

**Developing solvent:** Toluene, chloroform, acetone, absolute alcohol in the ratio of 40 : 20 : 30 : 5.

**Preparation of standard catechin solution:** Catechin was purified by crystallisation from an ethanolic solution of *katha*. Pure epi-catechin was also similar to the former in respect of chromatographic behaviour. One gram of catechin was dissolved in 100 ml of methanol.

**Preparation of TLC plate:** Silica gel (25 g) was slurried with 50 ml water and spread on thin glass plates (20 cm × 10 cm); allowed to set for one hour and dried at 100-105°C for 1 hr and cooled and stored in a desiccator before use.

**Preparation of standard curve:** Standard catechin solution of 10, 20 and 30 µl were spotted on TLC plate using the solvent. The solvent was run for 10 cm and dried. One of the spots was sprayed with spraying reagent leaving other spots not to get contact

with the reagent. The colour of the spot turns pink. The undeveloped spots at the same R<sub>f</sub> as the developed one were scraped separately, 5 ml acetone were added to each and centrifuged for 5 min at 3000 rpm. The supernatant was transferred to a 10 ml volumetric flask. The process was repeated with 2 ml acetone and the supernatant was transferred into the same 10 ml flask. To each flask 1 ml spraying reagent was added and the volume made upto 10 ml. Within 5 min of colour development the optical densities were measured at 515 nm; this was found to be the absorption maximum of the coloured species. A blank was prepared in the same manner by scraping portion of the silica gel of the plate, containing no catechin. A standard curve was prepared plotting absorbance against concentration. Lowest concentration tried was 0.1 µg but it can be detected even at 0.05 µg.

**Analytical procedure:** The test sample (1g) was taken in a 500 ml volumetric flask and 40 ml of solvent mixture (methanol, acetone, carbon disulphide, and solvent ether in the ratio of 1 : 1 : 1 : 1) was added, shaken vigorously for 30 min, filtered and the residue on the filter paper was washed twice with the solvent mixture and the pooled filtrate and washings were concentrated to 50 ml. A known amount of the concentrate was spotted on the plate and developed, and the area at the R<sub>f</sub> of catechin was scraped as described earlier. From the graph the amount could be estimated.

## Results and Discussion

The reference samples gave three spots on the chromatogram, the middle of R<sub>f</sub> 0.17 appearing as main and two other probably being impurities or isomers. The test samples also gave three pink spots on spraying with the reagent. The middle spot is the desired catechin (R<sub>f</sub> 0.17). Of the various extracting solvents tried

TABLE 1. EXTRACTION OF CATECHIN FROM *katha*

Solvents	Ratio	% recovery
Methanol : acetone	1 : 1	48
Methanol : acetone : ether	1 : 1 : 1	52
Methanol : carbon disulphide	1 : 2	61
Methanol : acetone : ether : carbon disulphide	1 : 1 : 1 : 1	77

(Table 1) a mixture of methanol, acetone, ethyl ether and carbon disulphide was found to be the most suitable as it extracts the full amount of catechin.

When ISI method was followed with the same sample the catechin extracted was only 71 per cent.

### Acknowledgement

Thanks are due to Dr. B. R. Roy, Director of the Laboratory, for suggesting the problem, providing facilities and guidance. Thanks are also due to Dr A. N. Singh, Indian Wood Products, Barielly, for supplying pure catechin.

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## Piperine and Related Compounds-II. Estimation of Pungency Stimuli and Correlation with Pungency in Pepper

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The sources of variation in the official UV spectrophotometric method for estimation of pungency stimuli of pepper extracts is examined in relation to the known properties of piperine and related compounds. A standardized procedure is described. The reproducibility of the defined procedure and its validation through correlation with sensory response by standardized threshold determinations is established by analysis of a number of pepper samples and pure piperine.

Pungency is the valued flavour quality of pepper in international trade. Many contracts stipulate the piperine content of pepper or its oleoresins and only generally the total volatile oil<sup>1</sup>. Piperine, 5-(3, 4-dioxy methylene phenyl)-2-trans, 4-trans pentadienoic acid piperidide, was identified as the principal pungency stimulus, quite early<sup>2</sup> though search for related compounds has continued. Minor amounts of trienoic<sup>3</sup>, monoenoic<sup>4</sup> piperidides and the pyrrolidine analog but not the structural isomers of piperine<sup>5</sup> have been reported. These aspects have recently been reviewed<sup>6</sup>.

It is significant that the spice trade in America specified pungency in Scoville units for capsicum (not per cent capsaicinoids) but prefers per cent piperine for pepper (not pungency) and makes no mention of pungency for yet another spice ginger, which also stimulates pungency. It would appear that the emphasis attached to pungency in standards and specification for quality is related to availability of reliable methods for the determination of the respective pungency stimuli. With the recent

proposal to include pungency as one of the basic tastes<sup>7</sup>, it is necessary to re-examine the stimuli and their pungency, sources of variation in the determination of the stimuli and establish correlations with the response.

The basis of estimation of piperine and related compounds has ranged from nitrogen estimation of the non-volatile ether extracts to colorimetric and spectral methods and have varying specificity and accuracy<sup>8</sup>. The currently favoured method<sup>9</sup> also under study for adoption by the International Standards Organization, is the measurement of the absorption of an extract of pepper or oleoresin solution at 342 nm, the characteristic absorption maxima of pure piperine with its conjugated diene system attached to an aromatic ring. Two of the related minor components identified in pepper, piperettine and pyrroperine, have this characteristic structure and will also be measured at 342 nm; piperettine, while having its maxima at 360 nm shows nearly as high an absorption as piperine at 342 nm. However, as these compounds are present in very small



amounts they are not likely to interfere in correlative work. Salzer<sup>10</sup> has stated that the absorption at 342 nm would be an underestimate measuring only piperine and not the related compounds and also reported this method to have poor reproducibility. He, therefore, recommended Labruyere's Hydrolysis-distillation method which measures all the piperidine compounds.

The estimates of piperine and related compounds need to be validated by their contribution to the pungency of pepper. While piperine in solution is known to stimulate pungency, the contribution of the analogs are known to be less and have not been adequately established. The observations in some studies are as varying as from being as pungent as piperine<sup>5</sup> to weakly pungent<sup>11</sup>.

It is also reported that pepper from some sources when adjusted to equal spectrophotometric piperine content, do not show equal pungency<sup>12</sup>. Pungency estimation by the Scoville tests have been considered very variable but with proper control of dilutions and using a trained, homogenous and defined panel, the method has been shown to be satisfactorily reproducible<sup>13</sup> and has been adopted to determine pungency of pepper and its extracts<sup>14</sup>.

We have, therefore re-examined the variables in the UV spectrophotometric methods affecting its specificity and reproducibility and established correlation of the instrumental values to the pungency in Scoville Units, both determined by standardized procedures. A number of samples of pepper and its oleoresins have been examined to validate the regression established between estimates of stimuli and pungency.

### Materials and Methods

Several pepper samples of different horticultural varieties, trade types and those from Ceylon and commercial oleoresins were used. Piperine with melting point of 127° - 128°C either repeatedly recrystallised in the laboratory or from K & K Labs, England were used.

(a) *Spectrophotometric measurement*: Suitable dilutions of the extracts (3-8 µg piperine/ml equivalent) were made and their absorption spectra between 370 and 280 nm were recorded. For routine estimation of piperine content, the absorption at its λ max 342 nm was recorded. Special care was taken to protect the solutions from direct light. This estimation is referred to as the direct UV method. The percentage of piperine was calculated from a standard graph using 1-10 µg pure piperine/ml.

The effect of diffuse, direct, sun and UV lights on the spectra of piperine solutions both at 1 and 0.1 per cent concentration in ethanol and benzene were determined at varying intervals. The effect was expressed

as the percentage change in 342 nm absorption. The shift in absorption maxima if any was also recorded. The pungency values were determined at each stage using the samples in ethanol and also expressed as percentage change from original.

(b) *Pungency valuation*: Pungency evaluation of pepper extract and pure piperine was there according to the standardized procedure using a trained homogeneous panel<sup>13</sup> and the results expressed in Scoville Units (SU). The panel sensitivity was  $1 \times 10^5$  SU for pure piperine. The precaution needed in this was the solubility of piperine (6.25 µg/ml) in water<sup>15</sup>, which is in the same range of the pungency threshold levels (6-8 µg/ml), of our panel. Therefore, the dilutions in salt solution were prepared from the alcoholic stock solution about 20-30 minutes before tasting to prevent possible errors from recrystallisation of piperine. The perception of pungency at threshold needs some time and the panel is to be informed about this. A 0.75 per cent salt solution was found better in pepper pungency estimation unlike 3 per cent sugar solution in pungency evaluation of capsaicin. The details and a set of convenient dilution tables are given in the Indian Standards publication<sup>14</sup>.

(c) *Correlation of pungency stimuli and pungency values*: The piperine content of pepper or extracts determined by the modified hydrolysis-distillation method<sup>16</sup> and direct UV method and pungency in SU (in thousands) were plotted to determine the correlation. A regression equation between pungency stimuli and response was worked out<sup>17</sup>.

### Results and Discussion

The influence of the following variables on the specificity and reproducibility of the UV spectrophotometric method of estimation were studied.

(a) *Solvent used for dilution in the spectrophotometric determination*: The solvents used for dilution in the spectrophotometric study influence the sensitivity and reproducibility. The  $E_{1\%}^{1\text{cm}}$  value of piperine in ethanol is 762.5 and in benzene 1056.25 (Fig. 1, A and B). Also, the absorption maxima of piperine in ethanol is found to vary between 335 and 342 nm. These observations have relevance in view of the proposal for measuring the absorption in ethanol by the International Standards Organisation. Tausig *et al.*<sup>18</sup> showed that the reduction in absorption of dilute solution is very rapid in chloroform compared to that in benzene. We have observed that there is no significant difference in the magnitude of reduction of absorption between solutions in benzene and ethanol. As dilution with benzene gives a higher absorption than ethanol it is recommended to use benzene for the final dilution.

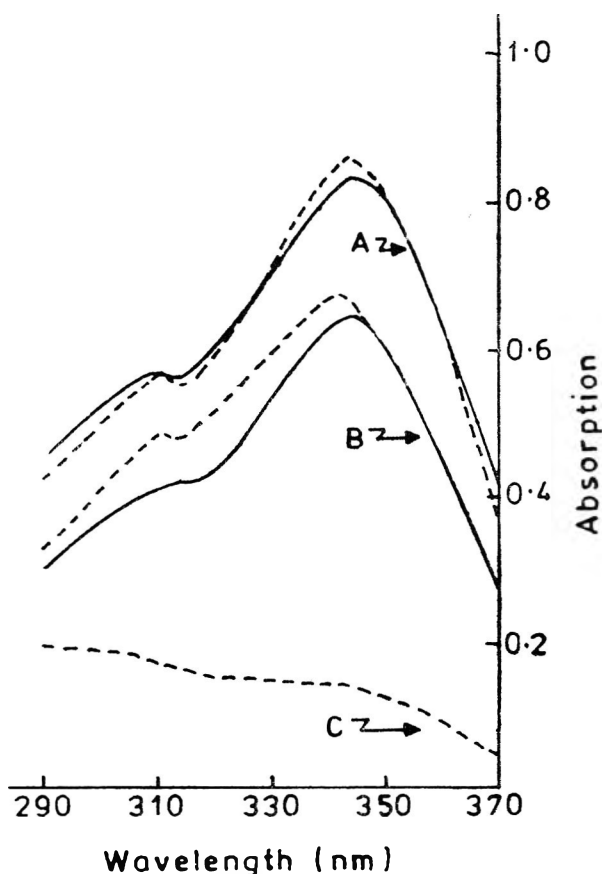


Fig. 1. UV spectra of pepper oleoresin, pure piperine and colour compounds

----, in benzene; —, in ethanol; A, Total pepper oleoresin; B, pure piperine; C, TLC isolated colour and resinous compounds of pepper oleoresin.

(b) *Contribution to the 342 nm absorption by components other than piperine and related compounds by TLC - UV method:* The TLC separated essential oil components at  $R_f$  0.3 and above (identified by the vanillin -  $H_2SO_4$  spray on a co-chromatographed spot<sup>19</sup>) when extracted, did not show any specific absorption in the UV region 370 to 280 nm; the coloured compounds (and possibly the resinous compounds) remaining at the starting point showed a continuously rising curve (Fig. 1. C). Its absorption at 342 nm contributed 3-5 per cent of the total absorption of the extract confirming earlier observation by Raghuvver and Ananthakrishna<sup>19</sup>.

(c) *Light induced isomerisation of piperine:* The change in spectra, both in absorption and the hypsochromic shift in  $\lambda$  max due to exposure to light have been shown by Tausig *et al.*<sup>18</sup> He has, however, tested high dilutions of piperine used for UV spectrophotometric measurement (0.0034 per cent - 0.068 per cent). The reduction started from 30 min after preparation of the dilutions and within a few hours it was as much

TABLE I. CHANGES IN PUNGENCY AND ABSORPTION OF PIPERINE ON EXPOSURE TO LIGHT

% Piperine	Mode of exposure	Duration of exposure	% Reduction		
			In ethanol Pungency SU	Absorption 342 nm	Absorption 342 nm
0.1	UV light	4 hr	58	16.4	16.0
	Sunlight	5 hr	50	17.2	23.3
1.0	Diffused light	12 days	0	0	0
		23 days	0	3.0	3.5
		34 days	6	7.4	8.5
	Sunlight	96 days	42	10.0	—
	Sunlight	6 hr	22	6.25	9.0

as 40-50 per cent reduced. Since this is an important source of error, we have studied this effect at higher concentrations of piperine (0.1 - 1 per cent) likely to be used for stock solutions, and also related them to the changes in pungency value. The results are given in Table I. It is clear that: *i*) 1 per cent solution of piperine either in ethanol or in benzene is stable in diffused light upto 23 days and after 34 days the loss in absorption, is 7-8 per cent; *ii*) this effect of diffused light is magnified when the piperine solution is exposed to direct sunlight or UV light and also when the concentration of the solution is reduced; and *iii*) the pungency reduction follows a pattern different from the 342 nm absorption. Besides, the correlation of pungency and estimated piperine (discussed later) does not persist once the reduction in absorption starts. Thus, the reduction in 342 nm absorption is only 10 - 13 per cent and the reduction in pungency is 45 - 50 per cent. This is obviously due to the differences in the molecular absorption and pungency of the isomers of piperine formed<sup>6</sup>. Verzele *et al.*<sup>20</sup> have shown the formation of an equilibrium mixture of the four isomers of piperine on its exposure to sunlight. The pungency of the resultant mixture has, however, not been reported. Earlier results on the pungency of the isomers vary from 'no pungency' to 'weak pungency'<sup>5,21</sup>. Preliminary observations on the TLC isolated isomers show that they have about half the pungency of piperine<sup>22</sup>. This would account for the fact that even after 96 days exposure to diffused light, the solution showed 50 per cent pungency of piperine. However, unless a method for quantitative estimation of the isomeric components and their individual pungency is developed, correlation of total pungency in mixtures of isomers cannot be made. It is thus essential to standardize the method, minimizing the effect of light.

(d) *Sampling of oleoresin:* Another potential source of error that can occur in the estimation of piperine

rine particularly in oleoresins is from sampling. Pepper oleoresin is a heterogeneous mixture of an oily phase and a solid phase. It has been shown in studies done at this laboratory<sup>22</sup> that piperine settles in the solid mass while the poorly pungent piperettine preferentially partitions in the oily layer. A thorough mixing and homogenisation by warming the sample is very important before sampling. The effect of improper sampling is further discussed under correlation of instrumental estimates of the stimuli with pungency.

(e) *Analogs of piperine in pepper*: It has been shown that the samples of pepper examined show definite presence of piperitine and possibly pyrroperine (syn piperylin), accounting for 7-15 per cent of piperine. According to the observation on TLC purified piperitine<sup>22</sup> has little or no pungency. When this is present in amounts of more than 10 percent of piperine it will contribute to the spectrophotometric value as piperine but not to pungency resulting in lower pungency value.

Pyrroperine also present at less than 2 per cent of piperine and reported differently to have equal<sup>5</sup> or only a fourth<sup>11</sup> of the pungency of piperine will be measured at 342 nm. The piperine (dihydropiperine) content of pepper is possibly less than 1 per cent of piperine. This is reported to have half the pungency of piperine.<sup>4</sup> However, this will not be measured at 342 nm because of the absence of the extended conjugated diene system. It has not found definitive evidence of this compound. These two components accounting together to only about 2 per cent equivalent of piperine and contributing poorly to pungency, will not affect the correlation of the spectrophotometric measure of the pungency stimuli and pungency values, especially as the variation in the determination of pungency has been shown to be 10 per cent.

*Correlation of pungency and estimates of piperine*: The proof of validity of any method of estimation of piperine and related pungency stimuli is by its relation to the determined pungency response. Therefore, the estimates of stimuli were correlated to pungency response with pure piperine and a number of pepper and oleoresin samples. The values of piperine and related compounds were determined by direct-UV method. The pungency was evaluated by the standardized Scoville tests<sup>14</sup>. These regressions are given in Fig. 2. The regression for pepper powder obtained in an earlier study by the modified hydrolysis-distillation method recommended by Salzer as accurate and reproducible is also included for comparison.

It is clear that there is a straight line relationship ( $r = 0.99$ ) between the pungency and estimate of the stimuli by the two methods indicating good reproducibility of the estimate of pungency stimulus by both methods. However, the hydrolysis-distillation proce-

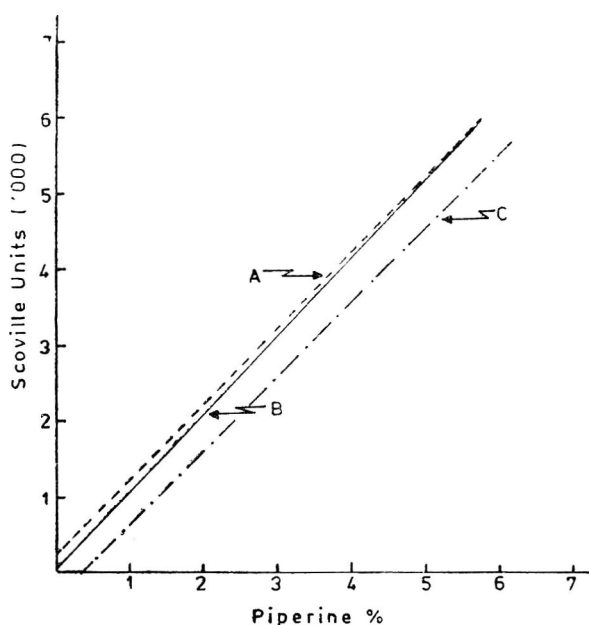


Fig. 2. Correlation of pungency (SU×'000) with estimated and pure piperine A —, Control curve for pure piperine; B - - - -, Direct UV method; C — . — ., Hydrolysis-distillation method.

dures yields a regression with a negative constant and is farthest removed from the control curve for pure piperine vs pungency. Considering the regression of SU on pure piperine as the control, the estimated values for piperine are higher than the values obtained by hydrolysis method. Results from the direct-UV method using samples with 1-6 per cent piperine content were not significantly different from the control curve except for an insignificant increase in the Y- intercept. This is possibly because of a bias created by the pepper aroma which is relatively higher with samples of low piperine content, requiring considerably less dilution in pungency threshold estimations.

The direct-UV method has thus been shown to be a quick and efficient method for estimating piperine and related compounds which correlates satisfactorily with pungency. The regression of SU on the piperine by UV method is given by the equation :

$$Y = 0.2766 + 1.0016 X, \quad \text{with } r = 0.997 \quad (P < .001)$$

where,

Y = predicted Scoville units in thousand  
X = percentage piperine.

The relationship of the predicted SU based on the above equation and the estimated SU are presented in Fig. 3 ( $r = 0.997$ ). Data from many more estimations covering the range 1 to 60 per cent of piperine are used to validate the regression as shown in Fig. 3. It can be seen that in two of the samples A and B, in Fig. 3 the estimated SU is only half of the predicted SU. These samples with 40-50 per cent piperettine were

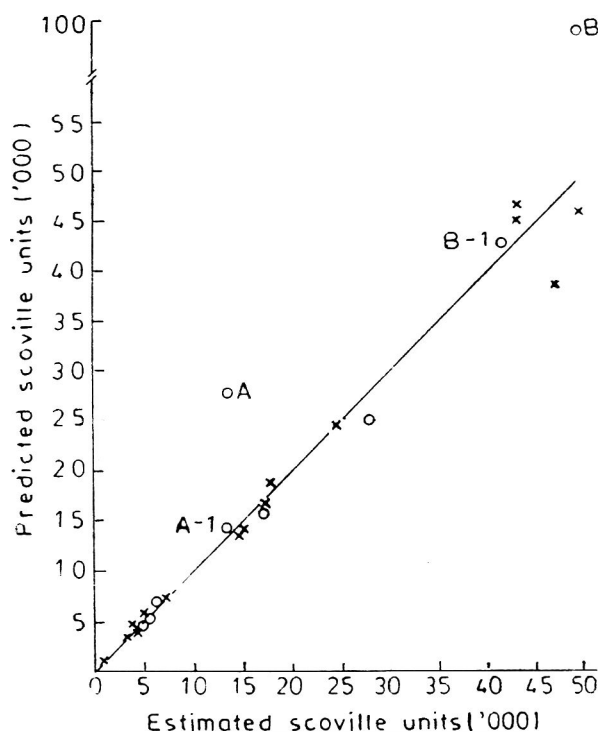


Fig. 3. Correlation of predicted and estimated scoville units (SU  $\times$  '000), Samples used in predicting equation; O Other samples to check the equation; A, B, supernatant of oleoresin; A-1, B-1, homogenized oleoresin.

from the supernatant portion of oleoresins. The piperine content of same oleoresin samples, when thoroughly homogenised by warming and sampled for estimation, agreed with predicted Scoville Units as seen in A-1 and B-1, in Fig. 3. This emphasises the importance of thorough mixing of oleoresin before sampling for estimation.

The method adopted (described below) which minimises the effect of variables was studied for reproducibility. Samples of 1, 2 and 3 g of a freshly ground stock (to pass 60 mesh sieve) were extracted in duplicates by refluxing with ethanol for 3 hr. The piperine content

TABLE 2. REPEATABILITY OF DIRECT UV ESTIMATION OF PIPERINE AND RELATED COMPOUNDS

Sample size (g)	Mean % piperine*	
	Extraction 1	Extraction 2
1	7.52	7.12
2	7.53	7.52
3	7.51	7.70
S. Em	$\pm 0.22$	

95% Confidence interval (relative)  $\pm 5.87\%$

\*Means of triplicate estimations; no significant differences were found among the means.

was estimated by direct-UV method in triplicates for each extract. There was no significant difference among the mean values of the samples of different weights (Table 2). It is seen that the mean value of piperine per cent is  $7.49 \pm 0.22$ . Relative standard deviation per cent for repeatability of the mean was 2.94 per cent. The  $2\sigma$  confidence interval about the mean was  $\pm 5.87$  per cent relative.

**Recommended method:** Reflux one gram of ground pepper (to pass 60 mesh sieve) in ethanol for 3 hr., filter through glass wool and wash repeatedly and make up the volume to 100 ml with ethanol to get the stock solution. Oleoresin samples are to be warmed on a waterbath stirring well to get a homogenous mass. Transfer to a weighing bottle while still warm and fluid, cool to room temperature and weigh. Make up to a 0.1 per cent solution in ethanol as a stock solution. Tightly stopper and keep the solution in cold.

Dilute the stock solution (1 ml to 100 ml) in benzene (AR) in triplicate. Protect the solution from undue exposure to light. Measure the absorption within an hour at 342 nm. Calculate piperine content from a standard graph made with pure piperine ( $1-10 \mu\text{g/ml}$ ) in benzene.

Use of ethanol in preparing the stock solution enables its use in Scoville tests to correlate with pungency values, when necessary.

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## Toxicity of Microquantities of Hexachlorocyclohexane Isomers to *Drosophila* using Topical Application

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**Toxicity of common hexachlorocyclohexane isomers  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ -, and  $e$ - was tested against *Drosophila oregon R* adults by topical application. The lowest limit of toxicity for the  $\gamma$ -isomer was found to be 1.2 ng/fly while that for  $\alpha$ - and  $\delta$ - isomers was 0.4  $\mu$ g/fly. The  $\beta$ - and  $e$ -isomers were found nontoxic even at 6.0  $\mu$ g/fly. The technique also confirmed isomerization of the  $\beta$ -isomer in water to biologically more active isomers. This was discernable due to high sensitivity of *Drosophila* to trace concentrations of HCH isomers.**

Topical application is recommended for determining the toxicity of pesticides<sup>1-3</sup>, against a variety of insects probably because it envisages uniform application of known doses of pesticides to individual insect. This method is, however, not used commonly against *Drosophila* probably for want of a convenient device to hold the test insect. During our *in vitro* studies on the transformation of hexachlorocyclohexane isomers, it was felt that the method of Pereira and Ahmed<sup>4</sup> can be used with modification for the topical application of HCH isomers to *Drosophila*. Further, in a recent publication from this laboratory Deo *et al*<sup>5</sup> have reported isomerization of the inert  $\beta$ -HCH in aqueous solution to biologically more active isomers. In view of this finding, it was decided to test the aqueous extract of  $\beta$ -HCH for its toxicity to *Drosophila*. The present paper describes our studies on the toxicity of common HCH isomers and of the aqueous extract of  $\beta$ -HCH to *Drosophila oregon R* adults using topical application.

### Materials and Methods

**Chemicals:** The HCH isomers were obtained from E. Merck, Germany.

**Insect rearing:** An homogeneous strain of *Drosophila oregon R* obtained from the Department of Botany, Mysore University, Mysore (India) was used for the toxicity studies. To meet the regular supply of adults of *Drosophila*, the insects were reared in wide mouth jars as per the procedure of Yule<sup>6</sup>. Population of insects emerging out from each jar every day was collected in separate jars so that it would be possible to use insects of the same age for toxicity studies. One to two days-old flies were used for topical application.

**Experimental:** Topical application of pesticides to *Drosophila* was done using an agla micrometer syringe following the method of Pereira and Ahmed<sup>4</sup> with slight modification. The diameter of both the fly holder tube and the vacuum pencil were reduced to suit the size of the insect. Solutions of  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ - and  $e$ -HCH isomers of desired concentration were prepared in acetone and applied topically to the abdomen of each fly.

To study toxicity of aqueous  $\beta$ -HCH to *Drosophila*, 2.0 mg of  $\beta$ -HCH were shaken with 125 ml of distilled water at  $25 \pm 1^\circ\text{C}$  for 24 hr, filtered, and the filtrate was left on the shaker for another 24 hr and 100 ml were then extracted with  $2 \times 25$  ml of ether. The ether

extract was dried and dissolved in acetone. The final volume of the acetone solution was reduced to 0.1 ml. This acetone solution at different dilutions was tested for its toxicity to *Drosophila*.

**Results and Discussion**

The results of topical application of  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ - and  $\epsilon$ -HCH isomers to adults of *Drosophila oregon R* are presented in Table 1. These data were subjected to probit analysis and the regression equation and LD<sub>50</sub> values are given in Table 2. The regression equation was further used to draw the probit kill vs. log. concentration graph (Fig. 1). The results in Table 1 give a fairly good idea about the toxicity of HCH isomers to the *Drosophila* strain under topical application. The  $\beta$ - and  $\epsilon$ -isomers were found nontoxic even at 6.0  $\mu$ g/fly. Both  $\alpha$ - and  $\delta$ -isomers were slightly toxic and showed 100 and 92 per cent mortality, respectively in 4 hr at a concentration of 0.4  $\mu$ g/fly. The most fascinating observation was the high toxicity exerted by microquantities of the  $\gamma$ -isomer. Application of 1.2 and 3.0 ng

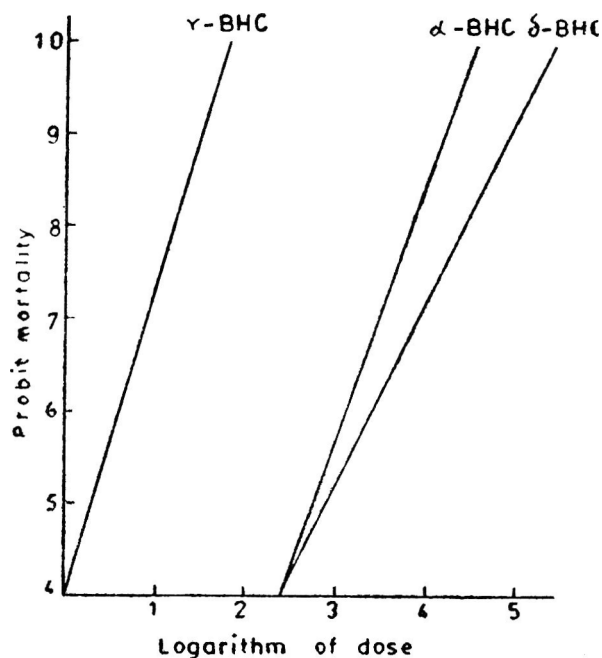


Fig. 1. Toxicity of HCH isomers to *Drosophila oregon R* adults

TABLE 1. TOXICITY OF HCH ISOMERS TO *Drosophila oregon R* ADULTS

HCH Isomer	Dose ng/fly	% mortality <sup>a</sup> at indicated hr			
		1	2	3	4
$\alpha$ -	400	19	32	72	100
	800	21	57	96	100
	1200	41	80	100	—
$\beta$ -	6000	nil	nil	nil	nil
$\gamma$ -	1.2	14	24	40	52
	3.0	40	60	82	90
	6.0	80	100	—	—
$\delta$ -	400	16	30	60	92
	800	18	50	82	100
	1200	35	67	95	100
$\epsilon$ -	6000	nil	nil	nil	nil
Control acetone only	0.61 $\mu$ l	nil	nil	nil	nil

a = Average of three replication each of 25 insects.

TABLE 2. LOG CONCENTRATION / PROBIT REGRESSION ANALYSIS OF DIFFERENT HCH ISOMERS

HCH isomer	Regression Equation	LD <sub>50</sub> (ng/fly)	Relative toxicity	Fiducial limits
$\alpha$ -	$Y = 2.3766 + 2.6383X$	588.8	-278.66	2.7007 2.8393
$\gamma$ -	$Y = 3.88 + 3.44X$	2.113	1	0.2618 0.3882
$\delta$ -	$Y = 0.9668 + 1.4527X$	758.6	-359.02	2.7955 2.9645

of  $\gamma$ -HCH per fly showed 52 and 90 per cent mortality, respectively in 4 hr. Toxicity at such low levels of  $\gamma$ -HCH against any insect has not been reported so far. Dresden and Oppenoorth<sup>7</sup> could find toxic effects with  $\gamma$ -HCH at as low as 9.0 ng/fly using skin application to *Drosophila*. This limit of detection is also about 4-5 times higher than that obtained with our strain of *Drosophila* using topical application.

The results of toxicity studies with the extracts of aqueous  $\beta$ -HCH are presented in Table 3. The high mortality values over the control strongly support earlier observations of Deo *et al.*<sup>5</sup> that aqueous  $\beta$ -HCH extract is quite toxic than  $\beta$ -HCH itself. Using the

TABLE 3. TOXICITY OF AQUEOUS  $\beta$ -HCH TO *Drosophila oregon R* ADULTS

Dose/insect ( $\mu$ l)	Vol. of acetone sol. obtained from 100 ml aq. $\beta$ -HCH (ml)	% mortality <sup>a</sup> at indicated hr			
		1	2	3	4
0.6	0.1	80	100	—	—
	0.2	60	72	76	84
	0.3	32	48	52	64
	0.4	nil	8	12	16
0.6	Control*	No mortality			

a = Av. of 3 replications, each of 25 insects.

\*Solution of fresh  $\beta$ -HCH in acetone (5 mg/ml)

ECGLC technique, these workers<sup>5</sup> have shown the presence of  $\alpha$ -,  $\gamma$ -, and  $\delta$ -isomers in the ether extract of aqueous  $\beta$ -HCH. Since the  $\gamma$ -isomer is about 50–10,000 times more toxic than other isomers, it is quite probable that the toxicity of aqueous  $\beta$ -HCH may be mostly due to the small quantities of  $\gamma$ -HCH resulting from isomerization of  $\beta$ -HCH. From Table 3, it is evident that 3.0  $\mu$ g of  $\beta$ -HCH extracted from its aqueous solution (presuming solubility of  $\beta$ -HCH as 5 ppm and efficiency of extraction 100 per cent) had the same mortality as that shown by 6 ng of  $\gamma$ -HCH/fly. From these results, if we presume that 6 ng of  $\gamma$ -HCH were formed from isomerization of 3.0  $\mu$ g of  $\beta$ -HCH, the isomerization of  $\beta$ -HCH to  $\gamma$ -HCH in water works out to be 0.2 per cent. This isomerization may be hazardous from environmental toxicology standpoint since  $\gamma$ -HCH is many times more toxic than other HCH isomers.

The data in Table 2 indicate that using topical application to *Drosophila*, it could be possible to detect very low levels of  $\gamma$ -HCH (2.113 ng) in the solution. The lowest limit of detection of  $\gamma$ -HCH using a GLC technique is normally around 1–2 ng, which is almost at the same level which we could detect by the present

bioassay method. Since the bioassay method, using topical application to *Drosophila*, does not involve the use of any sophisticated sensitive equipment, it can be easily used in any laboratory.

#### Acknowledgement

The authors wish to express their thanks to Mr. C. P. Natarajan, Director of the Institute for his keen interest in the present investigation.

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## A Reappraisal of Methods of Determination of Total Nucleotides in Sheep Postmortem Muscle

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Ultraviolet measurement of total nucleotides in postmortem muscles is an efficient and quick method to follow the breakdown of adenine nucleotides. Absorbance may be read at 250, 260 and 265 nm taking millimolar extinction of adenine and inosine nucleotides at 250 nm. For calculating total adenine and inosine nucleotides in muscle samples, the absorbance ratio of 265/250 appears to be most suitable.

Determination of high energy phosphates in meat is time consuming and cannot be used for rapid and routine analysis<sup>1</sup>. Different workers have used UV absorbance values and ratios to assess the postmortem breakdown of adenine nucleotides in the muscle tissues. Bendall and Davey<sup>2</sup> used Emax at 258 nm to calculate the nucleotide content using millimolar extinction values both for adenine (AN) and inosine (IN) nucleotides. They reported a total nucleotide (TN) content of 11.6  $\mu$  moles/g in rabbit muscles. Newbold and Scopes<sup>3</sup> used a modification of the method of Bendall and Davey<sup>2</sup> and obtained a value of 7.3  $\mu$  moles/g using

corrected absorbance readings at 250 nm for ox muscles. Although the TN content at different periods postmortem (p.m.) should not vary, there is a wide variation in TN content when calculated at 258 or 250 nm.

A critical assessment has been made of suitable wave-lengths and absorbance ratios, to give quick estimation of AN and more or less constant values for TN, have been suggested.

#### Materials and Methods

Semitendinosus (ST) muscle of a healthy male Bannur sheep (about 18 months old) was excised within 30 min

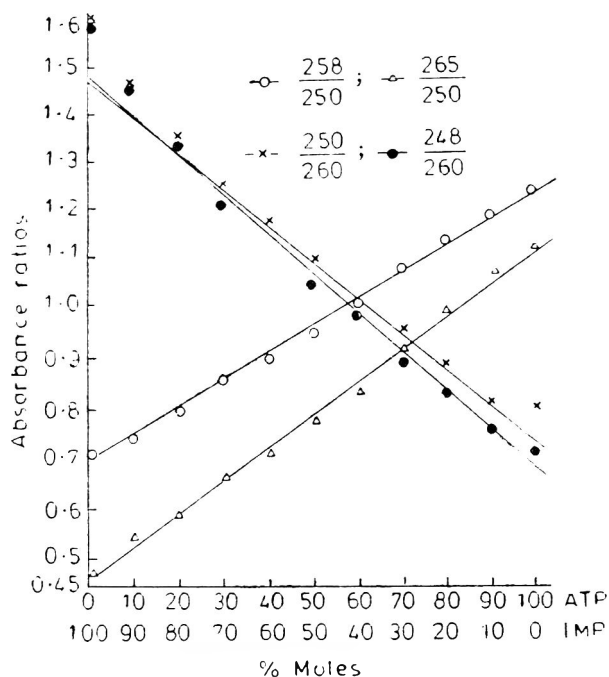


Fig. 1. Relation between absorbance ratios at percentages of pure ATP and IMP Mixtures (Concn of ATP and IMP varied from 0 to  $10 \times 10^{-5}$  M)

p. m. From this the samples were taken at regular intervals for analysis. One gram sample was thoroughly homogenised with 10 ml of 1M perchloric acid and filtered. 0.1 ml of filtrate, added to 4.9 ml of 0.1M phosphate buffer (pH 7.0), was used to measure absorbance at 248, 250, 258, 260 and 265 nm<sup>4</sup>. Standard curves were drawn using  $10^{-5}$ M solutions of ATP and IMP individually as well as mixtures<sup>5</sup>. TN content was calculated according to Bendall and Davey<sup>2</sup> taking millimolar extinction values for pure AN( $E_1$ ) and IN( $E_2$ ) at different wavelengths.

### Results and Discussion

Relationship between different absorbance ratios and percentages of pure ATP and IMP mixtures is shown in Fig. 1. All AN show an absorption maxima at

258-260 nm, whereas  $E_{max}$  for IN and hypoxanthine is at 248-250 nm. During conversion of AN to inosinic acid, a decrease in absorbance values at 258 nm is followed by a corresponding increase at 250 nm. The absorbance ratio 258/250<sup>1</sup> has also been found to change linearly with the ratio of AN and IN. Although the values of 258/250 and 265/250<sup>2,3</sup> change linearly, 248/260<sup>6</sup> and 250/260<sup>4</sup> do not change linearly especially below 20 per cent and above 80 per cent.

As seen in Table 1 TN contents show lower deviation when absorbance as well as  $E_1$  and  $E_2$  are taken at 250 nm (the isobestic point of AN and IN<sup>7</sup>). With this the TN content was almost constant throughout the p.m. period, whereas a higher variation was observed when TN content was calculated using absorbance at 258 nm. TN content based on absorbance ratio 248/260 and 250/260 exhibit no difference as also 258/250 and 265/250. It was also observed that a highly significant correlation ( $p < 0.01$ ) exists between the AN content calculated by the present method and the values of ATP estimated chemically.

### Acknowledgement

The authors thank Dr. P. K. Vijayaraghavan, Director, of the laboratory for his keen interest and for providing adequate facilities to carry out this work.

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TABLE 1. TOTAL NUCLEOTIDE\* ( $\mu$  MOLES/G) CALCULATED FROM DIFFERENT ABSORBANCE RATIOS

$E_1$ & $E_2$ at	Based on absorbance at 258 nm				Based on absorbance at 250 nm			
	258/250	265/250	250/260	248/260	258/250	265/250	250/260	248/260
250 nm	9.43 $\pm$ 1.93	9.42 $\pm$ 1.90	9.41 $\pm$ 1.84	9.41 $\pm$ 1.89	9.48 $\pm$ 0.16	9.49 $\pm$ 0.16	9.42 $\pm$ 0.16	9.48 $\pm$ 0.16
258 nm	9.65 $\pm$ 1.21	9.65 $\pm$ 1.30	9.88 $\pm$ 1.33	9.89 $\pm$ 1.22	9.77 $\pm$ 1.12	9.86 $\pm$ 0.85	10.03 $\pm$ 0.94	10.13 $\pm$ 1.04
$E_1$ at 258 nm $E_2$ at 250 nm	8.46 $\pm$ 1.40	8.43 $\pm$ 1.42	8.49 $\pm$ 1.40	8.52 $\pm$ 1.38	8.59 $\pm$ 0.46	8.55 $\pm$ 0.44	8.62 $\pm$ 0.47	8.53 $\pm$ 0.47

\*The total nucleotides represent the average of estimation at 8 intervals during 24 hr p.m.



# Effect of Fungitoxicants on Grape Must Fermentation and Wine Yeast Growth

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Six fungitoxicants namely, Difolotan, Bavistin, Calixin, Sclex, Dithane M-45 and Miltox were evaluated for two years for their effect on must fermentation and composition of red table wines when sprayed on 'Beauty Seedless' grapes. Only Difolotan was found to affect the rate of fermentation. However, the gross chemical composition of wines remained unchanged. All the fungitoxicants affected the growth of wine yeast *in vitro* at a concentration higher than 10 ppm but Difolotan was 10 times more toxic compared to others.

A large number of fungitoxicants are tested regularly to control the pathogenic fungi in field, during transportation and storage of grapes. The adverse effect on must fermentation by the residues of some of the fungitoxicants such as Captan, Phaltan, Eupareu, Mycodifol, Orthophaltan and Benomyl has been reported<sup>1,3</sup>. The systemic fungitoxicants were found to be less toxic compared to non-systemic<sup>4</sup>.

A large number of fungitoxicants like Captan, Phaltan, Difolotan Dichlofuanide, TMID and Benomyl, are inhibitory to a variety of yeast strains associated with grapes<sup>5-7</sup>. However, the interactions of a fungitoxicant with alcoholic fermentation of grape must largely depend upon the type of formulation, frequency, method of application, climatic conditions and type of yeast used for fermentation. Benomyl applied to grapes even 8 days before harvest has not been found to be toxic<sup>8</sup>, but was toxic in liquid peach fermentation at a concentration as low as 5 ng/ml<sup>3</sup>. Moreover, about 60 per cent of the fungitoxicants applied to grapes have been found to be transferred to must<sup>4</sup> and red wines contained more residues compared to white wines<sup>9</sup>. This is due to pulp fermentation process for red wine making. Therefore, attempts were made to examine the effect of commercially recommended fungitoxicants on Beauty Seedless grapes must fermentation, wine composition and *in vitro* growth of yeast.

## Materials and Methods

The fungitoxicants, Difolotan, Bavistin, Calixin, Sclex, Dithane M-45 and Miltox were obtained from Rallis India Ltd. (80 per cent Wettable powder (W.P.)), Ltd., BASF India Ltd. (50 per cent w.p.), BASF India (75 per cent emulsifiable concentrate), Sunito Chemical Co. Japan (30 per cent w.p.), Indofil Ltd. (75 per cent

w.p.) and Sandoz India Ltd. (49.5 per cent w.p.), respectively. Bavistin, Calixin, and Sclex are systemic, whereas others are non-systemic fungitoxicants. During 1975 vintage, Difolotan, Bavistin, Calixin and Sclex were tested, while in 1976 Dithane M-45 and Miltox replaced Calixin and Sclex. These fungitoxicants are being widely used in India for controlling pathogenic fungi on grapes.

The wine yeast *Saccharomyces cerevisiae* var. *ellipsoideus* (No. 522) used was from the Department of Enology and Viticulture, University of California, Davis, USA. The vines of cultivar 'Beauty Seedless' (*Vitis vinifera* L.) in the experimental vineyard of Haryana Agricultural University, Hissar, were sprayed with different fungitoxicants with 2 l of 0.2 per cent formulations/vine, 40 days before harvest in both the years. The control vines were sprayed with an equal quantity of water.

Grapes (10 kg) from the different fungitoxicant sprayed vines were destemmed and crushed gently with hands. The must was treated with 100 ppm of SO<sub>2</sub> (as potassium metabisulphite) and ameliorated with cane sugar to 24° Brix. The total acidity was adjusted to 0.75 per cent with tartaric acid. For initiating the fermentation an actively growing yeast at 5 per cent level ( $\approx 2 \times 10^7$  cells/ml) prepared in pasteurized grapes was added. The course of fermentation was measured by using calibrated Brix hydrometers. The pomace was removed at 8° Brix and the juice was allowed to ferment further to dryness (-1.5° Brix). The red wines were processed by the methods reported elsewhere<sup>10</sup>. The composition of grape musts and red wines were determined by the procedures described by Amerine<sup>11</sup>.

Effect of different fungitoxicants on the growth of wine yeast was examined *in vitro* using Difco yeast extract nitrogen base (YNB) containing 2 per cent

glucose. To 50 ml of sterilized YNB broth various concentrations (0.1, 1, 10 and 100 p.p.m.) of fungitoxicants were added. To test residual toxicity which increased with solubility, in one set 5 per cent ethanol was also added to the sterilized YNB broth. All the flasks including the control without fungitoxicant were incubated with an actively growing 24 hr old yeast culture at one per cent level ( $\approx 5 \times 10^7$  cells/ml) and incubated on a rotary shaker at  $30 \pm 1^\circ\text{C}$ . The growth was measured by determining optical density at 520 nm using Bousch and Lomb Spectronic-20 after 24 hr of incubation using uninoculated respective fungitoxicant YNB broth as control.

TABLE 2. FALL IN °BRIX DURING FERMENTATION OF GRAPE MUST

Fungitoxicant	year	Fermentation time (hr)					
		0	12	36	60	84	108
Control	1975	24.0	20.0	6.0	0.0	-1.5	—
	1976	24.0	18.5	1.5	1.0	-1.5	—
Difolotan	1975	24.0	21.0	10.0	4.0	1.0	-1.5
	1976	24.0	19.5	8.0	2.0	0.0	-1.5
Bavistin	1975	24.0	20.5	7.0	0.5	-1.5	—
	1976	24.0	18.5	1.0	-1.5	—	—
Calixin	1975	24.0	21.0	7.5	0.5	-1.5	—
Sclex	1975	24.0	20.0	6.5	0.5	-1.5	—
Dithane M-45	1976	24.0	18.5	1.5	-1.5	—	—
Milttox	1976	24.0	12.5	1.0	-1.5	—	—

TABLE 3. EFFECT OF FUNGITOXICANTS ON YEAST GROWTH *in vitro*

Fungitoxicants	Concn. (p.p.m.)	Optical density at 520 nm at diff. times			
		Without ethanol 24 hr	Without ethanol 48 hr	With ethanol 24 hr	With ethanol 48 hr
Control	0.0	0.96	1.47	0.46	1.44
Difolotan	0.1	0.94	1.37	0.24	1.11
	1.0	0.05	0.05	0.03	0.02
	10.0	0.06	0.06	0.01	0.03
	100.0	0.03	0.03	0.02	0.02
Bavistin	0.1	0.89	1.32	0.42	1.32
	1.0	0.88	1.23	0.35	1.35
	10.0	0.80	1.12	0.36	1.35
	100.0	0.50	0.84	0.18	0.15
Calixin	0.1	1.03	1.38	0.45	1.30
	1.0	0.62	1.04	0.33	1.05
	10.0	0.48	0.69	0.19	0.35
	100.0	0.05	0.05	0.05	0.05
Sclex	0.1	0.97	1.51	0.43	1.40
	1.0	0.98	1.47	0.44	1.43
	10.0	0.99	1.48	0.32	1.31
	100.0	0.77	1.38	0.31	1.32
Dithane M-45	0.1	0.96	1.44	0.45	1.36
	1.0	0.95	1.45	0.35	1.33
	10.0	0.03	0.05	0.05	0.03
	100.0	0.03	0.04	0.04	0.03
Milttox	0.1	0.98	1.48	0.48	1.46
	1.0	0.87	1.39	0.41	1.40
	10.0	0.03	0.05	0.04	0.04
	100.0	0.03	0.04	0.04	0.04

TABLE 1. MUST COMPOSITION OF FUNGITOXICANTS TREATED GRAPE

Fungitoxicant	Year	pH	Total acidity %	°Brix	Balling acid ratio
Control	1975	3.2	0.75	18.0	24.0
	1976	3.1	0.87	15.5	17.8
Difolotan	1975	3.2	0.75	18.0	24.0
	1976	3.1	0.87	15.5	17.8
Bavistin	1975	3.2	0.72	16.0	22.2
	1976	3.1	0.88	14.5	16.7
Calixin	1975	3.2	0.75	18.0	24.0
Sclex	1975	3.2	0.75	17.0	22.7
Dithane M-45	1976	3.1	0.87	15.5	17.8
Milttox	1976	3.1	0.88	15.5	17.6

TABLE 4. ANALYSIS OF FRESH RED WINES PREPARED FROM FUNGITOXICANTS TREATED *BEAUTY SEEDLESS* GRAPES

Fungitoxicants	Year	Optical density (520 nm)	pH	Total acidity (%)	Volatile acidity (%)	Alcohol (% v/v)	Total aldehydes (ppm)	Sugar (%)	Total phenols (%)
Control	1975	2.20	3.4	0.69	0.024	11.6	19.8	0.40	0.094
	1976	1.84	3.2	0.78	0.017	10.0	22.0	0.11	0.090
Difolotan	1975	1.96	3.3	0.63	0.020	11.2	14.2	0.15	0.086
	1976	1.92	3.2	0.78	0.017	9.3	66.0	0.13	0.060
Bavistin	1975	2.10	3.4	0.62	0.021	11.2	19.8	0.30	0.086
	1976	1.92	3.0	0.78	0.017	9.1	24.2	0.13	0.060
Calixin	1975	1.87	3.4	0.65	0.026	11.4	26.4	0.40	0.088
Sclex	1975	1.96	3.3	0.70	0.023	11.6	17.6	0.30	0.086
Dithane M-45	1976	2.19	3.2	0.78	0.015	9.3	33.0	0.11	0.090
Milttox	1976	1.49	3.3	0.77	0.017	8.9	33.0	0.13	0.047

broth. The delay in fermentation rate was more pronounced between 24 and 36 hr by Difolotan because at this stage of wine fermentation about 5 per cent of ethanol is produced in the must. Therefore, the effect of fungitoxicants was also examined in 5 per cent ethanol.

The yeast growth was completely inhibited at a concentration of 1 p.p.m. of Difolotan, 10 p.p.m. of Dithane M-45 and Milttox and 100 p.p.m. of Calixin (Table 3). There was no significant effect of Sclex and Bavistin on growth. Added ethanol showed synergistic response on yeast growth inhibition. Therefore, the delay in fermentation rate can be attributed to higher toxicity of Difolotan than by other fungitoxicants. The fungicidal/fungistatic effect on large number of yeast strains and fruit microflora has been well documented in literature<sup>1,3,5,8</sup>. However, the extent of inhibition differs from strain to strain. The fresh red table wines prepared from different fungitoxicant sprayed grapes were analysed chemically and sensorially (Table 4). The fungitoxicants used did not show any appreciable change in gross chemical composition and organoleptic tests. Similar observations have been reported by several workers<sup>13,14</sup>. The present studies suggest that Difolotan spray on 'Beauty Seedless' grapes intended for red table wine making should be avoided. The other five fungitoxicants can be used safely provided their residual concentrations do not exceed 10 p.p.m.

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# Use of Simple Solar Dehydrator for Drying Fruit and Vegetable Products

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Two models of solar dehydrators were constructed with mixed functions of direct and indirect dryers. In model I, the trays were placed in a separate chamber and in model II, the trays were adjusted inside the collector. The model I and model II attained 10–20°C and 20–25°C, respectively, higher temperature than the open atmosphere. Samples of mango slices, mango leather, green peas, okra and three potato products were satisfactorily dehydrated. On an average, model II reduced the moisture to 6 per cent in 7 hr and model I to 7 per cent in over 8 hr compared to about 9 hr needed in open atmosphere for getting 8 per cent moisture. The products were considered qualitatively superior to the open sun-dried products. The solar dehydrators are simple to fabricate and are well suited to rural conditions and small scale food processing industries.

The use of solar energy for processing of agricultural produce would be most suitable in a country like India provided the system is simple and economical. Sun-dried products are usually of inferior quality, containing dust and dead insects. But some of the sun-dried foods are superior to the ones dried in hot air ovens<sup>1</sup>.

With the objective of harnessing solar energy for food processing, two simple models of solar dehydrators, which could be useful for small scale units in rural areas, were assembled and number of mango and potato products, green peas and okra were dehydrated satisfactorily.

## Materials and Methods

Two models of solar dehydrators were fabricated. These are mixed type of dehydrators, operating as direct and indirect solar dryers, although in one model, the material to be dehydrated is exposed more to direct solar radiation than the other.

**Model I:** This solar dehydrator (Fig. 1) is a modified version of the model developed by the University of Hawaii, USA<sup>2</sup>. Basically, it has a solar collector and a chamber to accommodate eight aluminium mesh trays of 50 × 90 cm size. There is a clearance of about 8 cm between the trays. The air inlet is controlled by means of sliding plates (6 × 60 cm) at the lower end of the solar collector and the outlet is similarly regulated by sliding plates (4 cm wide) on all the four sides at the top of the chamber. All the apertures are fitted with fine aluminium mesh to prevent the entry of insects,

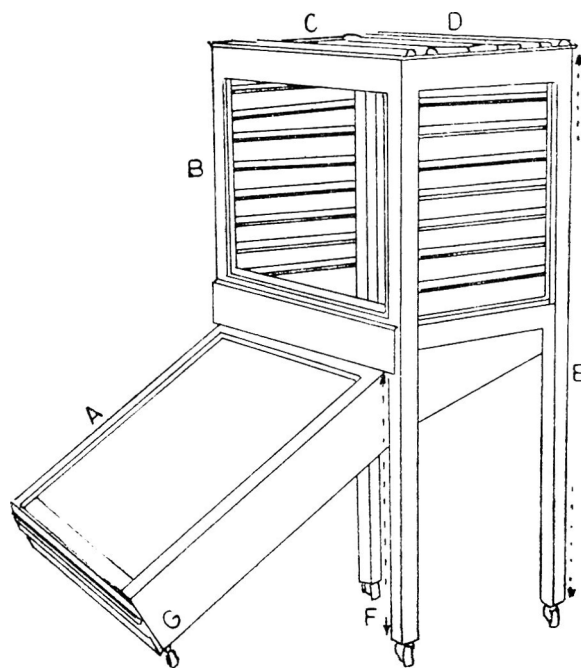


Fig. 1. Solar dehydrator, model I. The dimensions (cm) are A = 150, B = 74, C = 60, D = 100, E = 174, F = 90 and G = 15.

etc. The chamber is reported to have a chimney effect and may increase the air flow. During the operation, air inside the chamber can attain a temperature of 10–20°C higher than the outside air at full opening of the aperture. It has a total loading area of 3.6 sq m and costs about Rs. 800.

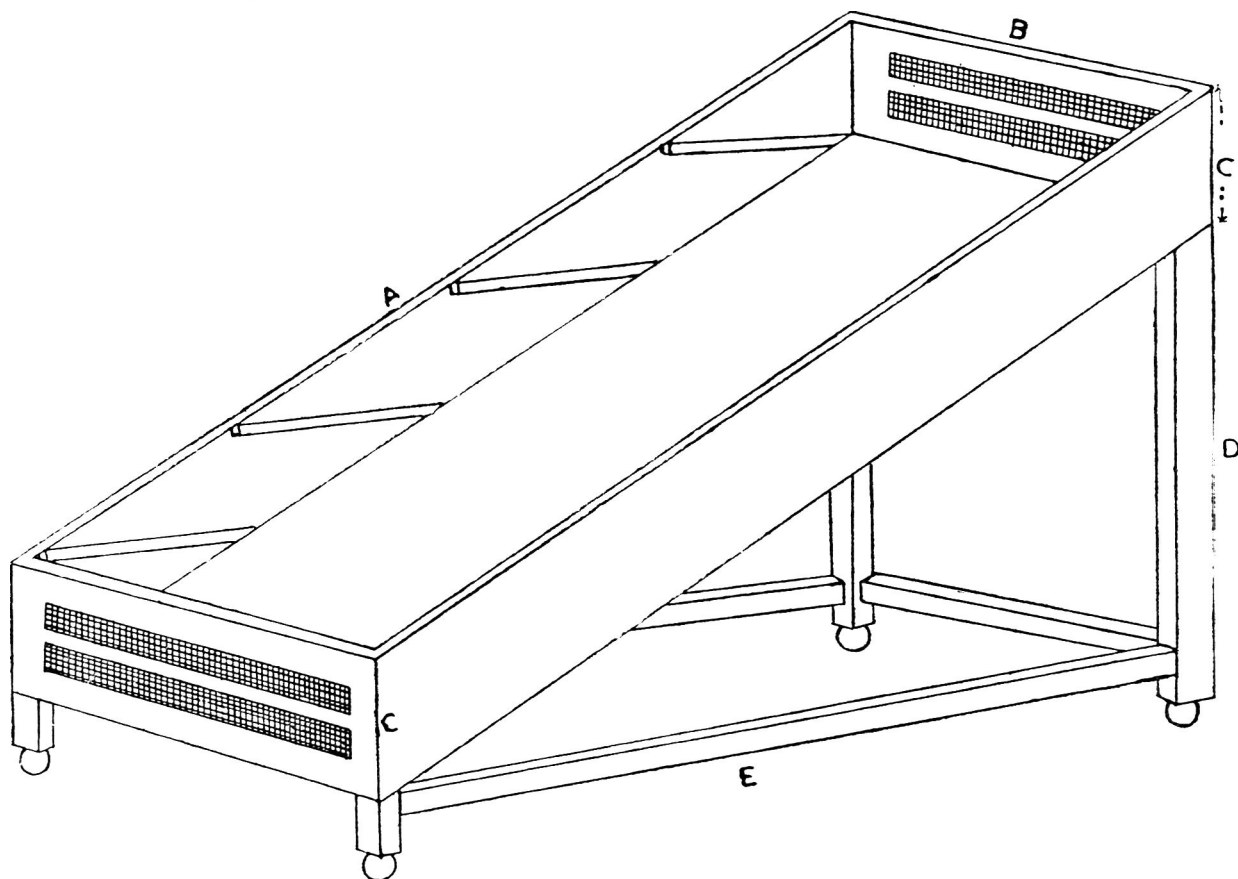


Fig. 2. Solar dehydrator, model II. The dimensions (cm) are A=173, B=94, C=30, D=157 and E=143.

**Model II:** This appears similar to the flat-plate solar collector<sup>3</sup> but it has been so designed that the same chamber could act as solar collector as well as dehydrator (Fig. 2). It can accommodate upto seven trays. In the present model, however, only four trays (38 × 90 cm, each) are placed inside the collector at equal distances and parallel to the ground. The air inlet and outlet are provided at the lower and upper ends of the collector, respectively. The temperature attained inside this dehydrator is 20–30°C higher than that of the outside air at full aperture opening. The drying area is 2.4 sq m and the cost of construction is approximately Rs. 550.

Both the solar radiation collectors are made up of single glass plates at the top while the bottom, is provided with black painted iron sheet facing the sun, insulated underneath with thermocol and plywood. The depth of collectors is 15 cm in model I and 27 cm in model II.

Mangoes, peas, okra and potatoes, grown at the Experimental Station of the Central Mango Research Station, were dehydrated.

**Green mango powder:** Raw mangoes from a seedling tree were washed, peeled and cut into slices, of 4–5 mm thick and 30–40 mm long and were immersed for 10 min in 0.2 per cent potassium metabisulphite (KMS) solution. After dehydration, the slices were powdered.

**Mango slices:** Fully ripe but not soft 'Dashehari', 'Langra' and 'Chausa' mango fruits were peeled and sliced into pieces of 5–6 mm thick and 6–10 cm long. The slices were dipped for 5 min in a solution of 0.4 per cent CaCl<sub>2</sub> and 1.5 per cent KMS to maintain texture and inhibit bacterial growth<sup>4</sup>.

**Mango leather:** The pulp from "Dashehari and 'Langra' fruits was homogenized with KMS (equivalent to 150 ppm SO<sub>2</sub>) and spread on the aluminium trays of the solar dehydrator.

**Green peas:** Fresh pods were manually shelled, slit with a pin-brush and treated for 5 min with 0.8 per cent KMS solution.

**Okra:** Fresh green lady's finger were washed, wiped and cut into approximately 2 cm long pieces and dehydrated.

**Potato chips:** Potatoes were washed and peeled manually. One millimetre thick slices were prepared with hand slicer and blanched for one minute in hot water at 80–85°C. The slices were then dipped for 10 min in a solution containing 0.3 per cent sodiumbisulphite, 0.4 per cent CaCl<sub>2</sub> and 1.0 per cent NaCl. They were spread in single layer on the trays of the dehydrator.

**Potato Fries:** After washing and peeling, the potatoes were cut into French Fries of 5 mm thick and 5–7 cm

long and were blanched for 10 min in hot water at 80-85°C and treated in a solution containing 0.3 per cent sodiumbisulphite, 0.2 per cent CaCl<sub>2</sub> and 1.0 per cent NaCl for 10 min.

*Potato 'Papad'*: Potatoes were washed, boiled for 30 min, peeled manually, and were mashed with 1 per cent fat and a small quantity of salt and some spices. 'Papad', of approximately 0.5-1 mm thickness and 10 cm diameter were prepared and spread on trays of the dehydrator.

The moisture content of fruits and vegetables was determined by drying in an oven at 70-72°C to a constant weight.

### Results and Discussion

The prepared fruit and vegetable products were dehydrated in either of the two models of solar dehydrator. One lot was also dried in the open air for comparison of the efficiency of dehydration.

*Dehydration of fruit products*: The dehydration data for fruits are presented in Table I. In the case of raw mango slices, model II dehydrator was more efficient. It took about 12 hr to reduce the moisture content from 83 to 2.5 per cent while in model I it required nearly 14 hr to bring down the moisture to 3 per cent. After 7 hr of drying of ripe mango slices in model II, there was only 8 per cent moisture as against 13 per cent in the open air-dried samples. Mango leather with two layerings took 7 hr in model II to bring the moisture to 8.9 per cent as compared to 9 hr needed to reduce the moisture to 10 per cent in open air. The efficiency of model I was in-between those of model II and open air drying (Table I).

*Dehydration of vegetable products*: Green peas are harvested during winter months and in that period, solar dehydrator, model I attained a temperature of 15-20°C higher than that of the atmosphere. The

moisture content of green peas was reduced from 75 to 5 per cent in less than 9 hr of drying as compared to more than 10 hr needed in open air drying (Table I). Further,

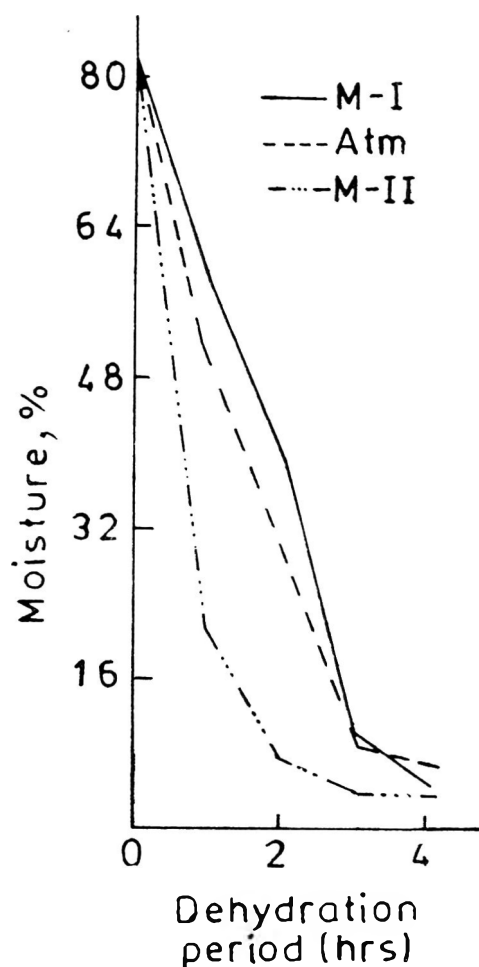


Fig. 3. Dehydration pattern of potato chips in solar dehydrator. M-I Model I (57°C), M-II, Model II (73°C); Atm, open air (44°C)

TABLE I. SOLAR DEHYDRATION TEST AND CONDITIONS

Material	Loading (kg/m <sup>2</sup> )	Model I			Model II			Open air		
		Temp (°C)	Drying time (hr)	Moisture (%)	Temp (°C)	Drying time (hr)	Moisture (%)	Temp (°C)	Drying time (hr)	Moisture (%)
Mango slices (raw)	4.4	52	14	3.0	70	12	2.5	41	14	4.0
Mango slices (ripe)	4.9	55	7	12.0	70	7	8.0	42	7	13.0
Mango leather	8.5	55	8	9.0	70	7	8.9	43	9	10.0
Green peas	4.8	42	9	5.0	nil	nil	nil	28	10	7.0
Okra	3.6	51	10	9.5	68	9	7.8	45	10	10.9
Potato chips	1.7	57	4	5.6	73	3	4.4	44	4	6.0
French fries	2.8	52	10	3.3	63	8	5.1	43	10	7.0
Potato 'papad'	0.8	48	5	7.0	55	4	5.0	35	5	8.0

The data are the average of 3 observations.

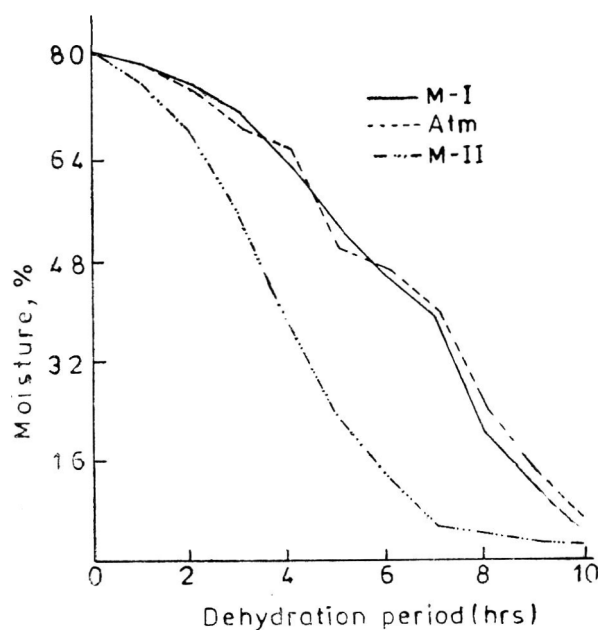


Fig. 4. Dehydration pattern of French Fries M-I, Model I (52°C); M-II, Model II (63°C); Atm. open air (43°C).

the colour and general quality of the dehydrated product were also better.

In case of dehydration in model II, okra took 9 hr, potato chips 3 hr, 'Paped' 4 hr and French Fries 8 hr (Table I). In all these cases, model II was more efficient than model I which was only slightly better than open air drying. The rate of dehydration and open air drying with time are presented in Fig. 3, 4 & 5 for potato

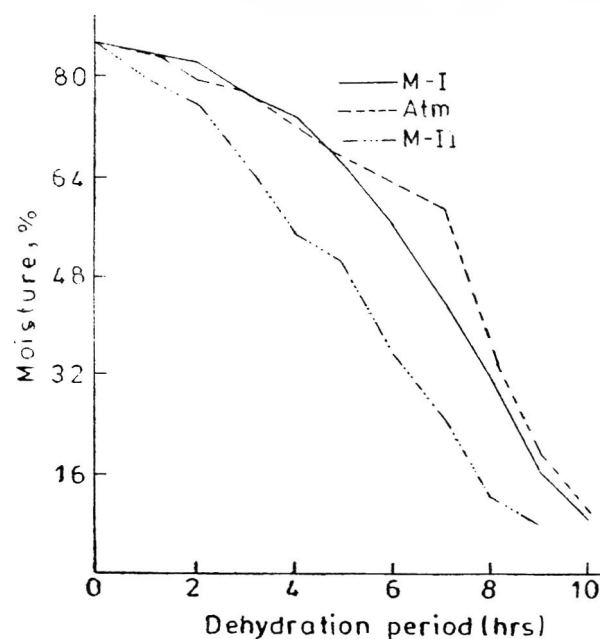


Fig. 5. Dehydration pattern of okra. M-I, Model I (51°C); M-II, Model II (68°C); Atm, open air (45°C)

chips, french Fries and okra, respectively. The higher dehydration efficiency of model II is by and large due to the higher temperature attained (70°C) as compared to that of model I (52-55°C) and of the atmosphere (41-43°C).

Solar dehydrators are not only more efficient dryers but also yield products which are clean, free from insect infestation and of better quality<sup>4-6</sup>. Even fruit products like mango which contain high concentration of sugars, can be dehydrated under better hygienic conditions free from the attack of birds, rodents, etc. The temperature in the solar dehydrator depends upon the external atmospheric temperature. Windy and cloudy weather tends to limit the difference between inside and outside temperatures. The heat as well as the air current in the dehydrator can be controlled, to some extent, by adjusting the sliding windows. Although increasing the aperture will slightly lower the temperature, but it may accelerate the air flow and hence, the rate of dehydration. The collector has to be manually rotated so as to receive direct solar radiation.

Such simple dehydrators are particularly suited to rural conditions in India because there is non availability of electrical or any other form of additional energy. Their design is simple and easy to fabricate with less cost even by a local carpenter. Many of the food products require temperatures less than 80°C for drying and the solar collectors are highly efficient in this range<sup>7,8</sup>. As such, the model dehydrators based on solar energy only are likely to be highly useful for small scale drying of fruits and vegetables and some of their products.

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# Effects of Freeze-concentration on Chemical and Sensory Qualities of Apple Juice

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The objectives of the present study were to investigate the effects of freeze-concentration on the chemical, physical and sensory qualities of apple juice concentrate prepared from two varieties of apples. Golden delicious and Starking apple juice samples were enzymatically clarified and freeze-concentrated. The reconstituted juice samples were tested for chemical and sensory properties. On freeze-concentration of apple juice to five fold, the acid content increased less than four fold, indicating that acids were selectively lost during freeze-concentration. Ascorbic acid was completely lost during freeze concentration. However, in the enriched juice, 23% of the ascorbic acid was retained in Golden apple juice and 15% in Starking apple juice. The results of sensory tests showed that ascorbic acid addition improved both the color and flavor of reconstituted apple juice. The taste panel showed preference for the clarified apple juice.

The search for improved methods of concentration of thermolabile juices to avoid objectionable flavour changes led to the use of vacuum concentration and freeze-concentration methods. The moisture is removed in the latter process, after freezing the juice as ice crystals<sup>1,2</sup>.

The objectives of this study were to freeze-concentrate apple juice obtained from two local varieties of apples, namely, 'Starking' and 'Golden Delicious', and to study the effects of freeze concentration, clarification, and ascorbic acid enrichment, on chemical and sensory qualities of the concentrated juice.

## Materials and Methods

Two apple (*Pyrus malus*) varieties were used viz., 'Golden Delicious' and 'Red Starking'. The fruits were washed, pressed in a laboratory size juicer; juice strained through two layers of cheese cloth, heated at 87°C for 2 min and cooled rapidly. Enzymatic clarification of apple juice was carried out by adding 0.15 g/l of pectic enzyme (Pectinol R-10 from Rohm and Haas Co.). The juice was kept at 15°C for 12 hr and filtered. Ascorbic acid enriched juice was prepared by adding 60 mg of ascorbic acid per 100 ml of juice immediately before clarification. Freezing of the juice was done in plastic containers (capacity 170 ml); and the juice was kept in a freezer at -24°C for 3-5 hr. Freeze-concentration of the juice was done by centrifuging the frozen juice at 4500 rpm for 7 min. During centrifugation, supernatant was separated from the solid mass and was recovered as much as possible by decanting. The solid mass was mostly ice and most of the juice solids separated into the supernatant.

The supernatant was tested for total soluble solids (TSS) content, and then placed in the freezer at -24°C until frozen. The whole process of centrifugation of the refrozen supernatant and separation of the consequent supernatant was repeated several times until a concentrate of the desired solids content was obtained.

## Results and Discussion

On freeze concentration, the 'Golden Delicious' apple juice (clarified and unclarified) was concentrated by five folds (from 12° to 60° Brix), while the acid content increased only 3-4 folds. On reconstituting the concentrate back to the original strength, the acid content of the reconstituted 'Golden Delicious' apple juice was less than that of fresh Juice (0.25 per cent) as shown in Table 1. This shows that a decrease in acid content had taken place during freeze-concentration.

Similar results were also obtained with 'Starking' juice on freeze-concentration. These results agree with those of Charley<sup>1</sup> and Heiss and Schachinger<sup>3</sup> who found that organic acids such as malic, tartaric and citric tend to remain in capillary spaces of the solid components. They found that the TSS increased by 4-5 folds while the acidity increased only 3 folds; and on reconstitution the acid content was less than that of the fresh juice.

Ascorbic acid was completely lost on freeze-concentration and reconstitution to the single strength juice, in both varieties, as shown in Table 2. Previous studies by Heiss and Schachinger<sup>3</sup> indicated that a very small quantity of ascorbic acid accumulated in the ice



TABLE 1. CHEMICAL CHARACTERISTICS OF UNCLARIFIED AND CLARIFIED APPLE JUICES

Type of Juice	Total soluble solids (%)		Acidity (as malic acid %)		Ascorbic acid (mg/100 ml)		pH	
	GD	St	GD	St	GD	St	GD	St
<i>Unclarified</i>								
Single strength	11.8	13.9	0.32	0.33	6.5	8.74	3.81	3.86
Freeze-concentrated	60.0	61.0	1.20	1.29	—	—	3.75	3.79
Reconstituted	12.0	14.0	0.25	0.25	nil	nil	3.81	3.87
<i>Clarified</i>								
Single strength	12.3	13.7	0.38	0.36	5.13	5.47	3.80	3.86
Freeze-concentrated	59.9	60.3	1.32	1.27	—	—	3.75	3.87
Reconstituted	12.0	14.0	0.31	0.22	nil	nil	3.81	3.87

GD = Golden Delicious; St = Starking.

TABLE 2. EFFECT OF FREEZE CONCENTRATION ON ASCORBIC ACID RETENTION OF RECONSTITUTED APPLE JUICE

Type of juice	Unenriched (mg/100 ml)		Enriched (mg/100 ml)		Retention (%)	
	GD	St	GD	St	GD	St
<i>Unclarified</i>						
Single strength	7.45	9.90	65.8	67.71	—	—
Freeze-conct. reconstituted	—	—	14.9	9.64	23	14
<i>Clarified</i>						
Single strength	4.32	5.18	63.9	65.29	—	—
Freeze-conct. reconstituted	—	—	14.0	10.20	22	16

GD = Golden Delicious; St = Starking.

TABLE 3. SENSORY SCORES\* OF RECONSTITUTED APPLE JUICE USING HEDONIC SCALE

Treatment	Colour		Odour		Cloudiness		Taste		Overall rank	
	GD	St	GD	St	GD	St	GD	St	GD	St
Unclarified-unenriched	4.7	4.0	7.0	7.4	5.5	4.3	6.4	7.0	6.5	6.5
Unclarified-enriched	7.4	7.0	7.4	7.0	6.5	6.1	6.8	7.7	6.7	7.2
Clarified-unenriched	8.4	7.3	6.4	7.3	8.4	8.4	7.4	7.6	7.5	7.8
Clarified-enriched	8.4	8.3	9.4	6.9	9.5	8.4	9.3	6.9	7.7	7.0

GD = Golden Delicious; St = Starking.

\*Average of 9 judges.

residue during freeze-concentration of juices because ascorbic acid does not exhibit capillary activity. Thus the plausible explanation for the loss is probably due to the auto-oxidation of ascorbic acid in the presence of oxygen when the frozen juice was thawed and refrozen several times during the concentration process. However, some ascorbic acid was retained in the enriched juice. It was found that 23 per cent of ascorbic acid was retained in the unclarified 'Golden Delicious' apple juice and 22 per cent in the clarified juice. The retention values were 14 per cent for the reconstituted 'Starking' unclarified juice and 16 per cent for the clarified juice. The loss of ascorbic acid in the enriched 'Starking' apple juice was greater than that of the enriched 'Golden Delicious' apple juice.

The pH clarified and unclarified, juice of both varieties decreased upon freeze-concentration and increased approximately to the same value as that of the fresh juice on reconstitution (Table 1).

The sensory analysis scores shown in Table 3 indicated that the maximum score for colour was given for clarified and clarified-enriched samples. Hence, clarification improved the colour of the reconstituted juice. Enrichment with ascorbic acid improved the colour of some samples and the odour of others. Cloudiness and taste were improved on ascorbic acid addition and clarification by enzyme.

Similar results were obtained for the reconstituted 'Starking' apple juices. The best ranking was given for the clarified-enriched juice sample. In general,

organoleptic scores for reconstituted juice were very close to those of fresh single strength juice, thereby indicating that freeze-concentration is an appropriate method which can be used to concentrate fruit juices and to maintain the same quality as that of the original juice.

It can be concluded from this study that freeze-concentration lowered the acid content of the juice. Enzyme clarification and ascorbic acid enrichment enhanced the sensory qualities of the juice by improving the colour and flavour, except in the clarified 'Starking' varieties. The ascorbic acid was totally lost in unenriched apple juice samples. Enriched 'Golden Delicious' apple juice

retained ascorbic acid more than the 'Starking' variety during freeze-concentration and reconstitution. This agrees with the findings of Bolin and Salunkhe<sup>4</sup> who compared different concentrating procedures and found freeze-concentration to be the best.

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## RESEARCH NOTES

### VARIETAL DIFFERENCES IN PUFFING QUALITY OF RAGI (*ELEUSINE CORACANA*)

Optimal conditions for puffing of Ragi (*Eleusine coracana*) were moistening to 19 per cent moisture and equilibration for 4 hr, followed by puffing in sand medium at 270°C. Wide varietal variation has been found in the puffing quality, among 14 varieties studied. No consistent relationship was observed between grain amylose, protein content or thickness of bran with puffing quality. 'Purna', 'Annapurna', 'Shakti', 'P. R. 202' and 'Indaf - 3' possess good puffing quality.

Puffing of cereals has been practiced since hundreds of years for use as a snack food either after spicing or sweetening<sup>1</sup>. Puffed material has a low bulk density and pleasing texture with a distinct appealing flavour. Wide varietal differences have been found in puffing quality of paddy<sup>2,3</sup>, sorghum<sup>4</sup> and other cereals. Varietal variation of ragi in this respect although generally known is not documented. An account of our study on this aspect is reported in this paper.

Market sample of ragi was initially used to study the effects of moisture content, equilibration time and temperature of puffing medium on puffing yield and expansion and to determine optimum process conditions. Fourteen varieties of Ragi (Table 1) obtained from Ragi Breeder, V. C. Farm, Mandya, were later studied

for their relative puffing characteristics as per the following method: After determining the moisture in the sample by oven drying method, water was sprayed on the grain to raise the moisture content to 19 per cent, mixed well and grain equilibrated in closed tin for 4 hr prior to puffing. Conditioned ragi samples (50 g) were puffed in an iron frying pan using fine (-40 B. S. S. mesh) sand as heat exchange medium, the temperature of which was maintained at 270°C. Immediately when puffing sound was stopped the pan was removed from the flame and sand was separated by immediate sieving through 40 mesh (B.S.S.) sieve. Unpuffed grains were separated by sieving through 8 (B.S.S.) mesh and the 18 mesh fraction representing puffed grain was weighed to determine the puffing yield and volume of puffed grains was measured in a measuring cylinder.

Protein was estimated by microkjeldahl method, total and water-soluble amylose by method of Sowbhagya and Bhattacharya<sup>5,6</sup>, husk and bran content by wet grinding method<sup>7</sup>. The grains were embedded in paraffin wax, cut into pieces and exposed to water for about 24 hr from which hand sections were taken and stained with Sudan-4 and the thickness of the bran and aleurone layers was measured microscopically.

From the data relating puffing yield to grain moisture, equilibration time and temperature of roasting medium (Fig. 1, 2 and 3), it can be seen that moisture content of about 19 per cent, equilibration of 4 hr and

TABLE 1. PUFFING CHARACTERISTICS OF RAGI VARIETIES

Ragi varieties	Puffing yield (%)	Bulk vol. (ml/g)	Bran content (%)	Bran thickness ( $\mu$ )	Protein (N $\times$ 6.25) (%)	Ether extractives (%)	Amylose (%)	
							Soluble	Insoluble
Purna	97	7.7	13.4	2.66	8.5	1.9	14.3	14.7
Annapurna	91	6.1	13.3	3.22	7.9	2.2	17.7	15.8
PR 202	90	5.6	13.9	3.36	8.1	1.8	17.4	13.3
Shakti	90	5.4	14.3	3.36	7.7	2.1	17.2	17.0
Indaf - 3	89	5.4	13.2	3.22	8.5	2.2	22.4	7.4
Indaf - 10	88	5.0	15.9	3.50	7.1	1.5	18.7	13.0
Indaf - 7	88	4.8	14.6	4.06	8.3	1.6	18.5	9.9
WB - 1*	64	4.4	12.4	3.64	8.6	1.9	18.5	15.0
Hamsa*	73	4.3	14.1	3.64	9.6	1.7	14.2	19.7
Indaf - 8	91	4.2	13.1	2.38	8.4	1.7	17.6	14.4
Indaf - 5	82	4.1	12.5	2.52	7.8	1.9	23.0	7.0
Indaf - 9	84	4.1	15.0	3.50	7.5	1.9	20.1	12.0
Indaf - 1	85	4.0	13.2	4.06	6.9	2.1	19.6	8.1
Indaf - 6	75	3.9	13.1	2.80	8.2	1.8	19.5	12.0

\*White ragi seeds.

Values are on dry weight basis

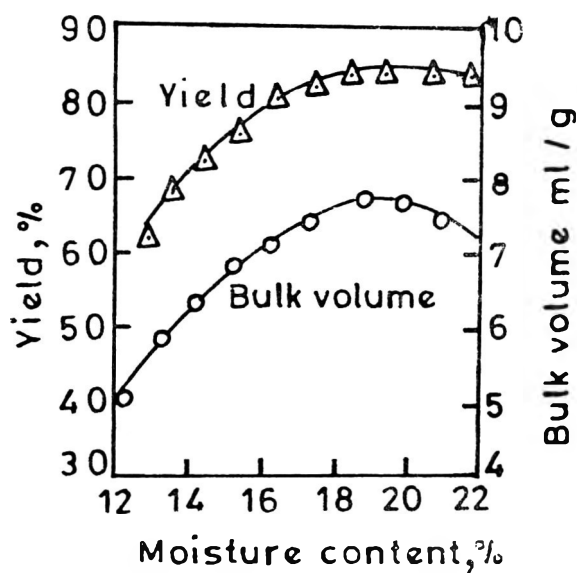


Fig. 1. Effect of moisture content on puffing of ragi.

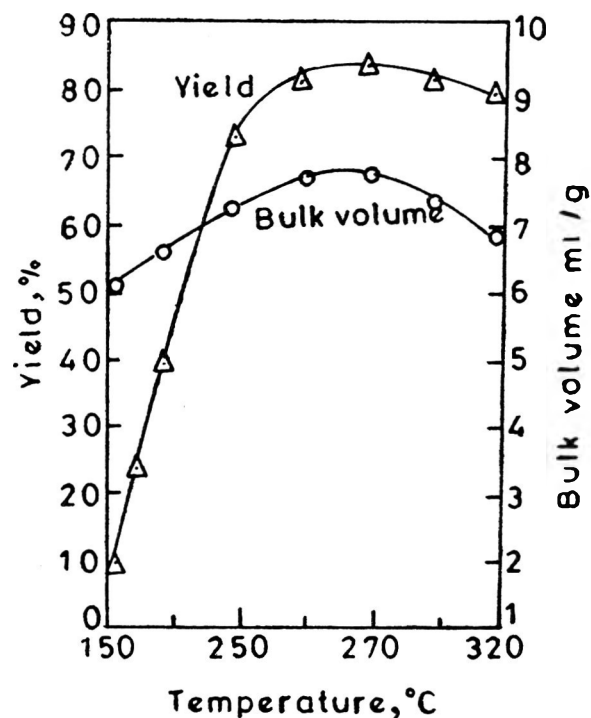


Fig. 3. Effect of temperature on puffing of ragi.

puffing temperature of 270°C are the optimum conditions for puffing of ragi. Deviation from these standard conditions either affected the puffing yield or specific volume. The time needed for completion of puffing process was about 25 sec.

Relevant data on the puffing characteristics of 14 varieties of ragi are presented in Table 1. The varieties are arranged in the descending order of the bulk volume of puffed product. Five ragi strains, viz., 'Purna', 'Annapurna', 'PR-202', 'Shakti' and 'Indaf-3' were considered good for puffing, the remaining being poor puffing varieties. Samples with bulk volume of more than 5 units are considered as good puffing varieties.

Puffing yield varied from 97 to 67 per cent and specific volume from 7.7 to 3.9. 'Purna' variety was found to be the best among these as it gave highest yield of puffed ragi with maximum expansion. Varieties with good puffing yield generally gave puffed material of higher specific volume.

Protein content ranged from 6.9 to 9.6 per cent, husk and bran content varied from 14.2 to 16 per cent whereas thickness of bran and aleurone layer varied from 2.38 to 4.06µ. There was very little difference in the amylose content among the varieties. No correlation between puffing quality and the grain parameters studied is apparent from the data. White ragi seeds were found not suitable for puffing.

The eating quality of puffed ragi products is adversely affected by the presence of dry bran. Therefore, refining of puffed ragi to obtain low bran product is essential to improve its eating quality. Studies in this direction are in progress.

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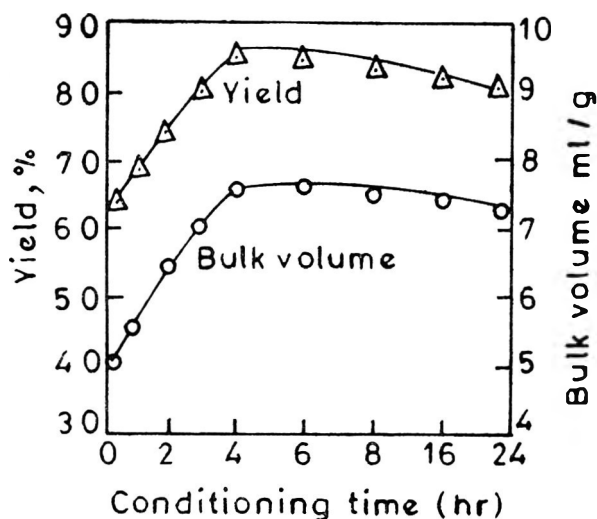


Fig. 2. Effect of conditioning time on puffing of ragi.

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## COOKING CHARACTERISTICS OF WINGED BEAN (*Psophocarpus tetragonolobus*)

Cooking characteristics such as hydration, dispersion of solids and cooking time of winged bean *dhal* were determined. Ammonium carbonate (0.5%) with either sodium carbonate or bicarbonate (0.5%) reduced the cooking time by nearly 50%. However, direct addition of these chemicals to the cooking water imparted an alkaline taste and undesirable colour. Coating of the *dhal* with the chemicals or presoaking in solution of these salts eliminated these disadvantages.

Winged bean (*Psophocarpus tetragonolobus*) is a minor tropical legume which contains high amount of good quality proteins<sup>1</sup>. It is free from any bitter or beany odour. It has been reported that the bean requires 2–3 hr to cook<sup>2</sup>. In this study, improvement in the cooking qualities of winged bean *dhal* by the addition of chemicals has been investigated.

Winged bean (*Psophocarpus tetragonolobus*) seeds obtained from National Botanical Research Institute, Lucknow, India were used. The preconditioned (moisture adjusted) winged bean seeds were dehusked and split into *dhal* in Satake polisher.

Ammonium carbonate, sodium carbonate, sodium bicarbonate and tri-sodium phosphate (each at 0.5 to 1 per cent level) used were of chemical purity grade. Cooking time and hydration were determined as described by Narasimha and Desikachar<sup>3</sup>. Dispersion of solids was determined as per the method of Subba Rao *et al*<sup>4</sup>.

Methods adopted for the treatment of the seeds were: (a) adding the chemicals to cooking water, (b) presoaking with the chemicals, and (c) coating the seeds with the chemicals.

Methods of chemical treatment of the Winged bean *dhal* was accomplished as described by Narasimha and Desikachar<sup>5</sup>. Winged bean *dhal* was directly added to the cooking water, containing the chemicals. In parallel studies, the winged bean *dhal* was coated with the chemicals by impregnating with solutions (5 ml/100 g) containing the same quantity of chemicals as added to the cooking water. After treating with the solutions, the *dhal* was mixed periodically to prevent lump formation. The chemical-coated bean was allowed to equilibrate in a closed container for 2 hr before drying in a current of air.

Presoaking was carried out by soaking the beans for 2 hr in 1.5 parts of water containing the chemicals and the excess soak water was drained off. The soaked *dhal* was rinsed twice with water before cooking.

The cooking time needed for winged bean *dhal* without the addition of chemicals was 150 min and the pH of the slurry was 6.6. The hydration and dispersal of solids at 50 min of cooking was 0.91 g/g and 33 per cent respectively. At the end of 150 min cooking, more than 77 per cent of solids were dispersed and the cooked slurry had whitish yellow colour.

The chemicals when tried individually did not give satisfactory results. Therefore, attempts were made to try these combinations. Of the several combinations of chemicals tried (Table I) ammonium carbonate along with sodium carbonate or sodium bicarbonate was suitable with respect to hydration, dispersion of solids, pH, colour, flavour and taste of the cooked *dhal* slurry. It lowered the cooking time to 70–80 min as compared to 150 min required for the control.

*Adding salts to the cooking water:* Cooking characteristics of winged bean *dhal*, with chemicals added directly to water and the results with the control have been presented in Table I. When the chemicals added were in the range of 0.75 to 1 per cent level the hydration and dispersion of solids were high and cooking time was reduced to 76–78 min; cooked *dhal* was, however, unacceptable due to its black colour, alkaline taste and high pH. These chemicals when used at 0.5 per cent level gave an acceptable cooked *dhal* without any significant increase in cooking time (75–80 min). There was no significant change in the hydration and dispersion of solids.

*Coating the seeds:* The chemicals were more effective when coated on winged bean *dhal*, than when added directly to the cooking water (Table I). This was probably due to better penetration, equilibration, and effective contact of the chemicals with the *dhal*. At 0.5 per cent level of both the chemicals, the cooked *dhal* had the desirable characteristics. Water uptake and dispersion of solids which are important parameters of cooking quality were also satisfactory. Chemical

TABLE 1. EFFECT OF CHEMICAL TREATMENT ON THE COOKING CHARACTERISTICS OF WINGED BEAN DHAL

Chemicals	Level (%)	Method of treatment	Cooking time (min)	Water uptake (at 50 min) (g/g)	Dispersed solids (at 60 min) (%)	pH of cooked dhal	overall acceptability
No chemical	—	—	150	0.91	33	6.6	Acceptable
Na <sub>2</sub> CO <sub>3</sub> + (NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub>	0.5+0.5	Direct addition	75	1.22	51	7.2	Acceptable
		Coated	73	1.24	60	7.1	Acceptable
		Presoaked	72	1.20	58	6.9	Acceptable
Na <sub>2</sub> CO <sub>3</sub> + (NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub>	0.75+0.5	Direct addition	76	1.24	55	7.9	Not acceptable
		Coated	72	1.28	65	7.8	Not acceptable
		Presoaked	70	1.26	60	7.0	Acceptable
NaHCO <sub>3</sub> + (NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub>	0.5+0.5	Direct addition	80	1.15	56	7.1	Acceptable
		Coated	76	1.20	60	7.0	Acceptable
		Presoaked	75	1.15	58	6.8	Acceptable
NaHCO <sub>3</sub> + (NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub>	1.0+0.5	Direct addition	78	1.23	60	7.8	Not acceptable
		Coated	75	1.24	64	7.6	Not acceptable
		Presoaked	72	1.22	60	6.9	Acceptable

treatment by coating was more effective than adding direct to the cooking water in reducing cooking time, and increasing the water uptake and dispersion of solids. Chemical coating can be practised in small scale but it may pose problems when large quantities are handled resulting in uneven mixing and non-uniform distribution of chemical on the bean, and formation of lumps.

*Presoaking of the seeds:* Studies on presoaking of winged bean *dhal* in chemical solutions were undertaken to overcome the above mentioned drawbacks. As in the earlier cases, combination of ammonium carbonate (0.5 per cent), either with sodium carbonate or sodium bicarbonate (0.5 per cent gave an acceptable product, besides reducing the cooking time to 72-75 min from 150 min required for control. Presoaking in chemical solution was a better method of addition of chemicals since pH was kept below 7.0.

In conclusion, the above studies suggest the possibilities of improving the cooking characteristics of the winged bean *dhal* such as reduction of cooking time, hydration and dispersion of solids by employing permissible chemicals. Both coating as well as presoaking in chemical solution can be adopted for reducing the cooking time of winged bean *dhal*.

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## SURVEY OF PESTICIDE RESIDUE ANALYSIS IN PADDY STRAW SAMPLES FROM SOME FARMS OF CHITTOOR DISTRICT OF ANDHRA PRADESH

Organochloride and organophosphate pesticide residues were determined by gas liquid chromatography and colorimetry in dried paddy straw samples collected from 36 farms of Chittoor district, Andhra Pradesh, India. A few samples showed high amounts of the residues which warrant careful application of the pesticides during paddy cultivation.

The production and usage of pesticides in agriculture results in contamination of the environment and especially the food products. The pesticide residues particularly the persistent ones affect the health and thus become a serious health problem. In India, the monitoring and surveillance of residues in various agricultural and food products are still inadequate. In the present investigation evaluation of pesticide residues is carried out in

the dried paddy straw. Paddy straw is largely consumed by milching cows and buffaloes in India and it is possible that these residues get into the milk as they are lipophilic. Thus the analysis of dried paddy straw was found essential in this respect.

Dried paddy straw were collected in the postharvest period from the village farms covering 150 km adjoining the Tirupati-Chittoor high road and Tirupathi - Madras high road. Dried paddy straw (100g) thus collected was analysed for pesticide residues after clean up of the samples.<sup>3,4</sup> Gas liquid chromatographic (GLC) analysis was carried out employing Varian aerograph 1400 series with the following conditions: EC detector equipped with 6' x 1/8" i.e. pyrex glass column packed with 5 per cent O. V. -17 chromosorb W with mesh 60-80 was used. Nitrogen (25ml/min) was used as carrier gas. EC detector was used with injector, column and detector temperatures at 195°C, 190°C and 210°C respectively at 10<sup>-10</sup> range with attenuator 32. Organophosphate residues were estimated by colorimetric method as reported earlier<sup>1-4</sup> after qualitatively identifying the organophosphorus pesticides by TLC-enzymatic method<sup>5,6</sup>.

The residue analysis by GLC for organochloride pesticides showed the presence of benzene hexachloride (BHC) in 33 samples out of 36 samples indicating the predominant application of BHC. The BHC residue ranged from 0-6 ppm (Table 1). BHC amounts ranged from 0-1, 1-2, 2-3 ppm in twenty, six and five farms respectively. In one of the farms it was as high as 6 ppm.

The organophosphate residues; methyl parathion, dimethoate and fenitrothion were detected<sup>5,6</sup> and determined<sup>1,2</sup>. The methyl parathion content ranged from 0.02 to 1.8 ppm in ten farms. Dimethoate content ranged from 0.3 to 2.36 ppm in eight farms. Fenitrothion contents ranged from 0.26 to 1.96 ppm in fifteen farm samples. In a few samples the methyl parathion content was found to exceed the recommended international maximum limits<sup>7</sup>. In a few samples both organochloride and organophosphate pesticides were found, thereby indicating the use of formulations containing both organochloride and organophosphates, either simultaneously or separately at different times to the paddy crop. The above results warrant the careful application of pesticides on the agricultural crops and periodical monitoring and surveillance programmes.

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7. *Recommended international maximum limits for pesticide residue*, Joint FAO / WHO Food Standards Programme, 1978.

TABLE 1. PESTICIDE RESIDUE AMOUNTS IN THE PADDY STRAW SAMPLES OF 36 FARMS (OBSERVATIONS ARE MEAN OF DUPLICATES)

Analysed pesticide detected	No. of farms analysed	No. of farms where detected under different ranges (ppm)				
		ND*	0-1	1-2	2-3	6
BHC	36	4	20	6	5	1
Methylparathion	36	26	6	4	—	—
Fenitrothion	36	28	6	2	—	—
Dimethoate	36	21	9	5	1	—

ND\* = Not detectable.

## BOOK REVIEWS

*Rice: Production and Utilization.* Edited by B. S. Luh, Avi Publishing Co., Westport, Conn. 1980, pp. xii + 925. Price US \$ 39 U. S. and Canada; \$ 54 other countries.

This book summarizes for the first time in a single volume the state of knowledge until 1977 on recent developments in rice production and utilization. One third of the book is on production and processing and the rest is on grain quality and utilization. The 24 chapters have 32 authors representing six nationalities, with five of the authors from the International Rice Research Institute in the Philippines and an equal number from the Food Industry Research and Development Institute in Taiwan. Chapter titles are: rice in its temporal and spatial perspectives; plant growth and development; genetics and breeding; culture; plant diseases; insect pests of rice plant; insect pests of stored rice; harvest, drying and storage of rough rice; milling; properties of the caryopsis; nutritional quality of endosperm; flours in baking; enrichment with vitamins and amino acids; parboiled rice; rice quality and grades; quick-cooking rice; canning, freezing and freeze drying; vinegar fermentation; hulls; riceoil; and ricebran.

The Asian emphasis of this book is most welcome and makes the information relevant to Asia, where the bulk of the rice is produced and consumed. Of particular interest to food scientists and technologists are the chapters on processed rice foods with emphasis on Asian foods. They answer, in part, the need to document the many traditional rice foods in the region which require specific varieties to make. Sake or Japanese rice wine and rice in brewing were not considered probably because these products were reviewed previously in "Rice Chemistry and Technology" edited by D. F. Houston and published by the American Association of Cereal Chemists, Inc. in 1972.

The price of the book can be made more attractive particularly to Asians if a paperback edition could be produced. Moreover, were the chapters kept uniformly concise, the cost could have been lowered.

B. O. JULIANO  
IRRI, LOS BANOS, PHILIPPINES

*Protein Crosslinking: Biochemical and Molecular Aspects:* Volume 86A in *Advances in Experimental Medicine and Biology*, by M. Friedman (Ed.), Plenum Press, New York, 1977; pp xiv + 760.

This volume represents the Proceedings of the Sympos-

ium on Protein Crosslinking held in September 1976 in San Francisco, under the aegis of the Protein Subdivision of the Division of Agriculture and Food Chemistry of the American Chemical Society, with some additional invited contributions. Crosslinks do occur in nature, and they impart special mechanical and functional properties to many macromolecules. Artificially engendered crosslinks provide the key to many applications of macromolecules (or cells) in analytical biochemistry and industry, affinity chromatography, enzyme linked immunoassays, analysis of aspects of structure of macromolecules, texturizing of articles like food and leather and a host of others. The volume under review contains 43 contributions, many from well-known investigators, and covers a wide crosssection of topics of interest. These include the role of disulphide bonds in the integrity of protein structure, antigenic structure of lysozyme and the functionality of wheat proteins, disulphide-thiol exchanges and turnover of proteins, renaturation of reduced proteins, thiolation and formation of disulphide crosslinked polymeric insulin, and introduction of disulphide crosslinks in fibrous proteins and serum albumin. Four articles deal with bifunctional reagents, and some others with alteration of antigens and lectins, protein-nucleic acid, ribosome, keratin and wool crosslinking, collagen in tanning, radiation, peroxydisulphate and photo-induced crosslinks, crosslinking of amino acids by formaldehyde, insolubilization of chymotrypsin, reduction of Schiff bases and electron microscopic studies on crosslinked proteins. One article deals with fish myofibrillar protein-lipid associations. Three contributions deal with gas chromatographic and other aspects of amino acid derivatives. As often happens with volumes of this nature, some of the contributions are well-seasoned and of great value. A few are of but passing interest. Nevertheless, this book encompasses a cross-section, which though by no means comprehensive, of the literature record on crosslinks, crosslinking and crosslinked proteins. To prospective entrants to this area of endeavour and to those interested in the chemistry and behaviour of proteins, it holds much that is wholesome. The companion volume (86 B) dealing with "Nutritional and Medical Consequences", which this reviewer has seen, would be of much interest to food and medical scientists.

L. K. RAMACHANDRAN  
DEPT. OF BIOCHEMISTRY  
OSMANIA UNIVERSITY, HYDERABAD



*JOJOBA: An assessment of prospects:* Tropical Products Institute, 56/62, Gray's Inn Road, London Wo 1X 8LU, 1979, pp 32; Price : £ 1.05.

Jojoba is a shrub which grows wild in semi-arid region of northern Mexico and south-western USA. The plant bears seeds which contain an unusual liquid wax. In recent years jojoba has attracted attention as the natural liquid wax has been suggested as a substitute for sperm whale oil.

This brochure is divided into five sections viz, production of jojoba, chemistry of jojoba and other waxes, uses of jojoba, economics of production and discussion

giving a bird's eye view of the problems in making jojoba cultivation and processing a commercial proposition.

The economics of production as outlined in the brochure may not be applicable to underdeveloped countries (as the authors admit), but the brochure contains useful analysis of the markets for sperm oil and animal and vegetable waxes and the price and volume parameters for jojoba oil to establish itself. The brochure is also available free of charge to public bodies in countries eligible for British aid.

J. V. PRABHAKAR  
CFTRI, MYSORE

## ASSOCIATION NEWS

### **Bangalore Chapter**

Following lectures were arranged by the Chapter during the year ending 1979-80.

*R & D Management and Appropriate Technology* on July 22, 1979, Dr. Arun Kilara, Department of Food Science, Pennsylvania State University, U. S. A. spoke on *Role of Immobilised Enzymes in Dairy and Food Industry* on August 20, 1979.

Dr. M. P. Vaidehi, Head of the Department of Home Economics, University of Agricultural Sciences spoke

on *Food Products from Processed Potatoes and Sweet Potatoes* on October 23, 1979.

Sri N. S. Saxena, Director, Indian Standard Institution spoke on *Food Flavours* on October 31, 1979.

Dr. K. T. Achaya, United Nations University spoke on *Indian Nutritional Realities* on December 28, 1980.

*Meda Kasturi Ranga Memorial Lecture* was delivered for the first time on September 9, 1979, by Dr. M. S. Swaminathan, Principal Secretary, Agricultural and Rural Development, Government of India. The topic was *Agricultural Growth with Stability*.

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*N. G. Malleshi and H. S. R. Desikachar*

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*Soaad H. Al-Bayati and Hadi Al-Rayess*

STUDIES ON THE ANTIMICROBIAL AND STIMULATORY FACTORS OF GARLIC (*ALLIUM SATIVUM* LINN)

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## Review Paper

GHEE: A RESUME OF RECENT RESEARCHES

*R. S. Sharma*

## INSTRUCTIONS TO CONTRIBUTORS

1. Manuscripts of papers should be typewritten in double space on one side of the paper only. They should be submitted in **triplicate**. The manuscripts should be complete and in final form, since no alterations or additions are allowed at the proof stage. The paper submitted should not have been published or communicated anywhere.
2. Short communications in the nature of letters to the editor should clearly indicate the scope of the investigation and the salient features of the results.
3. Names of chemical compounds and not their formulae should be used in the text. Superscript and subscripts should be legibly and carefully placed. Foot notes should be avoided as far as possible.
4. **Abstract:** The abstract should indicate the scope of the work and the principal findings of the paper. It should not normally exceed 200 words. It should be in such a form that abstracting periodicals can readily use it.
5. **Tables:** Graphs as well as tables, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. Nil results should be indicated and distinguished clearly from absence of data.
6. **Illustrations:** Line drawings should be made with *Indian ink* on white drawing paper preferably art paper. The lettering should be in pencil. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size. Photographs must be on glossy paper and contrasty; *two copies* should be sent.
7. Abbreviations of the titles of all scientific periodicals should strictly conform to those cited in the *World List of Scientific Periodicals*, Butterworths Scientific Publication, London, 1962.
8. **References:** Names of all the authors should be cited completely in each reference. Abbreviations, such as *et al.*, should be avoided.

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

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

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

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

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

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

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

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

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