JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Silver Jubilee Year

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS, INDIA

VOL. 25. NO. 1 JAN. / FEB. 1988

ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS

(INDIA)

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

Affiliated to the Institute of Food Technologists, USA

Objects:

1. To stimulate research on various aspects of Food Science and Technology.
2. To provide a forum for the exchange, discussion and dissemination of current developments in the field of Food Science and Technology.
3. To promote the profession of Food Science and Technology.

The ultimate object is to serve humanity through better food.

Major Activities:

1. Publication of Journal of Food Science and Technology (bi-monthly) and Indian Food Industry (IFI) (quarterly).
2. Arranging lectures and seminars for the benefit of members.
3. Holding symposia on different aspects of Food Science and Technology.

Membership:

Membership is open to graduates and diploma holders in Food Science and Technology, and to those engaged in the profession. As per the option exercised, each member will receive a copy of the Journal of Food Science and Technology or the Indian Food Industry. The Chapters of the Association are located at Bangalore, Bombay, Calcutta, Delhi, Hyderabad, Hissar, Jabalpur, Jammu, Karnal, Kanpur, Ludhiana, Lucknow, Madras, Nagpur, Parbhani, Poona, Palayamkottai, Pantnagar and Trivandrum.

Membership Fee		Admission Fee
Life Member	Rs 500	Rs 5
Life Member (Resident abroad)	$ 400	$ 1
Corporate Member	Rs 700	Rs 5
Member	Rs 40	Rs 5
Member (Resident abroad)	$ 25	$ 1
Affiliate Member	Rs 60	Rs 5
Student Member	Rs 25	Rs 5
Student Member (abroad)	$ 15	

Annual Subscription

Journal of Food Science and Technology

Inland	Rs. 180
Foreign: Surface Mail	$ 60
Air Mail	$ 80

Indian Food Industry

Inland	Rs. 125
Foreign: Surface Mail	$ 40
Air Mail	$ 50

Additional subscription for members for

JFST	Rs. 60
IFI	Rs. 40
Postage	Extra

For membership and other particulars kindly address

The Honorary Executive Secretary
Association of Food Scientists and Technologists, India
Central Food Technological Research Institute, Mysore-570 013, India

The Journal of Food Science and Technology is a bimonthly publication of the Association of Food Scientists and Technologists, India (AFST) issued in February, April, June, August, October, and December.

Manuscripts for publication and books for reviewing in the Journal should be addressed to the Editor, Journal of Food Science and Technology, AFST, Central Food Technological Research Institute, Mysore-570 013. The Editor reserves the privilege of editing the manuscript to make it suitable for publication in the Journal.

Correspondence regarding subscriptions and advertisements should be addressed to the Executive Secretary, AFST, Central Food Technological Research Institute, Mysore-570 013, India. Payments may be made by cheque, draft, postal or money order in favour of Exec. Secretary, AFST.

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Volume 25 Number 1 Jan./Feb. 1988

CONTENTS

Research Papers

Research Notes

EDITORIAL

The Journal of Food Science and Technology was started in 1964 to fulfil the needs of the emerging discipline of Food Science and Technology the importance of which was beginning to be realised in the developing countries. The Journal was meant not only for the publication of scientific and technical papers but also for publishing digests of technical information of interest to the food industries. The Journal is now entering the TWENTY FIFTH year of publication. During the first ten years, it was a QUARTERLY and subsequently, it was changed into a BIMONTHLY to accommodate the increased inflow of papers. The publication of digests of technical information from literature for the benefit of the Food Industries is now being taken care of by the sister journal INDIAN FOOD INDUSTRY which is in its seventh year of publication. The Journal of Food Science and Technology is now essentially devoted to the publication of Research Papers.

From a modest beginning of 16 research articles and a total of 106 pages in the First Volume, the Journal is currently publishing 100 to 110 articles covering about 350 pages. Several papers are contributed by authors outside India. The facts that: (i) the Journal is listed in Current Contents and is abstracted in internationally reputed Abstracting Journals such as Food Science and Technology Abstracts, Biological Abstracts, Chemical Abstracts, International Packaging Abstracts, Dairy Science Abstracts, and Nutrition Abstracts and Reviews, (ii) there are 125 foreign subscribers and (iii) the CFTRI library receives 52 journals in exchange for our journal are pointers to the status ot the JOURNAL among the Food Science Journals of the world.

The marked growth and success of the Journal is due to the authors of papers, the devoted work of the earlier editors, the members of the Editorial Boards, the referees and the cooperation of the Association's office staff and of the printers as also the keen interest of all the successive Directors of the Central Food Technological Research Institute. Mr. K. A. Ranganath deserves special mention for looking after the format of the Journal.

On the occasion of the Silver Jubilee Year of the Journal, it is my privilege to record my personal appreciation to all those who have worked for the Journal. With the continued co-operation of all concerned, there is no doubt that the Journal will do better in the years to come.

N. CHANDRASEKHARA

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 1–3

Reducing the Paste Viscosity (Dietary Bulk) of Roller Dried Weaning Foods Using Malt Flour or Fungal Amylase

N.G. MALLESHI AND H.S.R. DESIKACHAR
Discipline of Grain Science and Technology
Central Food Technological Research Institute, Mysore-570013, India

Received 27 *December* 1986; *revised* 26 *June* 1987

Addition of approximately 2.5% barley malt flour or 0.15% of fungal alpha-amylase to a proprietary brand of roller dried weaning food reduced the paste viscosity of its 20% slurry from 15,000 to 1,000 cp. Although storage of the weaning food containing 5% barley malt flour at 42°C for 200 days caused 28% loss in the enzyme activity, sufficient activity was retained in the stored sample to effect the desired lowering of its viscosity. Storage did not adversely affect the protein efficiency ratio and the sensory quality of the weaning food containing malt.

Most of the proprietary brands of weaning foods marketed in India and other developing countries are prepared by roller drying the slurries of cereals or cereal and legume blends[1,2]. Such foods swell considerably when stirred up with water and result in pastes of high viscosity or form high 'dietary bulk'. The high 'bulk' reduces the calorie intake by the child and therefore weaning foods that have low paste viscosity or low dietary bulk are desired and recommended[3]. The objective of the present study was to develop a simple method for reducing the paste viscosity of roller dried weaning foods using permitted starch hydrolysing adjuncts such as malt flour or fungal alpha-amylase[4] and also to know the effect of added malt on the sensory and protein qualities of the weaning food on storage.

Materials and Methods

Weaning food: A popular brand of weaning food prepared by roller drying the slurry consisting of cereal flour, skimmed milk powder, mineral salts and vitamins was purchased from the local market. The food was one month old when purchased as per the date of manufacture put on the unit packs. It contained 12 per cent protein, 2 per cent fat and 75 per cent carbohydrates.

Barley malt: Barley malt obtained from a commercial malt plant (Patiala, India) was powdered and the coarse husk (+ 60 fraction) was sieved off. The malt flour had an alpha-amylase activity of 13,000 maltose units/g. Fungal alpha-amylase used was from M/s. Anil Starch (Ahmedabad, India), which had an alpha-amylase activity of 1,05,000 maltose units/g.

Blending: The weaning food was blended with malt flour and fungal alpha-amylase in proportions varying from 0.5 to 5 per cent and 0.1 to 0.5 per cent respectively.

Viscosity measurement: To study the effect of added malt or fungal alpha-amylase on the paste viscosity of the weaning food, the blends were stirred with 4 parts of hot (95°C) water. The slurry was cooled to 30 ±2°C with occasional stirring and the viscosity was measured in a Brookfield synchrolectic viscometer (RVT model) at 100 rpm using appropriate spindles, according to Brandtzaeg *et al*[5].

Storage: The weaning food containing 5 per cent barley malt and 100 per cent weaning food as such (control) packed in unit pack tins were stored at 42°C. Samples were withdrawn after 40,100,150 and 200 days of storage and tested for alpha-amylase activity by measuring the amount of reducing sugars released[6], and for viscosity (20% slurry) as described earlier[5].

Sensory quality assessment: The slurries prepared from control and malt containing foods similar to that for viscosity determination were tested for sensory quality by a panel of 20 adult judges on each withdrawal from storage. The judges were asked to indicate the preferred samples for consistency (mouth feel), flavour (aroma and taste) and score for overall quality on a 5-point scale ranging from poor to very good[7].

PER determination: To study the effect of addition of malt flour and storage on the protein quality of the weaning food, PER of samples withdrawn after 100 days of storage was determined. The diets for PER experiment containing 79 g weaning foods, 10 g

peanut oil, 4 g salt mixture, 1 g vitamin mixture and 6 g corn starch to give 10 g protein per 100 g diet were prepared according to ISI guidelines[8]. Skimmed milk powder diet prepared similarly to contain 10 per cent protein served as reference protein. For PER determination, 21 day old male weanling rats (Wistar strain) weighing about 35 g were grouped into 3 groups of 8 rats each and were housed in individual metallic cages. Rats were fed with the diets moistened with hot water. Feed and water were given *ad libitum*. After four weeks of feeding, the PER was determined[8].

Results and Discussion

The viscosity of a 20 per cent slurry of the control weaning food was very high (15,000 cp). Addition of malt flour or fungal alpha-amylase lowered the viscosity of the weaning food considerably. There was a progressive reduction in viscosity with increasing levels of malt or fungal alpha-amylase in the blend (Fig. 1). A slurry of viscosity of about 1,000 cp, which is the consistency desired for feeding a one-year old child, was obtained by addition of approximately 2.5 per cent malt flour or 0.15 per cent of fungal alpha-amylase. Increasing the levels of these adjuncts further resulted in a marginal reduction in the viscosity. A slurry of such consistency that could even be bottle-fed (250-300 cp) could be obtained by increasing the concentration of barley malt flour to approximately 4.5 per cent or fungal alpha-amylase to 0.35 per cent. Addition of hot water to the weaning food blend and mixing results in a favourable condition (62°C) for the malt amylases to hydrolyse the pregelatinised starch of the weaning food, causing reduction in the viscosity[9]. This was revealed by the drastic change in viscosity within 5 min of addition of water. Cooling the slurry from 62°C to room temperature (30 ±2°C) did not produce substantial change in the viscosity.

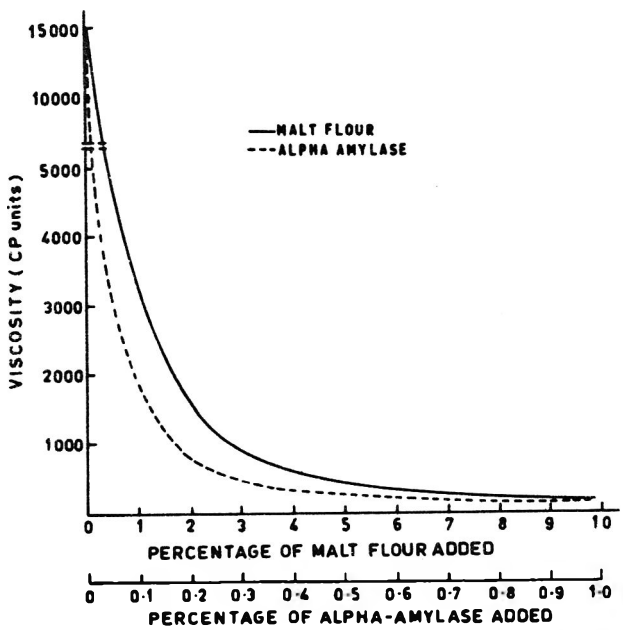


Fig. 1: Effect of addition of malt flour or fungal alpha-amylase on paste viscosity of weaning food.

TABLE 1. EFFECT OF STORAGE ON ALPHA - AMYLASE ACTIVITY AND VISCOSITY OF WEANING FOOD CONTAINING MALT.

Storage period (days)	α-amylase activity*	Viscosity (cp units)
Fresh	650	350
40	620	440
100	590	500
150	540	650
200	470	790

*mg of maltose released by the action of malt enzymes extracted from 1 g sample in acetate buffer on soluble starch.

The paste viscosity and alpha-amylase activity of sample containing malt stored at 42°C for different periods are presented in Table 1. There was a decrease in alpha-amylase activity from 650 to 470 maltose units and an increase in viscosity from 350 to 790 cp on storage for 200 days. This indicated that the malt alpha-amylase was reasonably stable in the blend. The small reduction in the activity on storage could be made up by overage. As expected, the alpha-amylase activity of control sample was nil and there was no change in its viscosity (15,000 cp) during storage.

The results of the sensory quality assessment tests of control as well as malt containing foods stored upto 200 days are given in Table 2. Malt containing sample showed highly significant preference for consistency throughout the storage period, whereas for flavour test, it showed significant preference upto 40 days of storage only and thereafter the preference between it and control sample did not attain statistical significance. However, at each storage period both the samples showed comparable overall quality; very good quality upto 40 days, good quality upto 150 days and fair quality thereafter. From this, it may be inferred that, addition of malt to weaning food does not necessarily improve its sensory quality but definitely reduces its dietary bulk and thereby enables the child to consume more food per sitting.

The data on PER experiments are summarised in Table 3. The PER values for the diets of control and malt containing samples were 2.27 and 2.31 respectively as compared to 3.30 for the reference protein diet. This revealed that the addition of barley malt to a roller-dried food and storing the blend for about 100 days even at 42°C did not affect its protein

TABLE 2. PREFERENCE‡ IN CONSISTENCY, FLAVOUR AND OVERALL QUALITY MEANS AMONG THE CONTROL (A) AND MALT CONTAINING (B) FOODS

Storage Period (days)	0		40		100		150		200	
	A	B	A	B	A	B	A	B	A	B
Consistency (mouth feel)	0	20**	0	20**	0	20**	0	20**	1	19**
Flavour (aroma & taste)	4	16*	5	15*	7	13^{NS}	8	12^{NS}	6	14^{NS}
Overall quality (means)	4.1^{efg}	4.6^{g}	3.8^{ef}	3.8^{fg}	3.1^{cd}	3.6^{de}	2.5^{ab}	2.7^{bc}	2.0^{a}	2.2^{ab}

‡ 20 panelists
NS: not significant
* $P \leq 0.05$
** $P \leq 0.01$
Limits for means: $1.5 \leq$ = poor
1.6–2.5 = fair
2.6–3.5 = good
≤ 3.6 = very good

Any two means carrying different superscripts in the row differ significantly ($P \leq 0.05$)

quality. Contrary to this, it has been shown by Venkat Rao *et al*[10]., that the addition of malt to the slurry of cereal and legume, prior to roller drying reduced the lysine availability and lowered PER of the food significantly.

The cost of the barley malt is generally less than the cost of the proprietary weaning foods; hence replacing the food by 5 per cent with barley malt will not affect their price structure. However, addition of fungal alpha-amylase may increase the price of foods marginally.

TABLE 3. PROTEIN EFFICIENCY RATIO OF WEANING FOOD SAMPLES STORED AT 42°C FOR 100 DAYS

Diets	Food intake (g)	Protein intake (g)	Wt gain (g)	PER
Control	196.6	19.1	43.4	2.27^{a}
Malt containing food	201.2	20.1	46.2	2.31^{a}
Skim milk powder	193.1	20.1	65.2	3.20^{b}
SEM (27df)				± 0.09

Means in the column with different superscripts differ significantly ($P \leq 0.05$) according to Duncan's new multiple range test.

In conclusion, it may be stated that, the present study established the feasibility of adding malt flour to roller dried weaning foods, to reduce their paste viscosity or 'dietary bulk' and increase the calorie density of the slurry without affecting their nutritional quality or shelf-life for about three months of storage at tropical temperatures.

Acknowledgement

The authors thank Mr S. Dhanaraj for advice while planning for sensory quality assessment tests and also for the statistical analysis of the data.

References

1. Chandrasekhara M.R., Krishnaiah, M.M., Chandrasekhar, H.N. and Shurpalekar S.R., Preparative trials on dried weaning foods. *J. Fd Sci. Technol.*, 1967, **4**, 117.

2. Desikachar, H.S.R., Weaning food formulations with low-hot paste viscosity suitable for home level application. In *Proceedings of the Symposium on Food Needs of Infants and Pre-school Children*, Madras, India, Nov. 1979, 54.

3. Ljungquist, B.G., Mellander, O. and Svanberg, U., Dietary bulk as a limiting factor for nutrient intake in pre-school children. I.A problem discription. *J. Trop. Pediatr.*, 1981, **27**, 68.

4. *Recommended International Standards for Weaning Foods.*, FAO/WHO Food Standard Programme, Codex Alimentarius Commission. FAO, Rome, Italy, 1976.

5. Brandtzaeg, B., Malleshi, N.G., Svanberg, U., Desikachar, H.S.R. and Mellander O., Dietary bulk as a limiting factor for nutrient intake with special reference to the feeding of pre-school children. III. Studies on malted flours from ragi, sorghum and greengram. *J. Trop. Pediatr.*, 1981, **27**, 184.

6. Bernfeld, P., In *Methods in Enzymology*, by S.P. Colovic and N.O. Kaplan., (Eds) Academic Press., Inc., New York, 1955, Vol. I, 149.

7. Amerine, M.A., Pongborn, R.M. and Roessler, E.B., *Principles of Sensory Evaluation of Food*, Academic Press, Inc., New York, 1965. 349.

8. *Methods for Determination of Protein Efficiency Ratio* IS-7481-1975 Indian Standards Institution, New Delhi.

9. Malleshi, N.G. and Desikachar, H.S.R., Formulation of a weaning food with low hot-paste viscosity based on malted ragi and green gram. *J. Fd. Sci. Technol.*, 1982, **19**, 193.

10. Venkat Rao. S., Vijayalakshmi, D., Kurien, S., Prasannappa, G., Chandrasekhar, H.N., Swaminathan, M. and Chandrasekhara, M.R., Nutritive value of weaning foods and malted milk powder. *J. Fd. Sci. Technol.*, 1976, **13**, 209.

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 4–6

Evaluation of a New Medium for Rapid Enumeration of Yeasts and Molds in Food

P. VENKATASUBBAIAH AND C.T. DWARAKANATH
Microbiology and Sanitation Discipline,
Central Food Technological Research Institute, Mysore-570 013, India.

Received 30 *December* 1986; *revised* 27 *April* 1987

Conventional methods of enumeration of yeasts and molds in foods and feeds involve long incubation periods (5 days) at ambient temperature. A rapid method has been evolved using a more nutritious medium consisting of malt extract or yeast extract, beef extract, dextrose, peptone and sodium chloride (pH 6.1) and incubation at 30°C for 48hr. Chlorotetracycline and chloramphenicol at 100 ppm levels were used for controlling bacterial growth. Comparative studies carried out using two storage fungi (*Aspergillus* sp., and *Rhizopus* sp.) and three strains of yeasts (*Saccharomyces cerevisiae*, *Hansenula anamola* and *Torulopsis holmii*) with six different conventional media have indicated better recovery. The modified method has also been found to help in reviving cultures that have been subjected to heat, chill, freeze or osmotic stress.

Development of new media for isolation and enumeration of yeast and molds from foods has been a continuing process. Acidified Potato Dextrose Agar has been used for many years for isolating yeast and molds from foods[1]. However, the growth of acid tolerant bacteria is rarely suppressed totally and media with low pH values may inhibit the growth of many molds especially if they have been sublethally stressed during the processing of the food. Several workers have found it advantageous to use media of higher pH values containing several antibiotics or other anti-bacterial agents[2–5]. There has been a growing need for a rapid method for enumeration of yeasts and molds in foods. A new medium with extra nutrients has been developed and it has been compared with several other media commonly used. The new media has also been evaluated both under normal and different conditions of stress.

Materials and Methods

Media: The newly compounded medium is a modified Dextrose, Yeast extract and Peptone agar (DYP) supplemented with beef extract and sodium chloride (DYPBS): it contained (g per cent) dextrose, 1.0; yeast extract, 0.47: peptone, 0.94; beef extract, 0.24; sodium chloride, 1.0 and agar, 2.0. The pH of the medium was 6.10 (unadjusted). The other media used for comparative studies were malt extract agar (MEA) (Oxoid composition), Malt extract agar (BBL composition), Potato dextrose agar (PDA), Czapek dox agar (CDA), Sobouraud dextrose agar (SDA) and Dextrose yeast extract peptone agar (DYA). The pH of all the media were adjusted to 6.10. Seitz filtered chlorotetracycline and chloramphenicol were added at 100 ppm levels after sterilization of the media.

Inoculum preparation: Aspergillus niger, A. flavus, A. oryzae and *Rhizopus oligosporus* were allowed to grow on PDA slants for 7 days at 26°C and the growth was washed with 10 ml of 0.1 per cent peptone water containing Tween 80 at 0.1 per cent level (PT). The spore suspension thus prepared was used as inoculum in various experiments.

Forty eight hour old cultures of *Saccharomyces cerevisiae, Hansenula anamola* and *Torulopsis holmii* in DYP broth were used as inocula.

Estimation of growth: Growth estimation was carried out by gravimetry and by counting colony forming units (cfu) on the plate. For gravimetric methods, 250 ml of different media were inoculated with 0.1 ml inoculum ($1\times/10^6$ cells or spores/ml) of different yeasts and mould cultures prepared as above. The inoculated flasks were incubated in a rotary shaker (230 rpm) for 96 hr. Cell biomass/mycelial mat from the flasks were harvested and dried to a constant weight.

The cfu were obtained by following the procedure of plate count as per APHA[6] procedure using PT as the suspending medium. The inoculum (0.1 ml) was inoculated on prepoured media surface and spread evenly by hockey sticks. The plates were incubated both at room temperature (25 ±1°C) and at 30°C for 48 hr. Colony forming units on different plates were counted after 48 hr. Average of five replications were recorded in both the methods.

Recovery experiments with inoculated grain samples: Grain samples were procured from the

local market. Attempts were made to select samples which had not been stored for long to avoid heavy contamination of native storage moulds. Surface sterilized (0.1% $HgCl_2$) samples of ragi (*Eleusine coracana*), rice (*Oryza sativa*) wheat (*Triticum vulgare*), green gram (*Phaseolus aureus*), Black gram (*Phaseolus mungo*), dolichos (*Dolichos lablab*), haraka (*Paspalum scrobiculatum*), Bengal gram (*Cicer arietenum*), Bengal gram (split) and sorghum (*Sorghum vulgare*) were mixed with *A. niger* spores (10 ml suspension containing 1×10^6 spores/ml). Ten grams of each were homogenized in a Colworth 400 stomacher with 90 ml of PT and kept at room temperature for 4 hr. Further dilutions were prepared as required with the same diluent. Using a sterile bent rod, a 0.1 ml sample from each material was spread evenly over the entire surface of a plate medium. Five replicates were maintained for each medium. The plates were incubated for 48 hr at 30°C. The colonies on different media were counted after 48 hr of incubation.

Growth of stressed A. niger spores in new medium: Fifty ml samples were subjected to one of the following treatments: (i) heated in a hot air oven at 50°C for 20 min (ii) held overnight at −18°C and then thawed, (iii) held at 4–6°C for 1–2 hr and (iv) was diluted ten times with peptone (0.1 per cent) Tween 80 (0.1 per cent) saline (0.85 per cent) (PTS) and homogenised, 0.1 ml of homogenate was transferred into 10 ml of PT diluent and allowed to stand for 5 min at room temperature; then 90 ml of PTS was added to salt-free sample suspension and the sample was analysed immediately.

Sample (0.1 ml) of suitable dilution was spread on different plate media as before, maintaining 5 replicates for each. The plates were incubated for 48 hr at 30°C and the recovered colonies were enumerated.

Results and Discussion

As indicated in Table 1 mycelial dry weight was higher with the new medium (DYPBS) compared to others. The cell and mycelial dry weight are the direct reflections of the total biomass due to growth. This was highest when the yeast and mould cultures were grown in DYPBS medium. The PDA medium has been found to be the next best. The experiments were conducted at room temperature incubation only in the shaker. It is presumed that high optimal incubation temperature (30°C) would have followed the same trend. The results obtained by the plate count method are shown in Table 2. As seen from the table. the DYPBS agar in comparison with other media recorded highest recovery of all the organisms tested at both room temperature and at 30°C. The recovery was,

TABLE 1. COMPARATIVE GRAVIMETRICAL ENUMERATION OF YEAST AND MOLDS ON DIFFERENT MEDIA

Medium	Mycelial dry wt. (mg)*						
	S. cerevisiae	*H. anamola*	*T. holmii*	*A. niger*	*A. flavus*	*A. oryzae*	*R. oligosporus*
MEA (Oxoid)	105.0 ± 7.6	265.0 ± 1.7	205.0 ± 2.5	236.6 ± 3.8	274.3 ± 3.8	264.6 ± 3.7	220.7 ± 0.6
MEA (BBL)	91.7 ± 4.4	271.3 ± 4.4	200.0 ± 2.9	233.3 ± 6.0	304.3 ± 3.4	307.6 ± 2.0	210.8. ± 2.0
CDA	50.0 ± 2.9	160.3 ± 1.5	121.0 ± 1.7	570.0 ± 3.6	402.0 ± 1.5	386.6 ± 0.3	331.3 ± 1.6
SDA	126.3 ± 2.7	317.7 ± 4.3	308.0 ± 3.0	473.3 ± 3.8	342.3 ± 2.6	303.0 ± 2.1	260.7 ± 0.7
DYA	210.7 ± 2.0	308.0 ± 1.2	410.3 ± 2.3	454.7 ± 1.5	311.3 ± 1.2	308.3 ± 1.8	310.0 ±1.2
PDA	199.3 ± 8.4	384.7 ± 4.3	349.3 ± 2.6	389.7 ± 2.6	514.0 ± 2.3	471.3 ± 1.8	251.3 ± 3.5
DYPBSA	234.3 ± 4.7	446.0 ± 3.2	361.3 ± 1.7	582.0 ± 2.9	609.0 ± 2.3	480.0 ± 2.3	580.0 ± 1.2

* Mean ± SD of 5 replicates

TABLE 2. RECOVERY (PER CENT) OF TEST CULTURES IN COMMONLY USED MEDIA IN COMPARISON WITH EXPERIMENTAL MEDIUM

Medium	*S. cerevisiae**		*H. anamola**		*T. holmii**		*A. niger*+		*A. flavus*+		*A. oryzae*+		*R. oligosporus*+	
	30°C	RT	30°C	RT	30°C	RT	30°C	RT	30°C	RT	30°C	RT	30°C	RT
MEA (Oxoid)	41.9	32.5	62.3	58.1	48.2	46.7	82.4	44.8	74.7	64.5	75.4	72.1	58.1	38.5
MEA (BBL)	62.0	41.7	61.8	68.6	60.8	58.0	72.9	52.4	71.3	56.9	73.7	49.1	45.0	38.8
CDA	37.2	24.7	36.2	32.0	12.2	10.5	55.8	26.1	63.7	43.1	50.8	29.5	39.3	20.1
SDA	43.5	40.8	75.7	55.8	55.6	51.0	78.1	58.1	72.4	63.8	63.9	59.1	50.0	38.8
DYA	47.2	37.0	76.8	69.2	47.8	33.4	64.7	63.9	63.8	70.7	77.1	68.9	43.8	35.6
PDA	81.9	61.5	85.6	68.5	57.1	55.8	88.6	63.3	70.7	60.4	62.3	50.8	50.6	31.3
DYPBSA	85.0	63.3	88.2	66.6	79.6	56.5	95.2	75.3	82.8	74.2	80.3	68.3	66.2	44.4

RT: Room temperature = 25 ± 1°C. Inoculum level: * = 10^4/ml, + = 10^3/ml.

TABLE 3. RECOVERY (PER CENT) OF ADDED *ASPERGILLUS NIGER* SPORES TO DIFFERENT FOOD GRAINS*

Raw materials	MEA (Oxoid)	MEA (BBL)	CDA	SDA	DYA	PDA	DYPBSA
Ragi	58.6	39.5	29.5	38.0	44.8	25.7	50.1
Rice	40.0	81.9	30.9	54.3	59.0	65.7	86.7
Wheat	44.2	48.9	25.7	35.3	56.2	44.2	76.7
Green gram	65.7	35.3	27.6	39.0	51.4	48.6	53.3
Black gram	61.9	40.9	30.9	40.2	47.8	36.4	56.0
Dolichos	45.3	56.1	40.5	47.1	46.2	34.2	51.0
Haraka	46.2	48.0	36.8	36.6	31.9	57.6	47.2
Bengal gram	35.3	43.8	33.3	44.8	47.6	55.2	60.0
Bengal gram (broken)	63.8	75.2	41.9	58.1	56.2	50.5	65.7
Sorghum	67.6	42.3	59.1	68.6	87.7	63.3	88.8

*Incubation temp. = 30°C, Inoculum level = 10^3/ml

however, very low in czapek dox agar, which is a synthetic medium normally recommended for qualitative taxonomic studies. The better growth on DYPBS might be attributed to the enriched nutrient composition of the medium.

Evaluation of the DYPBS medium in terms of per cent recovery of *A. niger* spores from artificially inoculated natural substrates like ragi, rice, wheat, green gram, black gram, dolichos, haraka, Bengal gram and sorghum was also done, the results of which are depicted in Table 3. Highest recovery of *A. niger* spores from 4 substrates (wheat, rice, Bengal gram and sorghum) was observed on the DYPBS medium. In case of other substrates, the medium ranked second to the best. DYPBS medium was also found to be superior to other media when the recovery of stressed cells was compared (Table 4).

Koburger and Rodgers[8] have questioned the use of one antibiotic in media to control bacterial growth during the routine evaluation of foods for their yeast and fungal populations. Subsequently, there has been sufficient data in the literature to indicate that a single antibiotic is inadequate to control bacteria during enumeration of yeast and fungi[9]. In the present study therefore, chlorotetracycline and chloramphenicol were used at 100 p.p.m. levels, with the presumption that in combination they would be able to control the broad spectrum bacterial growth; thus the newly evolved medium appeared to be highly promising and further detailed studies are advocated on the basis of which its use could be recommended for rapid estimation of yeast and moulds.

TABLE 4. RECOVERY (PER CENT) OF STRESSED *A. NIGER* SPORES TO DIFFERENT MEDIA*

Stress conditions	MEA (Oxoid)	MEA (BBL)	CDA	SDA	DYA	PDA	DYPBSA
Heat	42.6	43.7	12.4	32.7	39.4	44.2	61.5
Chill	34.8	39.3	21.6	41.4	42.6	48.1	71.4
Deep freeze	31.5	36.1	20.3	22.2	30.3	32.4	37.3
Osmotic	21.7	21.4	18.4	27.2	26.7	23.4	29.2
Control	82.4	72.9	55.8	78.1	64.7	88.6	95.2

*Incubation tem. = 30°C

Acknowledgement

The authors thank Dr. B.L. Amla, Director, Central Food Technological Research Institute, Mysore, for his keen interest in the present investigation.

References

1. Sharf, J.M., *Recommended Methods for the Microbiological Examination of Foods*, American Health Association., New York. 1966.
2. Jarvis, B., Comparison of an improved rose Bengal chlorotetracycline agar with other media for selective isolation and enumeration of moulds and yeasts in foods. *J. appl. Bacteriol.*, 1973, **36**, 723.
3. Koburger, J.A. and Farhat, B.Y., Comparison of media to enumerate yeast and molds. *J. Milk Fd Technol.*, 1975, **38**, 466.
4. Mossel, D.A.A., Vesser, M. and Mengesink, W.H.J., A comparison of media for the enumeration of molds and yeasts in food and beverages. *Lab. Pract.*, 1962, **11**, 213.
5. Overcast, W.W. and Weakley, D.J., An aureomycin rose Bengal agar for enumeration of yeast and mold in cottage cheese. *J. Milk Fd Technol.*, 1969, **32**, 442.
6. M.L. Speak (Ed) *Compendium of Methods for the Microbiological Examination of Foods* Prepared by APHA/Agency committee on Microbiological methods for Foods, Washington, D.C., 1976.
7. Hesseltine, C.W., De Camargo, R. and Rackis, J.J., A mould inhibitor in soybeans. *Nature*, Lond., 1963, **200**, 1226.
8. Koburger, J.A. and Rodgers, M.F., Single or multiple antibiotic-amended media to enumerate yeasts and molds. *J. Fd Prot.*, 1978, **41**, 367.
9. Smith, A.G. and Rohde, P.A., A new technique for isolating pathogenic fungi from mixed flora inocula. *Mycopath. Mycol. appl.*, 1972, **47**, 105.

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 7–10

Effect of Germination on the Functional Properties of Moth Bean (*Phaseolus aconitifolius* Jacq) Flours

V.D. PAWAR AND U.M. INGLE
College of Agricultural Technology, Marathwada Agricultural University, Parbhani, India

Received 27 *December* 1986; *revised* 23 *March* 1987

Moth bean was germinated for 0, 24, 48 and 72 hr, ground and functional properties viz., water and oil absorption capacity, gelation, nitrogen solubility, foaming and emulsion properties of flours were determined. All the functional properties were improved after germination. The nitrogen solubility profile of germinated moth bean flours was not significantly different from the control at any of the pHs studied except for a slight increase at pH 4.5. The foaming and emulsion capacities of germinated moth bean flours were improved at all pHs studied. The increase was more conspicuous at pH 4.5.

Food legumes are important in the diets of vegetarians in the developing countries. Legumes contain generally 20 to 25 per cent protein and a fair amount of B-complex vitamins and minerals. Several potential food proteins (oilseeds, microbial, leaf) are presently underutilised. Unlike these sources, dry beans are one source of food proteins with a significant potential for food application.

Moth bean is commonly grown in arid areas of India and is consumed either as such after cooking or after germination and cooking. There are reports on the cooking quality[1], effect of germination on polyphenols and *in vitro* protein digestibility[2] and carbohydrates, proteins, trypsin inhibitor, amylase inhibitor and hemagglutinin[3] and on a few functional properties of dry moth bean[4]. In this report, the effect of germination on functional properties of moth bean flour is presented.

Materials and Methods

Mature dry moth bean seeds (*Phaseolus aconitifolius* Jacq) grown and harvested in 1984 were purchased from the local market and cleaned.

Germination: One hundred gram seeds of uniform size were sterilized with 0.1 per cent mercuric chloride, soaked in water at 4°C for 6 hr, placed on a double layer of filter paper in dry clean petri plates and incubated at 30°C. The filter papers were moistened at regular intervals of 12 hr till 72 hr and seeds were allowed to germinate for 24, 48 and 72 hr. The germinated seeds were dried in a cabinet drier at 50°C and air velocity of 14 m/min till constant weight. The dried seeds were ground in a grinder, passed through a 60 mesh sieve and stored at 4°C till analysis.

Proximate analysis: Moisture, crude protein (N × 6.25), crude fat and ash contents were determined using AOAC[5] methods. Total carbohydrates were calculated by difference.

Functional properties: Water and oil (refined groundnut oil) absorption capacities were determined by the method of Beuchat[6]. Least-gelation concentrations of moth bean flours were determined by the method of Coffmann and Garcia[7], with slight modifications as described by Deshpande *et al*[8]. Nitrogen solubility of moth bean flours was determined in the pH range of 2–12, as described by Narayana and Rao[9]. Foaming capacity and stability of moth bean flours were studied according to the method of Coffmann and Garcia[7]. Emulsifying activity and emulsion stability were used as indices of emulsifying properties and were evaluated at room temperature (25°C ± 2) by the method of Yasumatsu *et al.*[10] with slight modifications as described by Deshpande *et al*[8].

Unless otherwise mentioned, all the measurements were made in triplicates and the values represent the average of three determinations.

Results and Discussion

Proximate composition: The data presented in Table 1 indicate the changes in proximate composition of moth bean flour due to germination up to 72 hr. There was a net increase in moisture and ash content and little increase in crude protein content during 72 hr germination. Similar findings were reported by Kylen and McCready[11] who attributed the increase to protein synthesis at the time of sprouting alfalfa, lentils, mung beans and soybeans. It was also possible

TABLE 1. EFFECT OF GERMINATION ON THE PROXIMATE COMPOSITION[a] OF MOTH BEAN FLOUR

Proximate composition (%)	Germination period (hr)			
	0	24	48	72
Moisture	10.50	12.00	12.80	13.60
Crude protein (N × 6.25)	23.50	24.10	24.40	24.90
Crude fat	1.61	1.44	1.43	1.42
Ash	3.32	4.18	4.32	4.44
Total carbohydrates	59.40	58.00	56.50	55.40

[a]on dry weight basis

that the increase in protein was due to changes resulting from the uptake of water during germination. Kakade and Evans[12] have reported increase in crude protein content in navy beans *(Phaseolus vulgaris)* on germination for 4 days. The observed decrease in carbohydrates and crude fat in moth bean flour during germination may be due to increased activity of carbohydrases and lipases.

Water and oil absorption capacity: It is seen from Table 2 that dry ungerminated moth bean flour had a water and oil absorption capacity of 2.4 and 1.7 g/g flour, respectively; the corresponding values for (in g/g) protein were 10.2 and 7.2. The values of water and oil absorption capacity of dry raw flours agree with those reported on moth bean by Borhade *et al*[4]. Germinated moth bean flours had higher water and oil absorption capacity than that of raw moth bean flour; 72 hr germination led to marked increase in water absorption capacity. This could be attributed to the increased protein content and changes in the quality of proteins upon germination as reported for mung bean[13]. The ability of proteins to bind fat is also important since fats act as flavour retainers and increase the mouth feel of foods. THe oil absorption capacity was also increased to 2.6 g/g flour and 10.4 g/g protein with an increase in protein content during 72 hr germination period. This could be attributed to the increased capacity of the flour to hold the fat globules as the amount of lipophilic proteins increases. del Rosario and Flores[13] also reported increase in oil absorption capacity in 36 hr sprouted mung bean flour. The lower oil absorption of the legume flours as compared to their water absorption values suggests that the major proteins in legumes are predominantly hydrophilic.

Gelation: Germination improved the gelation property of moth bean flours (Table 2). The least-gelation concentrations for the control dry bean and 72 hr germinated bean flours were 9 and 9.6 per cent, respectively. The increased least-gelation concentration of the germinated moth bean flours may

TABLE 2. EFFECT OF GERMINATION ON THE FUNCTIONAL PROPERTIES OF MOTH BEAN FLOUR

Functional properties	Germination period (hr)			
	0	24	48	72
1. Water absorption (g/g)				
Flour	2.4	2.8	3.0	3.6
Protein[a]	10.2	11.6	12.2	14.4
2. Oil absorption (g/g)				
Flour	1.7	2.1	2.4	2.6
Protein[a]	7.2	8.7	9.8	10.4
3. Least gelation concn (w/v)	9.0	9.1	9.6	9.6
4. Foaming properties				
Vol. increase (%)	27.0	30.0	36.0	40.0
Specific vol (ml/g)	1.30	1.33	1.38	1.43
% vol. decrease in 120 min	31.4	34.2	36.5	37.7
5. a) Emulsifying activity (g/g)				
Flour	20.2	23.0	25.0	27.5
Protein[a]	85.9	95.4	102.4	110.4
b) Emulsion stability[b]				
Flour	12.0	11.8	11.3	11.0
Protein[a]	51.0	48.9	46.3	44.1

[a] Expressed on crude protein basis.
[b] Per cent of the emulsifying activity after heating at 80°C for 30 min.

be due to increased protein content. During germination, seed coats are loosened, complex carbohydrates are minimised, the interference with carbohydrates in gel formation are also minimised and thus gelling capacity increased. The gelation capacity of legume proteins is mainly because of the globulin fraction which is the major protein fraction in moth bean flour. This finding and the hypothesis are supported by Fleming *et al*[14] on vegetable protein products.

Nitrogen solubility: As depicted in Fig. 1, nitrogen solubility was pH dependent. Raw moth bean flour had the minimum nitrogen solubility of 18 per cent, around pH 4.5; on either side of this pH, it increased. At pH 2.0 about 80 per cent of nitrogen was soluble and at pH 12.0 it was about 96 per cent. These results partly agree with the reports of Borade *et al*[4] who observed minimum nitrogen solubility at pH 4.5 and maximum at pH 2.0 and 9.0 in moth bean. The decrease in nitrogen solubility beyond pH 9.0 observed by Borhade *et al* may be due to salting-in effects of extractants used by them. Narayana and Rao[9] observed a minimum nitrogen solubility of 23 per cent around pH 4.5 and maximum of 95 per cent at pH 10.5 in case of winged beans.

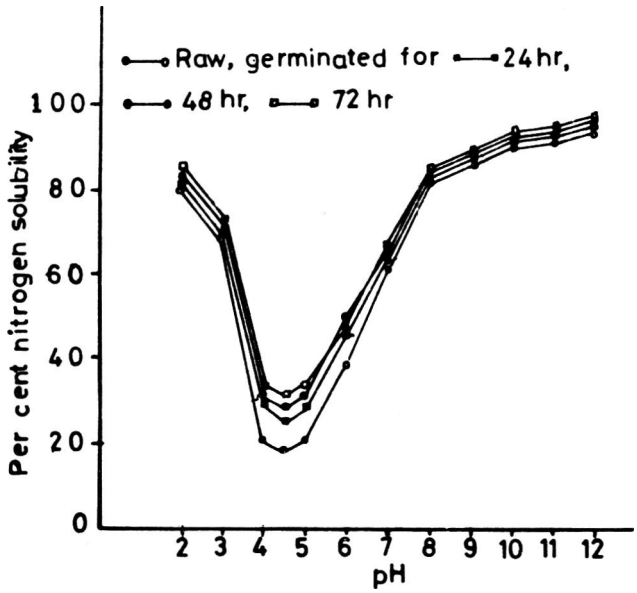


Fig. 1 Nitrogen solubility Vs pH profile of raw and germinated moth bean flours.

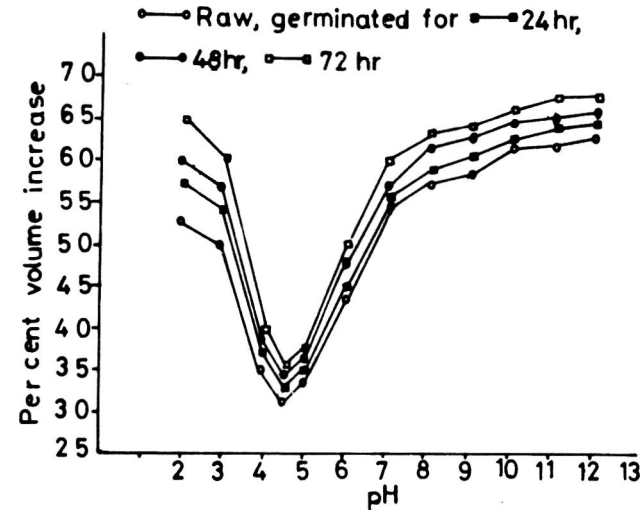


Fig. 2 Foaming capacity Vs pH profile of 2 per cent (w/v) aqueous dispersion of raw and germinated moth bean flours.

The nitrogen solubility increased in germinated moth bean flours at all pH's studied (Fig. 1). The increase was more conspicuous in 72 hr germinated than in 24 and 48 hr germinated moth bean flours. The main difference in nitrogen solubility was between pH 4 and 6. At pH of minimum nitrogen solubility i.e. 4.5, there was significant difference in nitrogen solubility among all test samples. A minimum nitrogen solubility of 23, 28 and 31 per cent was observed in 24, 48 and 72 hr germinated moth bean flours respectively compared to 18 per cent with raw flour. Nitrogen solubility, however, is affected by germination which results in protein synthesis. The results fairly agree with those reported on sprouted mung bean[13].

Foaming properties: Germination improved foaming properties (Table 2). Volume increase on whipping of 1 per cent aqueous dispersions of the raw moth bean flour was 27 per cent which increased progressively to 40 per cent as the germination period increased to 72 hr. This suggests that foaming property was dependent on the solubilized proteins. During germination, the solubilized protein increases. Germination also improved the specific volume of foams, indicating better foaming and air uptake during whipping. The lower air uptake during whipping of ungerminated moth bean flour was accompanied by a decrease in its foam volume. In the present investigation, though the protein content during germination was apparently increased, foam stability was poorer than the raw moth bean flour indicating thereby that the foam stability is dependent on native proteins present. The same observations were also reported by Yasumatsu *et al.*[10] and Lin *et al*[15] wherein they correlated foaming capacity and stability with the amount of native proteins present.

Foaming capacity of raw and germinated moth bean flours was pH dependent (Fig. 2). The foam capacity versus pH profile of raw and germinated moth bean flours closely resembled the nitrogen solubility versus pH profile (Fig. 1) suggesting that foaming property was also dependent on the solubilized protein. The trend of foaming capacity at different pH levels varied with the period of germination. At all the pH values investigated, 72 hr germinated moth bean flour had a greater increase in volume than did the corresponding 24 and 48 hr germinated and raw moth bean flours. At pH 4.5, an apparent isoelectric pH of moth bean proteins, minimum foaming was observed for all samples. Invariably, the greatest increases in foam volumes of raw and germinated moth bean flours were observed at pH 2,3,8,9,11 and 12. The increased foaming capacity in both acidic and alkaline pH values is attributed to the dissociation of seed proteins and increased solubility of major storage globulins during germination. Such pH dependence of foaming characteristics was also reported for winged bean[9] and sunflower proteins[15].

Emulsion properties: Emulsifying activity of raw moth bean flour and protein was 20.2 and 85.9 (g/g), respectively, which was increased during germination at all periods (Table 2). The increase may be ascribed partly to more protein being in the suspension for a given amount of oil and partly to the lipolytic products (mono-di-and triglycerides) formed during germination as lipase activity increase considerably. Moreover, increased amount of solubilized proteins during germination has positive correlation with emulsifying activity. In winged bean proteins, similar relationship between emulsification capacity and

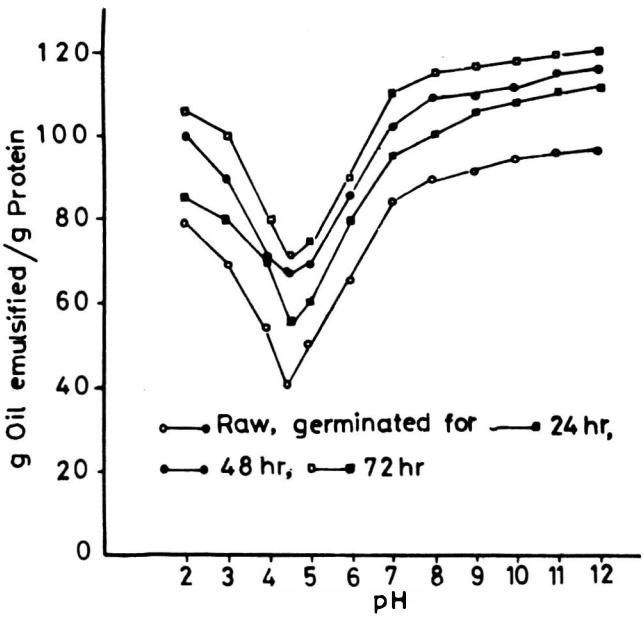


Fig. 3 Emulsification capacity Vs pH profile of raw and germinated moth bean flours

solubilized proteins has been reported[9]. The emulsion stability of germinated flours, however, dropped from 12.0 to 11.0 and 51.0 to 44.1 per cent for flour and protein, respectively, upto 72 hr germination. The decrease in emulsion stability during germination may be due to increased proteolytic activity which is responsible for protein hydrolysis. The emulsion properties of flours cannot be solely attributed to the proteins but other food components such as carbohydrates and lipids may also contribute appreciably, possibly through protein-carbohydrate and protein-lipid interactions. These interactions are reduced during germination with concomittant decrease in the emulsion stability. This explanation is also supported by the observation of McWatters and Cherry[16] on cowpeas.

The effect of pH on the emulsification capacity of the raw and germinated moth bean flour (Fig.3) showed that emulsification capacity of germination flours was higher than raw flour. The emulsification capacity of 72 hr germinated flour was 106 g/g protein at pH 2.0, 72 g/g protein at pH 4.5 (the pH of minimum solubility) and 115 g/g protein at pH 12. The emulsification capacity increased progressively with increase in germination time. At all pH's studied, the emulsification capacity of germinated flours was higher compared to raw flour. The graph of emulsification capacity versus pH profile closely resembled the nitrogen solubility versus pH profile (Fig.1). This suggested that emulsification capacity was due to solubilized proteins. The observations are supported by the reports of Narayana and Rao[9] on winged bean.

References

1. Kadam S.S., Subramanyam, P., Jawale, H.K., Satwadhar, P.N. and Jadhav, S.J., Improvement in cooking quality of horse gram (*Dolichos biflorus*) by pre-soaking treatment with salt solution. *Qualitas Pl. Pl. Fd. Hum. Nutr.*, 1981, **31**, 171.
2. Satwadhar, P.N., Kadam, S.S. and Salunkhe, D.K., Effects of germination and cooking on polyphenols and *in vitro* protein digestibility of horse gram and moth bean. *Qualitas Pl. Pl. Fd. Hum. Nutr.*, 1981, **31**,71.
3. Subbulakshmi, G., Ganeshkumar, K. and Venkataraman, L.V.. Effect of germination on the carbohydrates, proteins, trypsin inhibitor, amylase inhibitor and hemagglutinin in horse gram and moth bean. *Nutr. Rep. Int.*, 1986, **13**, 19.
4. Borade, V.P., Kadam, S.S. and Salunkhe, D.K., Solubilization and functional properties of moth bean (*Vigna aconitifolia* Jacq.) Marechal and horse gram (*Macrotyloma uniflorum* Lam.) Verdc proteins. *J. Fd. Biochem.*, 1984, **8**, 229.
5. *Official Methods of Analysis.* Association of Official Agricultural Chemists, St. Paul MN., 1975, 12th Edn.
6. Beuchat, L.R., Functional and electrophoretic characteristics of succinylated peanut flour proteins. *J. agric. Fd. Chem..*, 1977, **25**, 258.
7. Coffmann, C.W. and Garcia, V.V., Functional properties and amino acid content of a protein isolate from mung bean flour. *J. Fd. Technol.* 1977, **12**, 473.
8. Deshpande, S.S., Sathe, S.K., Cornforth, D. and Salunkhe, D.K., Effects of dehulling on functional properties of dry beans (*Phaseolus vulgaris*) flours. *Cereal Chem.*, 1982, **59**, 396.
9. Narayana, K. and Rao, M.S.N., Functional properties of raw and heat processed winged bean (*Psophocarpus tetragonolobus*) flours. *J. Fd. Sci.*, 1982, **47**, 1534.
10. Yasumatsu, K., Sawada, K., Moritaka, S., Misaki, M., Toda, J., Wada, T. and Ishil, K., Whipping and emulsifying properties of soybean products. *Agric. biol. Chem.*, 1972, **36**, 719.
11. Kylen, A.M. and McCready, R.M., Nutrients in seeds and sprouts of alfalfa, lentils, mung beans and soybeans. *J. Fd. Sci.*, 1975, **40**, 781.
13. del Rosario, R.R. and Flores, D., Functional properties of four types of mung bean flour. *J. Sci. Fd. Agric.*, 1981, **32**, 175.
14. Fleming, S.E., Sosulski, F.W. and Hamon, N.W., Gelation and thickening phenomena of vegetable protein products. *J. Fd.Sci., 1975, 40, 805.*
15. Lin, M.J.U., Humbert, E.S. and Sosulski, F.W., Certain functional properties of sunflower meal products. *J. Fd. Sci.*, 1974, **39**, 368.
16. McWatters, K. H. and Cherry, J.P., Emulsification, foaming and protein solubility properties of defatted soybean, peanut, field pea and pecan flours. *J. Fd. Sci.*, 1977, **42**, 444.

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 11–15

Functional Properties of Peanut Flour as Affected by Different Heat Treatments

E.H. RAHMA AND M.M. MOSTAFA
Food Science and Technology Department, Faculty of Agriculture, University of Monoufeia, Shibin El-Kom, Egypt.

Received 28 *October* 1986; *revised* 2 *March* 1987

Peanut kernels were heated at 100, 120, 140 and 160°C for 15, 30, 45 and 60 min, autoclaved at 121°C for 5 to 30 min and exposed to different doses of radiation. The effect of these treatments on emulsification capacity (EC), water and fat absorption capacities (WAC, FAC) foam capacity (FC) and foam stability (FS) of the defatted flours was studied. Water and fat absorption by flour increased due to heat treatments. The increase was more at higher temperatures and longer times of heating. Radiation also increased both these parameters. Dry heat, moist heat and radiation decreased the EC of the flour, the effect being most pronounced with moist heat. Foaming properties were decreased due to dry heat and moist heat treatments but the decrease was less in irradiated samples.

Functional properties of specific proteins from oil seeds have been studied for many years. Soybean has been a primary source of vegetable proteins for use as a functional ingredient in many food products[1]. Cottonseed and groundnut protein have also found some uses [2–4].

Groundnut or peanut is a good source of oil and protein. The emulsification properties of groundnut flour were studied by Ramanatham *et al*[5]. Functional and electrophoretic characteristics of succinylated peanut flour protein were investigated by Beuchat[6]. Also solubility and PAGE pattern and chemical composition of peanut protein isolate were reported[7]. The effect of dry heat on some antinutritional factors and functional properties of peanut protein were studied by Perkins and Toledo[8].

In the present investigation, the functional properties such as emulsification capacity (EC), water and fat absorption capacities (WAC, FAC), and foam capacity (FC) and stability (FS) of raw, dry heated, autoclaved and irradiated peanut flour were determined.

Materials and Methods

Peanuts (*Arachis hypogaea*, L) variety 'Giza 4', grown at El-Ismaelia Governorate during the year 1985 were obtained from the Seed Department, Ministry of Agriculture at Giza. Egypt. The peanuts were depodded manually and the sound seeds were subjected to different treatments as indicated in Fig. 1.

Emulsification capacity: The EC of the flour was determined by the procedure of Beuchat *et al*[9] at room temperature (~ 25°C). A 2 g flour sample and 25 ml distilled water were blended for 30 sec in a

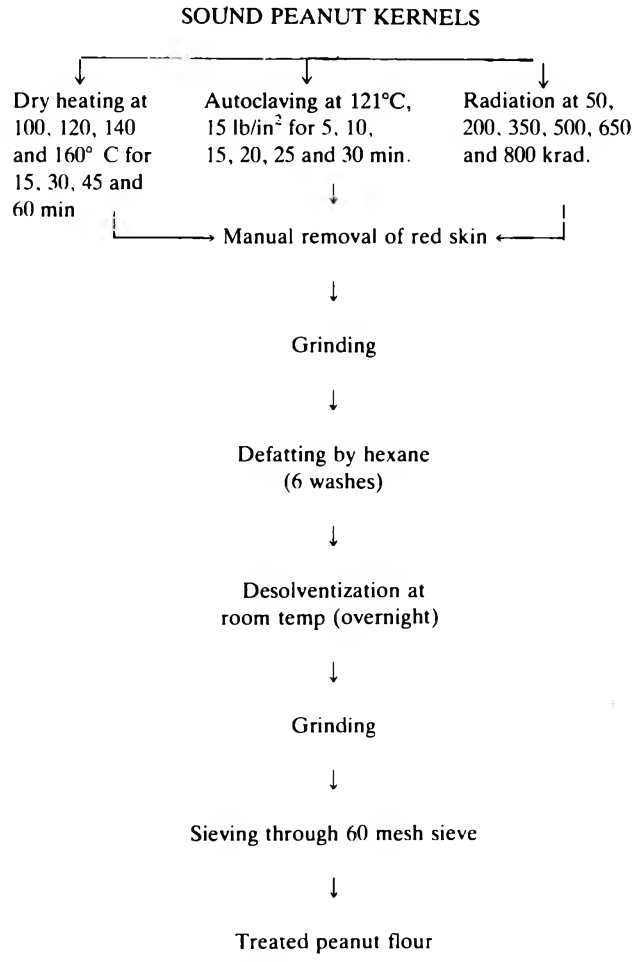


Fig. 1: Flow sheet for preparing dry and wet heat and irradiated peanut flours

Braun Blender at 1600 rpm. After complete dispersion, refined corn oil was added from a burette and blending continued until there was separation into two layers. The EC values are expressed as ml of oil emulsified by 1 g of flour.

Water and fat absorption capacities: For these determinations, 15 ml of distilled water was added to 1 g of the flour in a weighed 25 ml centrifuge tube. The tube was agitated on a vortex mixer for 2 min and centrifuged for 20 min at 4000 rpm; the clear supernatant was decanted and discarded. The adhering drops of water were removed and the tube weighed. Water absorption capacity is expressed as the weight of water bound per 100 g of dry flour[10]. Fat absorption capacity was estimated in the same way as water absorption capacity except that 10 ml of refined corn oil was added to 1 g of the flour and is expressed as ml oil bound per 100 g of dry flour[11].

Foaming capacity and foam stability: These were determined as described by Lawhon *et al*[2]. A 2 g flour sample and 50 ml distilled water were mixed in a Braun Blender at room temperature. The suspension was stirred for 5 min at 1600 rpm and the contents along with the foam were poured into a 100 ml graduated measuring cylinder and the total volume was recorded after 30 sec. The percentage increase in volume after 30 sec is expressed as foam capacity. The volume of foam only (Total volume – Liquid volume) after 30 min of standing at room temperature is taken as foam stability.

The data reported are an average of duplicate determinations which agreed closely.

Results and Discussion

A) *Effect of dry heating:* Water absorption capacity of unheated and dry heated peanut at different temperatures and times is shown in Fig. 2. The raw sample had a water absorption capacity of 110 g H_2O/ 100 g flour. Heat treatment decreased the ability of the flour to bind and retain the water up to heating time of 15 min. However, the water absorption capacity increased thereafter as the heating was continued. The increase was very considerable at the higher temperatures and longer times of exposure to heat. The absorption capacity of water after 60 min. of heating at 100, 120, 140 and 160°C was 110, 115, 125 and 145 g H_2O/100 g flour respectively exceeding the initial water absorption capacity. Wu and Inglett[12] have reported that water absorption capacity of soy flour increased due to heat processing. The same observation was also reported for heated winged bean flour[13]. Catsimopoolas *et al*[14] have mentioned that proteins consist of subunit structure and the proteins are dissociated upon heating. It is possible that the

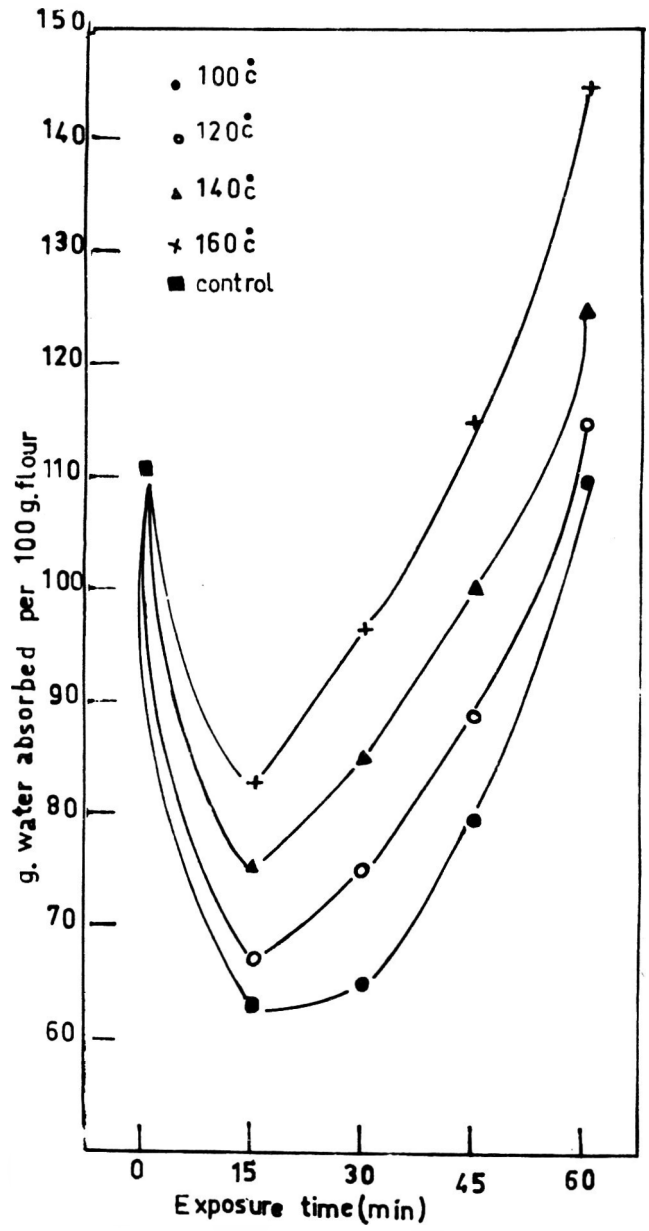


Fig 2 Effect of dry heat on the EC of peanut flour.

dissociated subunits have more water binding sites than the undenatured protein. This could be the reason for the observation that the extensively heat treated peanut has higher water absorption capacity.

The ability of proteins to bind fats is important since fats act as flavour retainers and improve the mouth feel of foods[15]. The raw peanut flour had fat absorption capacity (250 ml oil/100 g flour) and this value increased to 295 and 340 ml oil/100 g flour after heating for 60 min. at 140 and 160°C respectively (Fig. 3). Mild heat treatment at 100 and 120°C for shorter times (15 and 30 min) decreased the fat absorption capacity slightly but it increased on prolonged heating.

Generally, more hydrophobic proteins show superior binding of lipids[15], implying that nonpolar

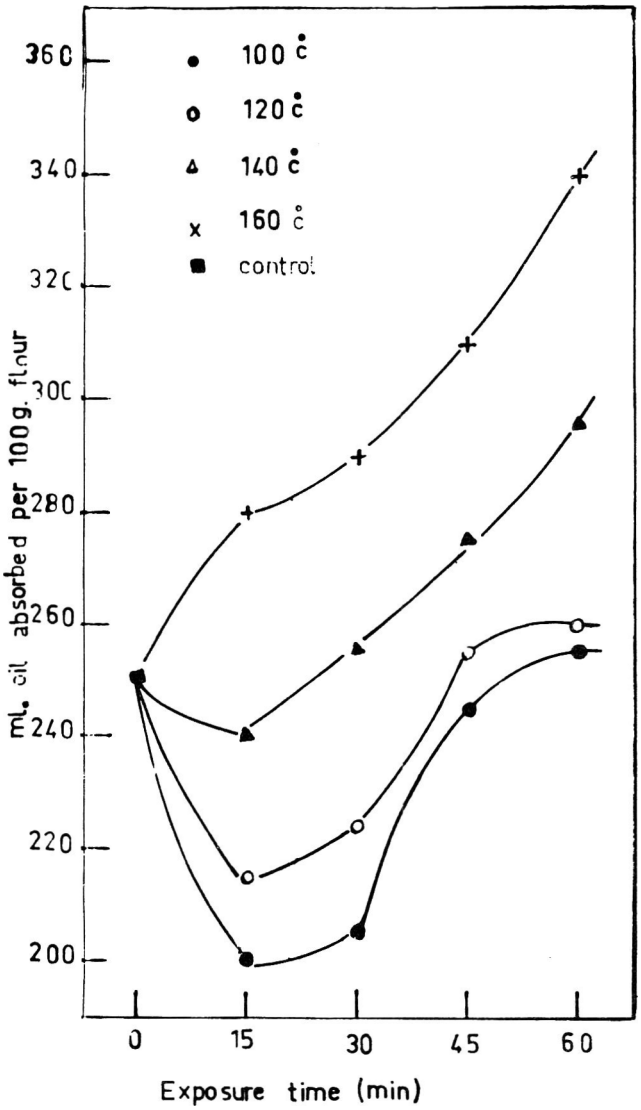


Fig. 3 Effect of dry heat on the OAC

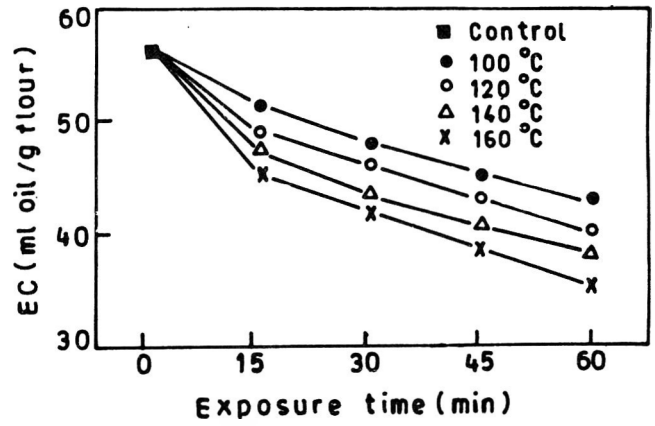


Fig. 4 Effect of dry heat on the EC of peanut flour.

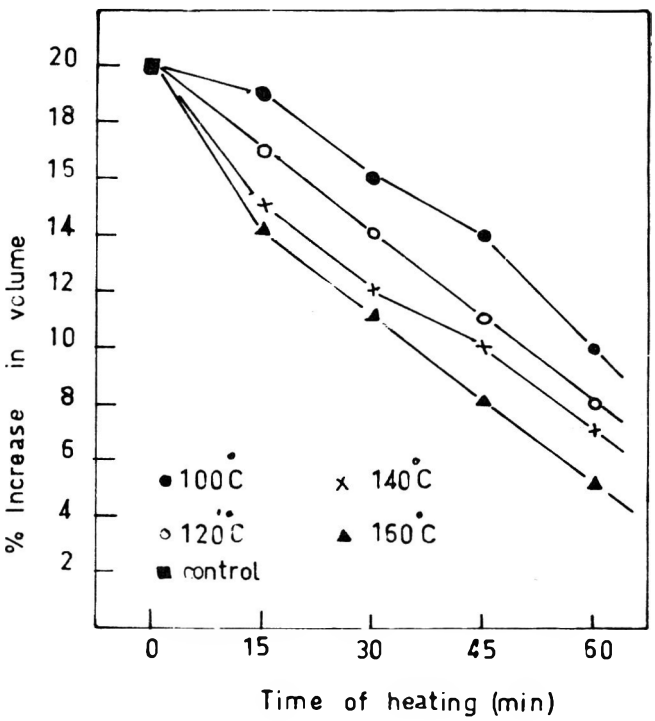


Fig. 5 Effect of dry heating at different times and temperatures on the foaming capacity of peanut flour.

amino acid side chains bind the paraffin chains of fats. Therefore, the increase in fat absorption of heated peanut could be due to both the dissociation and denaturation of the proteins by heat which may unmask the nonpolar residues of the protein molecules.

Emulsification capacity of peanut flour as affected by dry heat treatment is shown in Fig. 4. The EC of unheated sample was 56.5 ml oil per g flour; however, it decreased as the temperature or the time of heating increased. The EC was decreased to 35 ml oil per g flour after being heated at 160°C for 60 min. The lowering effect of heat processing on the EC of winged bean flour was also reported[13]. A decrease in protein solubility due to heat treatment was also observed[3].

Dry heat processing reduced the foam capacity of peanut flour (Fig. 5). The decrease was higher at high temperature. Also increasing the time of heating lowered the foam stability at all the temperatures used. The maximum decrease was at 160°C for 60 min (Table, 1). The same findings were reported for winged and soy bean flours respectively[13,16].

It has been suggested that foaming properties are related to protein denaturation. Native proteins have higher foaming capacity and stability than denatured protein[16]. The observed decrease in foaming properties of peanut flour due to dry heating appears to be due to the decrease in protein solubility caused by heat denaturation of protein.

B) *Effect of autoclaving:* Moist heat or autoclaving

TABLE 1. EFFECT OF DRY HEATING AT DIFFERENT TEMPERTURES AND TIMES OF PEANUT ON THE FOAMING STABILITY OF THE FLOUR.

Heating period (min)	Foaming stability (ml) at indicated temp.			
	100°C	120°C	140°C	160°C
Unheated	10	10	10	10
15	9	7	6	5
30	7	6	4	3
45	5	4	3	2
60	4	3	2	1

of peanut flour showed the same increasing trend in water absorption capacity of the flour (Table, 2). The rate of increase was very high as compared to the effect of dry heat (Fig. 2). This was expected since pressure may also play a role in gelatinization of the starch, swelling of the crude fiber and dissociation rate of the protein. Fat absorption capacity of autoclaved peanut showed an increasing trend similar to that observed for water absorption capacity. The ability of flour to bind and retain oil increased with the time of autoclaving at 121°C (15 lb/in^2). It reached a value of 340 ml oil/100 g flour after moist heating for 30 min (Table 2).

Autoclaving of peanut had a more marked effect in decreasing the EC from 56.5 ml oil to 18 ml oil per g than dry heating (Table, 2). Similar decrease in the EC of winged bean flour due to autoclaving was reported[13]. McWatters and Holmes[17] have observed that soy and peanut flours are sensitive to moist heat and heating time was the primary determinant in the reduction of nitrogen solubility and emulsification capacity. Our results agree well with their findings.

Wet or moist heat treatment of peanut also decreased the foaming properties of peanut flour (Table, 2). Autoclaving had a marked effect in lowering the foaming properties of peanut flour than dry heat. Foam stability also decreased as the time of autoclaving increased. This decrease is mainly due to denaturation of the protein which became less soluble.

C) *Effect of irradiation:* Peanut was irridiated for different doses and its effect on the functional properties of the flour is given in Table 3. Both water and fat absorption capacities were increased as the radiation dose increased. However, the increase in the fat absorption capacity was higher than for water. Dissociation and denaturation of the protein usually cause an increase in fat and water absorption when compared to the native protein.

Emulsification capacity of the irradiated samples was lower than that of the raw flour (Table, 3). This could be due to protein denaturation as a result of exposing to radiation which reduced nitrogen solubility. The maximum decrease was observed in 800

TABLE 2. EFFECT OF AUTOCLAVING OF PEANUT AT 121°C FOR DIFFERENT TIMES ON THE FUNCTIONAL PROPERTIES OF THE FLOUR.

Heating period (min)	WAC (g H_2O/100 g flour)	OAC (ml Oil/100 g flour)	EC (ml Oil/g flour)	FC (% vol. increase)	FS (ml)
Unheated	110.0	250.0	56.5	20.0	10.0
5	116.9	275.3	45.0	17.0	4.0
10	128.3	290.4	36.8	13.0	3.0
15	139.5	300.1	30.5	9.0	2.0
20	150.6	310.7	23.3	7.0	1.0
25	158.4	325.3	20.2	5.0	1.0
30	163.6	340.8	18.4	5.0	1.0

TABLE 3. EFFECT OF DIFFERENT DOSES OF IRRADIATION OF PEANUT ON THE FUNCTIONAL PROPERTIES OF THE FLOUR.

Dose (Krad)	WAC (g H_2O/100 g flour)	OAC (ml Oil/100 g flour)	EC (ml Oil/g flour)	FC (% vol. increase)	FS (ml)
Untreated	110.0	250	56.5	20.0	10.0
50	104.0	280	50.4	16.0	8.0
200	105.0	285	46.5	14.0	7.0
350	109.0	290	46.0	11.0	5.0
500	115.0	294	45.1	7.0	3.0
650	120.0	300	44.3	6.0	2.0
800	127.0	315	41.2	6.0	1.0

Krad treated sample. Mori *et al.*, [18] and Yamagishi *et al.*,[19] have reported that microwave heating caused irreversible disruption of the quaternary structure of soybean globulin.

Radiation treatment lowered both the foam capacity and stability of peanut flour. The decrease was considerable at the higher doses. The reduction rate in foam capacity reached its maximum (75 per cent) at 650 Krad (Table 3). Foam stability also showed the same trend and decreased with the increase of radiation rate.

Acknowledgement

This research project was carried out under Grant No. 851027 by the FRCU.

References

1. Wolf, W.J., In *Food Proteins*, by Whitaker, J.R. and Tannebaum, S.R., (Eds) AVI Publishing Co. Inc. Westport, Connecticut, 1977, 291.
2. Lawhon, J.T. and Cater, C.M., Effect of processing method and pH of precipitation on the yields and functional properties of protein isolates from glandless cottonseed. *J. Fd Sci.*, 1971, **36**, 372.
3. McWatters, K.H. and Cherry, J.P., Emulsification foaming and protein solubility properties of defatted soybean, peanut, field pea and pecan flours, *J. Fd Sci.*, 1977, **42**, 1444.

4. Rahma, E.H., UNU report World Hunger Programme. Central Food Technological Research Institute, Mysore, India, 1983.

5. Ramanatham, G., Ran, L.H. and Urs, L.H. Emulsification properties of groundnut protein, *J. Fd. Sci.*, 1978, **43,** 1270.

6. Beuchat, L.R., Functional and electrophoretic characteristics of succinylated peanut flour, *J. Agric. Fd Chem.*, 1977, **25,** 258.

7. Mahaboob Basha, S.M. and Cherry, J.P., Composition, solubility gel electrophoretic properties of proteins isolated from florunner (*Arachis hypogaea* L.) peanut seeds, *J. Agric. Fd Chem.*, 1976, **24,** 359.

8. Perkins, D. and Toledo, R.T., Effect of heat treatment for trypsin inhibitor inactivation on physical and functional properties of peanut protein, *J. Fd Sci.*, 1982, **47,** 917.

9. Beuchat, L.R., Cherry, J.P. and Quinn, M.R., Physico-chemical properties of peanut flour as affected by proteolysis,/ *J. Agric. Fd Chem.*, 1975, **23,** 616.

10. Sosulski, F.W., The centrifuge method for determining flour absorption in hard red spring wheats, *Cereal Chem.*, 1962, **39,** 344.

11. Sosulski, F.W., Humbert, E.S., Bui, K. and Jonnes, J.D., Functional properties of rapeseed flours, concentrates and isolate, *J.Fd. Sci., 1976, 41, 1349.*

12. Wu, Y.V. and Inglett, J.F., *Denaturation of plant proteins related to functionality and food applications. A review. J.Fd. Sci.*, 1974, **39,** 218.

13. Narayana, K. and Narasinga Rao, M.S., Functional properties of raw and heat processed winged bean (*Psophocarpus tetragonolobus*) flour, *J.Fd Sci.*, 1982, **47,** 1534.

14. Catsimopoolas, N., Funk, S.K. and Meyer, E.W., Thermal aggregation of glycinin subunits. *Cereal Chem.*, 1970, **47,** 331.

15. Kinsella, J.E., Functional properties of proteins in foods: A survey, *Crit. Rev. Fd Sci. Nutr.*, 1976, **7,** 219.

16. Yasumatsu, K., Sawada, K., Moritaka, S., Misaki, M., Toda, J., Wada, T. and Ishii, K., Whipping and emulsifying properties of soybean products, *Agric. biol. Chem.*, 1972, **36,** 719.

17. McWatters, K.H. and Holmes, M.R., Influence of moist heat on solubility and emulsification properties of soy and peanut flours, *J.Fd Sci.*, 1979, **44,** 774.

18. Mori, T., Nakamura, T. and Utsumi, S., Gelation mechanism of soybean 11s globulin: Formation of soluble aggregates as transient intermediates, *J.Fd Sci.*, 1982, **47,** 26.

19. Yamagishi, T., Tamarchi, F. and Shibasaki, K., Isolation and partial characterization of heat-denatured products of soybean 11s globulin and their analysis by electrophoresis, *Agric. biol. Chem.*, 1980, **44,** 1575.

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 16–19

Foaming Properties of Sunflower Seed Protein

M. KABIRULLAH
Institute of Food Science and Technology, BCSIR, Dhanmondi, Dhaka-5, Bangladesh.
and
R.B.H. WILLS
School of Food Technology, University of New South Wales, Kensington, NSW-2033, Australia

Received 9 *January* 1987; *revised* 25 *May* 1987

Foaming properties of sunflower seed and sunflower seed protein isolate (SPI) were studied under different conditions. The foaming properties of SPI was similar to that of soy protein isolate. But sunflower seed flour showed lower foaming properties than that of SPI perhaps due to lower amount of protein and the presence of carbohydrate and chlorogenic acid. With the increase of pH and protein concentration, the foaming properties increased. Stirring for longer time increased foam stability and increased stirring speed increased foam expansion. Sodium chloride increased foaming properties perhaps by increasing protein solubility. Sugar decreased the foaming properties of SPI significantly.

There is a growing interest in the use of oilseed meal protein for human consumption. The interest grew out of the need to overcome a perceived protein shortage in the developing countries; the increasing cost of animal protein compared to plant protein and increased knowledge of the nutritional and functional qualities of oilseed meal proteins associated with improved processing technology.[1]. Of the plant proteins, most work has been done on soybeans[2–4] and peanut protein[5]. Though sunflower seed is the second largest source of vegetable oil and an important economic crop in a number of countries[6], its application for human consumption on a commercial basis has been relatively unexplored.[7]. Sunflower oilseed meal is currently used as animal feed and fertilizer. However, the meal has some unique nutritional and functional qualities which could lead to some application for human use[7–11]. Foaming properties of sunflower seed flour,[12,13] diffusion extracted[14] and acid-alcohol-treated sunflower seed protein concentrates[15] were studied under various conditions. But no work was done on untreated sunflower seed protein isolate. In this paper, we report on the foaming properties of untreated sunflower seed protein products.

Materials and Methods

Sunflower seeds (*Helianthus annuus* 'Commander') were dehulled, defatted using petroleum ether (40-60°C) and ground to pass through a 60 mesh screen. Protein was isolated from this flour (SF) by the method of Gheyasuddin *et al.*[16] The sunflower seed protein isolate (SPI) was then freeze-dried. The products were analysed for proximate composition by the methods of AOAC.[17].

The foaming properties were determined according to the procedure described by Puski[18] with foam expansion (FE) and foam stability (FS) taken as indices of foaming properties as proposed by Yasumatsu *et al*[19] A 1% (w/v) protein suspension (50 ml) was adjusted to the required pH and stirred in a 250 ml cup in a Sorvall Omni Mixer for the desired time. The foam was transferred to a 250 ml cylinder to determine the foam volume which is defined as foam expansion. After standing for 30 min, the residual foam volume was recorded as foam stability. The effect of pH, protein concentration, stirring speed, stirring time and additives on foaming properties were studied. Soy protein isolate (Promine-D) was used as standard protein for comparison.

Results and Discussion

Table 1 shows the proximate composition of sunflower seed flour (SF), sunflower seed protein isolate (SPI) and soy protein isolate (Promine-D). Fig. 1 shows the effect of pH on the foaming properties. The foam expansion of SF was- not appreciably affected by the change of pH and the foam stability decreased with increasing pH. SF showed lower foaming properties compared to SPI perhaps due to lower amount of protein and the presence of carbohydrate and chlorogenic acid which has a depressing action on foaming properties.[1,20] Foaming properties of SPI and soy protein isolate at pH $\geq$7 were similar. With the increase in pH, the foaming

TABLE 1. PROXIMATE COMPOSITION (G/100 G DRY WT) OF SUNFLOWER SEED FLOUR AND PROTEIN ISOLATE

Component	Sunflower flour	Protein isolate	Soy protein isolate
Moisture	9.5	5.2	4.0
Fat	0.3	0.0	0.0
Protein (N×5.75)	55.3	88.2	90.2
Fibre	3.5	0.1	0.1
Chlorogenic acid	3.8	1.8	0.0
Ash	6.2	3.9	4.2
Nitrogen solubility index (NSI)	60.5	71.9	70.1

Values are means of five determinations
Soy protein isolate – Promine-D.

properties of SPI increased probably due to the increase in protein solubility at higher pH.[1,19,21] Foaming properties of all the samples increased with an increase in protein concentration (Fig. 2). The effect may be explained similar to that of pH. With the increase in protein in solution, more protein becomes available to form and to stabilize the foam. However, the rate of increase with concentration of protein was not linear over the whole range examined. At lower concentration of protein, foaming properties of all the samples were more affected by a change in concentration excepting the foam stability of SF which was relatively insensitive at low concentration. Soy protein concentrate showed a similar increase of foam expansion upto 3 per cent protein concentration and further increase in protein concentration resulted in a decrease in foam expansion.[21] Canella[15] reported

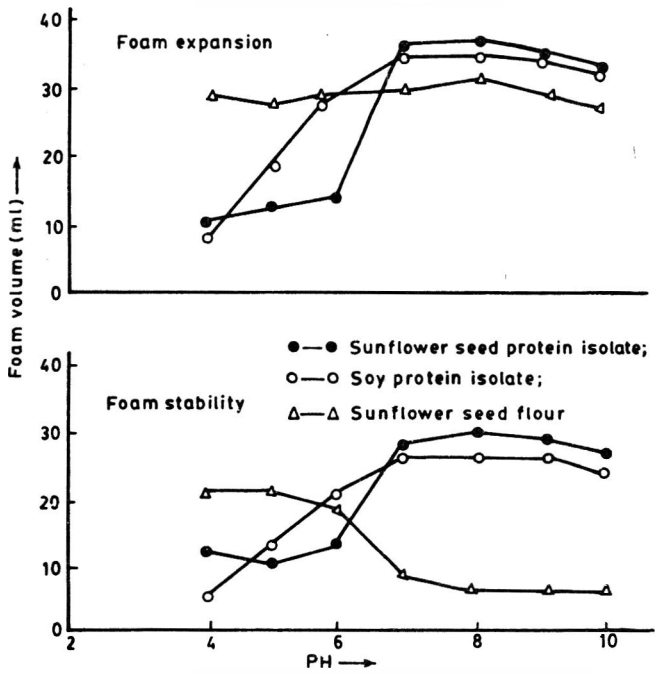


Fig. 1. Foaming of sunflower proteins as affected by change of pH. (50 ml of 1% protein, stirred at 10,000 rpm for 1 min)

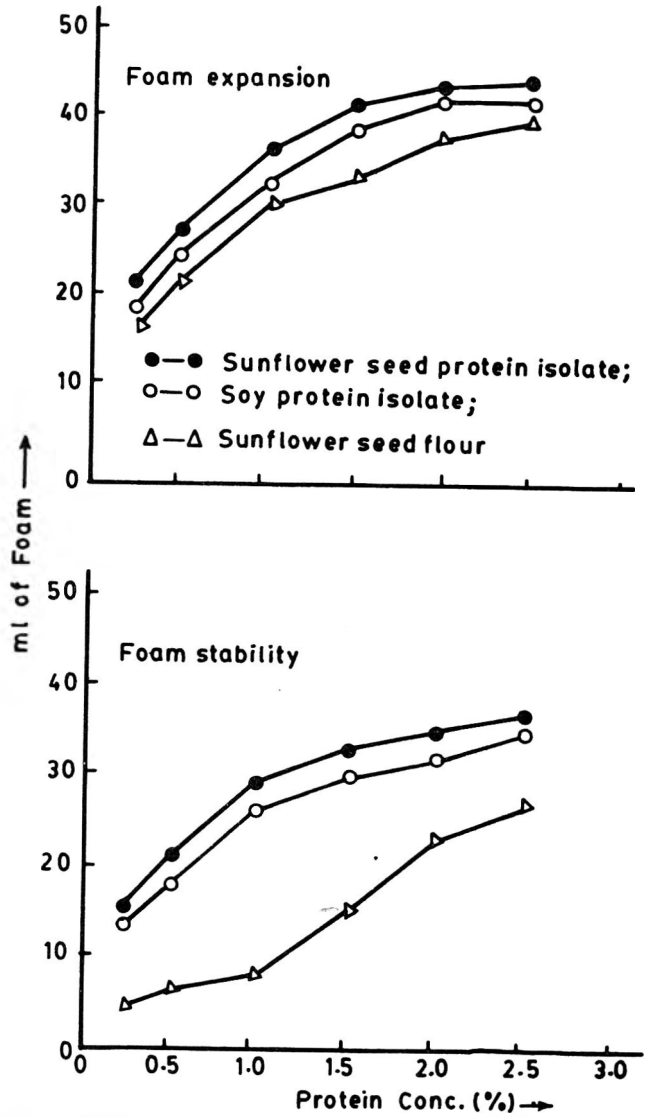


Fig. 2. Effect of protein concentration on the foaming properties. (50 ml of protein suspension at pH 7.0, stirred at 10,000 rpm for 1 min).

maximum foam expansion of acid-butanol-treated SF and SPI and soy protein isolate at 1 per cent level. On the other hand, Huffman *et al*[12] observed an increase in foam expansion up to 8 per cent concentration of SF at pH 9.0. From the above observations, it appears that foaming properties may increase with the increase in protein concentration up to a certain level, presumably due to the saturation point of protein solubility under the experimental conditions.

The effect of stirring time and stirring speed has been shown in Fig. 3 and Fig. 4 respectively. The effect may be explained in terms of shear force applied on the system. But both factors produced different effects. Increased stirring time did not appreciably effect foam expansion but increased foam stability with SF showing the highest effect. Increased stirring

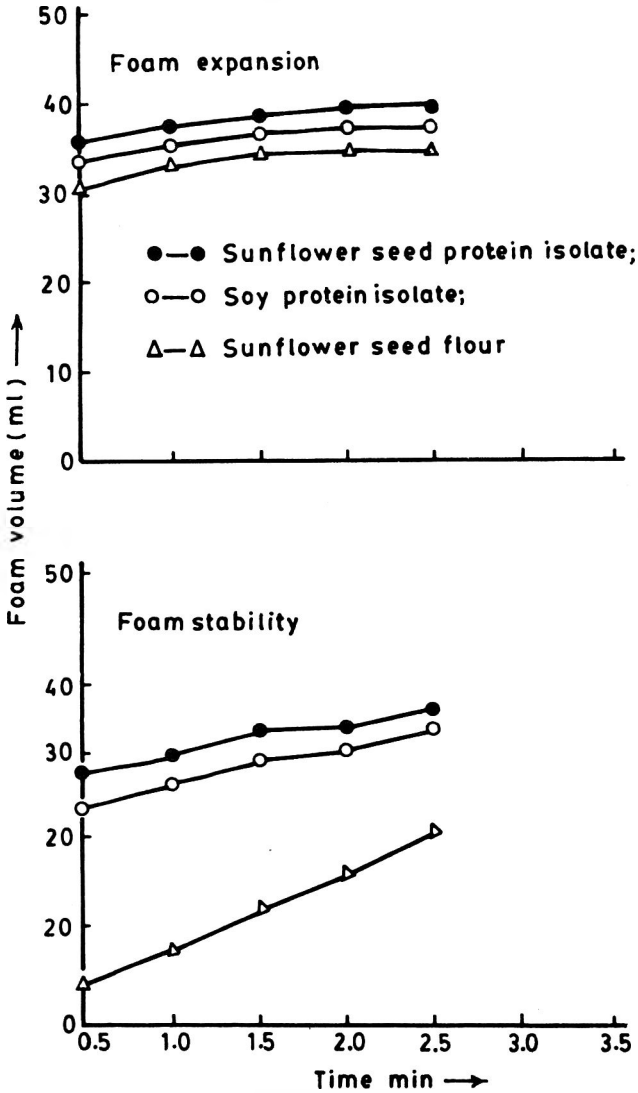


Fig. 3. Effect of time of stirring on the foaming properties of sunflower proteins. (50 ml of 1% protein suspension, pH 7.0, stirred at 10,000 rpm)

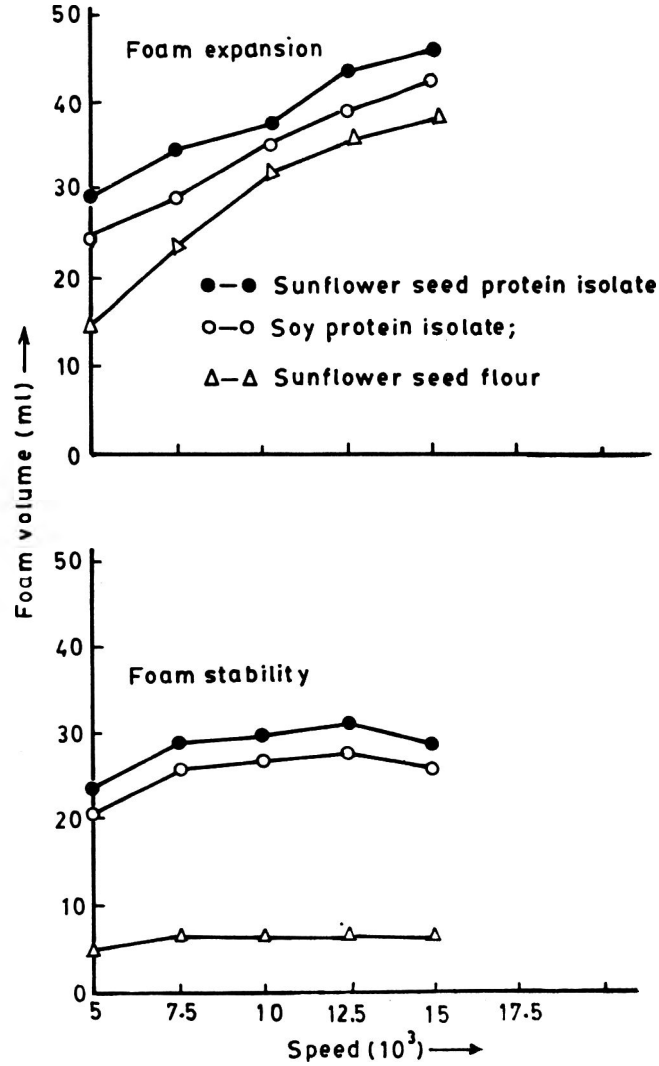


Fig. 4. Effect of stirring speed on the foaming properties of sunflower proteins. (50 ml of 1% protein suspension, pH 7.0 stirred for 1 min.)

speed increased foam expansion but only slightly increased foam stability. Similar variable effects were also reported for soy protein,[21], acid-butanol-treated SF[15] and sunflower seed flour.[12] With the increase in shear force applied, the foam gradually becomes smaller and increases the surface area of the foam requiring more protein to cover it. If the protein is not limiting in the system, the foaming properties should increase[1]. The difference in the effects may be due to the different nature of the applied force, one being a constant force applied for a longer time resulting in increased foam stability and the other being a higher force applied for a constant time resulting in increase foam expansion.

The effect of the additives on the foaming properties was variable (Table 2). NaCl increased foaming properties perhaps by increasing the protein solubility.[16]. The effect was more on SF than on SPI or soy protein isolate. A similar effect was also observed by Canella[15] and Huffman *et al.*[12] with various sunflower products. Rahma and Rao[20] reported that a 2 per cent NaCl treated SF possessed

TABLE 2. EFFECT OF ADDITIVES ON THE FOAMING PROPERTIES OF SUNFLOWER SEED FLOUR (SF) AND SUNFLOWER PROTEIN ISOLATE (SPI)

Protein	No additive		NaCl		Sugar	
	FE (ml)	FS	FE (ml)	FS	FE (ml)	FS
SF	31	8	86	26	24	12
SPI	36	29	75	40	11	9
Soy protein isolate	35	28	75	32	22	16

50 ml of 1% protein dispersion at pH 7.0, stirred for 1 min at 10,000 rpm. (FE, Foam expansion, FS- Foam stability. Soy protein isolate – Promine-D.

lower foaming properties although NSI was higher. However, Wang and Kinsella[22] reported little effect on foaming properties of leaf protein. Eldridge *et al*[21] showed a decrease in foam stability of soy protein whereas Franzen and Kinsella[23] showed increased foaming properties of soy protein in the presence of NaCl. Similar variable results were also observed with the addition of sugar[21,13] but in most cases the effect was a decrease in the foam volume[1], but foam stabilities were not affected significantly.

References

1. Kinsella, J.E., Functional properties of proteins in food – A survey *Crit. Rev. Fd. Sci. Nutr.*, 1976, **7,** 219.
2. Bird, K., Plant proteins progress and problems. *Fd. Technol*, 1974, **29,** 31.
3. Kinsella, J.E., Functional properties of soy proteins. *J. Am. Oil Chem. Soc.*, 1979, **56,** 242.
4. Wolf, W.J., Soybean protein: Their production, properties and food uses. A selected bibliography. *Proc. Int. Soya Protein Fd. Conf.*, Singapore, 1978, 121.
5. Natarajan, K.R., Peanut protein ingredients: Preparation, properties and food uses.. *Adv. Food Res.*, 1980, **26,** 215.
6. *Production Year Book*, FAO of the UN, Rome, 1977.
7. Sosulski, F.W., Food uses of sunflower proteins. *J. Am. Oil Chem. Soc.*, 1979, **56,** 438.
8. Sosulski, F.W. and Fleming, S.E., Chemical, functional and nutritional properties of sunflower protein products. *J. Am. Oil Chem. Soc.*, 1977, **54,** 100.
9. Burns, E.E., Talley, L.J. and Brummett, B.J., Sunflower utilization in human food. *Cereal Sci. Today*, 1972, **17,** 288.
10. Wills, R.B.H., Kabirullah, M. and Heyhoe, T.C., Enrichment of biscuits with sunflower protein. *Lebensm-Wiss. –Tech.* 1984, **17,** 205.
11. Wills, R.B.H. and Kabirullah, M., Use of sunflower protein in sausages. *J. Fd. Sci.*, 1981, **46,** 1657.
12. Huffman, V.L., Lee, C.K. and Burns, E.E., Selected functional properties of sunflower meal. *J.Fd. Sci.*, 1975, **40,** 70.
13. Lawhon, J.T., Cater, C.M. and Mattil, K.F., A comparative study of the whipping potential of an extract from several oilseed flours. *Cereal Sci. Today*, 1972, **17,** 240.
14. Lin, M.J.Y., Humbert, E.S. and Sosulski, F.W., Certain functional properties of sunflower meal products. *J. Fd. Sci.*, 1974, **39,** 368.
15. Canella, M., Whipping properties of sunflower protein dispersion. *Lebensm-Wiss. –Technol.*, 1978, **11,** 259.
16. Gheyasuddin, S., Cater, C.M. and Mattil, K. F., Effect of several variables on the extractibility of sunflower seed protein. *J. Fd. Sci.*, 1970, **35,** 453.
17. *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, D.C., 1980, 13th Edn.
18. Puski, G., Modification of functional properties of soy protein by proteolytic enzyme treatment. *Cereal Chem.*, 1975, **52,** 655.
19. Yasumatsu, K., Sawada, K., Moritaka, S., Missaka, M., Toda, J., Wada, T. and Ishi, K., Whipping and emulsifying properties of soybean products. *Agric. biol. Chem.*, 1972, **36,** 719.
20. Rahma, E.H. and Rao, N.M.S., Removal of polyphenols from sunflower meal by various solvents: Effect on functional properties. *J. Fol. Sci.*, 1981, **46,** 1521.
21. Eldridge, A.C., Hall, P.K. and Wolf, W.J., Stable foam from unhydrolysed soybean protein. *Fd. Technol.*, 1963, **17,** 1592.
22. Wang, J.C. and Kinsella, J.E., Functional properties of alfalfa leaf proteins: Foaming. *J. Fd. Sci.*, 1976, **41,** 498.
23. Franzen, K.K. and Kinsella, J.E., Functional properties of succinylated and acetylated soy protein. *J.agric. Fd. Chem.*, 1976, **24,** 788.

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 20–22

Dehydration Characteristics of Winter Vegetables

B.L. MANDHYAN, C.M. ABROAL AND H.R. TYAGI
College of Agricultural Engineering, J.N. Krishi Vishwa Vidyalaya, Jabalpur-482 004, India

Received 6 *August* 1986; *revised* 15 *July* 1987

Winter vegetables like peas, spinach, carrot and cabbage were dehydrated in the sun and in the solar cabinet dryer and drying constants were calculated. It was observed that the rate of moisture depletion in all the vegetables was high in the beginning and declined later. The moisture depletion with time indicated a straight line function. Reduction in the drying time was observed to be 15 to 20 per cent when solar cabinet dryer was used in place of direct sun-drying.

Vegetables not only decorate the dining table but also enrich the health of human beings. They constitute the nutritive menu of man and tone up his energy and vigour.[1] Traditional market places with their long established methods of buying and selling vegetables are faced with new problems which are likely to produce many changes in the general pattern of marketing. Vegetables are highly seasonal and are usually available in plenty at a particular part of the year. In the peak season, the selling price becomes too low leading to heavy losses to the grower. Also, due to the abundant supply during the season, there is a glut in the market resulting in the spoilage of large quantities. Preservation of these vegetables can prevent the huge wastage and make them available in the off season at remunerative prices.

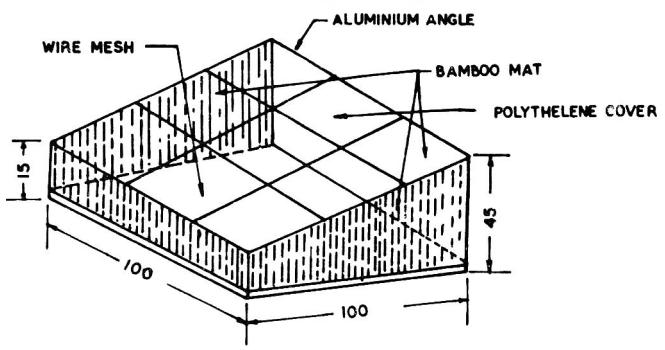
Sun-drying is the oldest and easiest method of preservation based on reducing the moisture content of the vegetables to a very low level. The concentration of soluble solids becomes so high in the product that the product becomes relatively chemically stable. It is no longer a suitable substrate for the growth of moulds, yeasts and bacteria, thus preventing spoilage during storage.[1] Even today the process is preferable to canning because it lowers the cost of packaging, storing and transportation by reducing both the weight and volume of the final product[2], whereas canning of vegetables is very expensive due to the prohibitive cost of cans and chemicals used for steeping.[3]

Adoption of the standard vegetable dehydration process in the industry is out of reach for the small enterpreneurs due to the huge investment. Moreover, the information on drying constants and moisture content ratios is lacking in spite of extensive work done on the design of solar and mechanical dryers. The present study on dehydration characteristics of vegetables was, therefore, taken up with a view to test the feasibility of a simple solar cabinet dryer for drying and arrive at optimum conditions.

Materials and Methods

Four vegetables viz. peas, carrot, spinach and cabbage were selected for the study. Fresh peas were procured from the local market and peeled. Carrots were scraped and sliced into 3 mm thick slices. Spinach was used as whole leaves; only the thick twig attached to the bottom of the leaf was removed. Cabbage was also sliced into pieces of 1.5 to 2 cm overall dimension. Each vegetable was then divided into ten equal parts. Five parts were exposed to the sun on a polythylene sheet whereas the other five parts were kept in five solar cabinet dryers designed and fabricated at the Harvest and Post Harvest Technology Centre, College of Agricultural Engineering, J.N. Krishi Vishwa Vidyalaya, Jabalpur. The solar cabinet dryer is made of M.S. angle iron frame with perforated sheet metal having a floor area 1m × 1m. The height of solar cabinet dryer at one end is 45 cm and at other it is 30 cm (Fig. 1).

The peas and carrot samples (500 g each) as also the



All Dimensions are in mm
Scale 1:20

Fig. 1. Solar cabinet dryer

cabbage and spinach samples (250 g each) were spread in single layers for sun-drying as well as in the solar cabinet dryer. Hence the principle of single layer drying held good. There was a little natural air draft, due to perforated bottom of cabinet dryer. Relative humidity, temperature and moisture content were noted at intervals of one hr for carrot and peas and at intervals of $\frac{1}{4}$ to $\frac{1}{2}$ hr for cabbage and spinach. The average of five readings of temperature and moisture content taken from five samples of each vegetable at the indicated intervals was then calculated. The average value of moisture contents (db) were plotted on semi-log graph paper, with time along abscissa. The drying constant and the moisture ratio were calculated by the formula

$$\frac{M - M_e}{M_o - M_e} = e^{-Ko}$$

where M = moisture content of the sample at any time, M_o = initial moisture content of sample, M_e = equilibrium moisture content, o = time in hours, K = drying constant, hr.

The values of equilibrium moisture content of the vegetables were taken from literature[4] Peas, carrot and cabbage were blanched as follows:[5]

Peas were kept in boiling water solution, containing 0.5 per cent potassium meta-bi-sulphate, 0.1 per cent magnesium oxide, 0.1 per cent sodium-bi-carbonate for four minutes.

Carrot and cabbage were kept in boiling water for three minutes[6].

Results and Discussion

Drying Characteristics: Fig 2, 3, 4 and 5 show the drying curves of green peas, cabbage, carrot and spinach respectively. When plotted on semilog paper, near straight line relationships were observed between drying time and moisture content. Depending upon their initial moisture content and structure these vegetables responded to drying differently. Although the initial moisture content for carrot, cabbage and spinach was very high, i.e. 614, 1200 and 977 per cent (db) respectively, their drying ratio was found to be very high possibly due to the thin slices of carrot (3.00 mm) and paper like structure of spinach and cabbage. The drying ratio increased when the vegetables were dried in the solar cabinet dryer. It has been found that three hours can be saved if peas and cabbage are dried in the solar cabinet dryer instead of sun-drying. Sun-drying took additional one hour for carrot and 2.5 hours for spinach as compared with drying time taken in the solar cabinet dryer. Observations show that the first hour of sun-drying reduced the initial moisture content drastically by 451, 673 and 556 per cent for

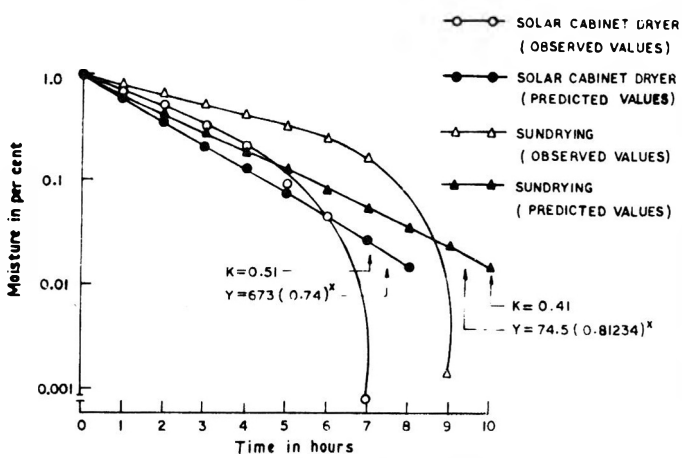


Fig. 2. Moisture depletion pattern in peas

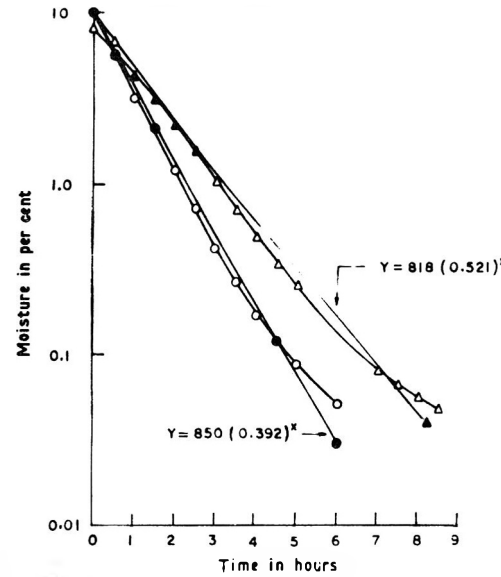


Fig. 3. Moisture depletion pattern in spinach

Legend as in Fig. 2.

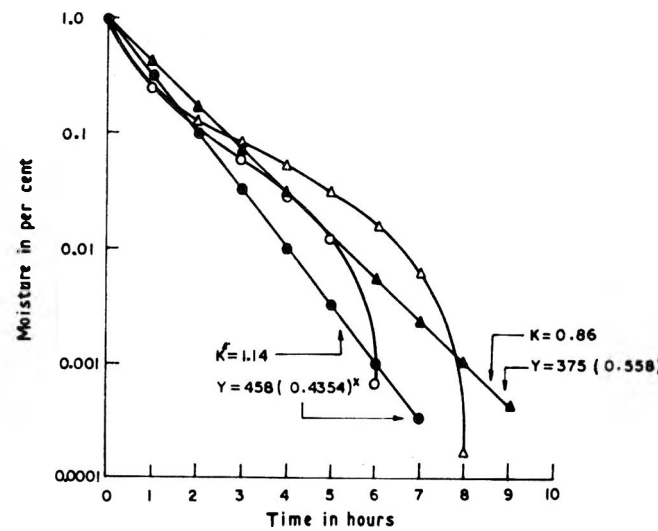


Fig. 4. Moisture depletion pattern in carrot

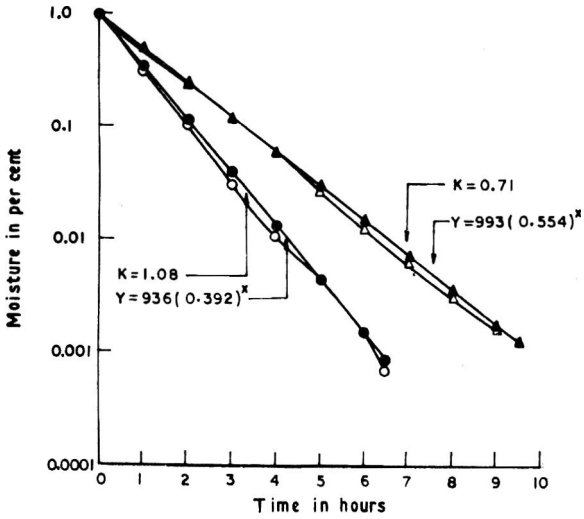


Fig. 5. Moisture depletion pattern in cabbage

Legend as in Fig. 2.

carrot, cabbage and spinach respectively. The solar cabinet dryer was effective in reducing the initial moisture content by 461, 838 and 658 per cent for carrot, cabbage and spinach respectively during the first hour of drying. Very little reduction in moisture content was observed during the first hour of drying for peas, i.e. from 68.64 to 59.38 per cent (db) for solar cabinet drying. Peas behaved differently from the other three vegetables because of low initial moisture content and the compact structure of pea cotyledon along with covering skin, which restrict moisture diffusion. Emperical equations were developed to predict the moisture content of peas, carrot, cabbage and spinach at any hour of drying under the sun or in the solar cabinet dryer. The values predicted by the equations were very close to the observed values. The equations are shown in Table 1.

Moisture ratio: The moisture ratio variation in the four vegetables follows a straight line relationship on semi-log graph. There is very little difference between observed values and predicted values as determined with the help of the equations.

TABLE 1. EMPERICAL EQUATIONS FOR PREDICTING MOISTURE CONTENT IN VEGETABLES

Vegetable	Sun-drying	Solar cabinet dryer
Peas	$Y = 74.5\ (0.81234)^X$	$Y = 673\ (0.74)^X$
Carrot	$Y = 375\ (0.558)^X$	$Y = 458\ (0.4354)^X$
Cabbage	$Y = 993\ (0.544)^X$	$Y = 936\ (0.392)^X$
Spinach	$Y = 818\ (0.521)^X$	$Y = 849\ (0.302)^X$

Y = moisture content (%, db)
X = drying time in hr

TABLE 2. MOISTURE REDUCTION AND DRYING TIME FOR VEGETABLES DRIED BY SUN-DRYING AND SOLAR CABINET DRYING

	Sun-drying		Solar cabinet drying	
Vegetable	Drying time (hr)	% moisture reduction	Drying time (hr)	% moisture reduction
Peas	11.0	88.06	8.0	87.82
Carrot	8.0	610.80	7.0	612.38
Cabbage	9.50	1,195.06	6.5	1116.20
Spinach	8.50	973.30	6.0	1300.00

Sun-drying: Drying temp 34–39°C and RH, 40–75%
Solar cabinet dryer: Drying temp. 56£61°C and RH. 35–60%

Total moisture reduction: The total moisture loss was observed to be 60.45 per cent in sun-drying and 60.28 per cent of the initial moisture of 68.64 per cent in solar cabinet dryer for peas. The time taken was 10 hr in sun-drying and 8 hr in the solar cabinet dryer (Table 2).

The total moisture removed from cabbage in 9.5 hr was 1195.06 per cent under sun-drying while it was 1116.2 per cent in the solar cabinet drying.

The total moisture reduction was observed to be 973.30 per cent in spinach by sun-drying and 300 percent by solar cabinet dryer. This moisture reduction was achieved in 8.5 and 6 hr respectively.

Acknowledgement

The authors are thankful to the Indian Council of Agricultural Research for financing the study through AICRP on Post Harvest Technology.

References

1. Chauhan, D.V.S., *Vegetable Production in India*, Ram Prasad and Sons, Agra, 1965, Vol 1, 69.
2. Luh, B.S. and Woodroof, J.G., *Commercial Vegetable Processing*. The AVI Publishing Company, 1975.
3. Tekchandani, C.K. and Jain, S.K., Pea dehydration technology- A home based industry, *Paper presented at the XXI Annual convention of ISAE*, Agra, 1965.
4. Marris, T.N., The dehydration of food, D.Van Nostrand Co. New York, 1947.
5. Sethi, V. and Anand, J.C., Preserving fresh peas with mild heat treatment, *Veget. Sci.* **10**, 1.
6. Tandon, G.L. and Virmani, R.S., Determination of blanching time of vegetables, *Indian J. Hort.*, 1949, 6(1).

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 23–27

Growth and Production of Thermostable Deoxyribonuclease by *Staphylococcus Aureus* in *Shrikhand*

M.C. VARADARAJ* AND B. RANGANATHAN
Department of Dairy Bacteriology
National Dairy Research Institute, Bangalore–560 030, India

Received 14 *November* 1986; *revised* 24 *July* 1987

The ability of *Staphylococcus aureus* strains to grow and produce thermostable deoxyribonuclease (TD Nase) in *Shrikhand* when used in combination with *Streptococcus lactis, Str. cremoris, Str. thermophilus* and *Str. lactis* subsp. *diacetylactis* was evaluated. Cultures of *S. aureus* when inoculated in milk at levels of 1×10^5 CFU/ml produced detectable levels of TD Nase. Growth and production of TD Nase by *S. aureus* was less in the presence of *Str. lactis*.

Indigenous milk products have been implicated in several staphylococcal food poisoning outbreaks. Often, milk used in preparing these products contain a high microbial load including enterotoxigenic strains of *Staphylococcus aureus*[1–4]. Cells of *S. aureus* grow and produce enterotoxins as well as thermostable deoxyribonuclease (TDNase). A high correlation between TDNase and enterotoxin production by *staphylococci* have been reported[5–6]

In a mixed microflora, *staphylococci* for the most part are inhibited by other competitive microflora, particularly by those of lactic acid bacteria[7–10]. However, strains of *S. aureus* inoculated together with normal or phage inhibited starter cultures were able to grow and produce enterotoxins in normal cheeses as well as in those with starter failures[11–14]

Shrikhand – a sweetened lactic fermented milk product is widely consumed in different parts of India. Considering the presence of lactic acid bacteria in *shrikhand*, and the use of milk in which *staphylococci* would be present, an attempt has been made in the present study to find out the ability of *S. aureus* to grow and produce TDNase in *shrikhand*. Production of TDNase has been considered in view of its close correlation with enterotoxin production.

Materials and Methods

Cultures: The cultures used were (i) two wild strains of *S. aureus* K-283 and K-192 producing enterotoxins A and E, respectively and two standard strains of *S. aureus* A_{100} and E_{326} elaborating enterotoxins A and E, respectively, and (ii) strains of *Streptococcus lactis* S_1R, *Str. cremoris* SC_1, *Str. thermophilus* (Wisconsin) and *Str. lactis* subsp. *diacetylactis* DRC_1 obtained from the stock culture collection of Dairy Bacteriology Division of this Institute. The two wild strains of *S. aureus* were isolated from samples of market khoa[15]

Preparation of Shrikhand: This involved production of curd by lactic fermentation of milk, followed by draining out of whey from the curd through a muslin cloth bag tied to a stand hung for 6-7 hr. The resulting curd (*Chakka*) was uniformly mixed with sugar and made into a semi-solid mass to which saffron and cardamom were added.

In *shrikhand* preparation, each test culture of *S. aureus* was used individually in combination with each of the *Streptococcus* species.

One litre lots of cows' milk were collected from the Institute's farm, steam sterilised for 25 min at 100°C and cooled to room temperature (25°C). The milk was inoculated simultaneously with the lactic test culture and *S. aureus* at a level of 1×10^5 CFU/ml of milk in the above mentioned combinations. The inoculated milk samples were uniformly mixed and incubated at 37°C for 18–20 hr. The curd obtained was transferred to a clean muslin cloth bag and hung for 6–7 hr to drain out the whey. The semi-solid matter (*chakka*) was then transferred to a sterile glass container and used as the base material for preparation of *Shrikhand*.

For 100 g of *chakka*, about 40 g of clean sugar was added and kneaded with the help of a clean spoon

*Present address: Discipline of Food Microbiology and Sanitation
Central Food Technological Research Institute
Mysore: 570013, INDIA

until it was completely dissolved. The final product, *Shrikhand* was stored in a clean glass container for a period of 24 hr at room temperature (20–30°C).

Enumeration of staphylococci and lactic acid bacteria: Samples of curd, fresh *chakka* and one day old *shrikhand* were examined for staphylococcal and lactic counts. *Staphylococci* were enumerated according to the method of Baird-Parker[16] by surface plating appropriate dilutions of the samples on egg yolk-tellurite-glycine-pyruvate agar (ETGPA). The petri plates were incubated for 24–48 hr at 37°C. Staphylococcal counts were expressed as CFU/ml or g. Lactic counts were determined by pour plate method using whey agar (Hi-media). The petri plates were incubated at 30 and 37°C for 24–36 hr depending upon the lactic culture used for the preparation of *Shrikhand*. The lactic counts were expressed as CFU/ml or g.

Determination of TDNase: Samples of curd, *chakka* and *shrikhand* in 10^{-1} dilutions (10 g in 100 ml) were centrifuged at 10,000 rpm for 20 min, followed by neutralisation of the supernatants to pH 7.0 using IN NaOH. The neutralised supernatants were steamed for 15 min at 100°C cooled to room temperature and filtered through Whatman No. 1 filter paper. The final supernatants were examined for the presence of TDNase using toluidine blue-deoxyribonucleic acid (TB-DNA) agar plate method of Lachica *et al*[17]. TDNase activity of the test cultures was expressed as zone diameter in millimeters.

For estimating TDNase in whey, samples of whey were neutralized to pH 7.0 using 1N NaOH. The neutralized samples were centrifuged at 10,000 rpm for 20 min. The resulting supernatants were used for determining the presence of TDNase as described above.

Results and Discussion

The results presented in Tables 1 and 2 show that among the four strains of *S. aureus*, two wild strains K-283 and K-192 and one standard strain E_{326} grew and produced TDNase better than the standard strain A_{100}. These test cultures of *S. aureus* grew well in milk in association with the lactic cultures as could be seen from the cell numbers of *staphylococci* (10^7-10^8 CFU/ml) reached in 20 hr of incubation at 37°C. A higher *S. aureus* cell population of 450×10^7 and 720×10^7 CFU/ml of curd were obtained in culture combinations involving (i) *S. aureus* and *Str. thermophilus* and (ii) *S. aureus* and *Str. lactis* subsp. *diacetylactis*, respectively. However, in the culture combinations of *S. aureus* and *Str. lactis* and *S. aureus* and *Str. cremoris*, the cell numbers of *S. aureus* (42×10^6 and 360×10^6 CFU/ml of curd) were comparatively less. At the same time, the lactic cultures also grew well in the curd attaining numbers ranging between 40×10^6 and 650×10^6 CFU/ml of curd, depending upon the *Streptococcus* species used in the combinations (Tables 1 and 2). As compared to the three strains of *S. aureus* K-283, K-192 and E_{326}, *S. aureus* A_{100} showed no appreciable increase in cell numbers in the curd from the initial number of 1×10^5 CFU/ml, except for a cell population of 18×10^6 CFU/ml when present in combination with *Str. lactis*

TABLE 1. GROWTH AND PRODUCTION OF TDNASE BY S. AUREUS IN SHRIKHAND PREPARED WITH STR. LACTIS AND STR. CREMORIS

	K-283			A_{100}			K-192			E_{326}		
	STPC[a]	LC[b]	TDNase[c]	STPC	LC	TD Nase	STPC	LC	TD Nase	STPC	LC	TDNase
						S. aureus/Str. lactis						
Curd	18	120	14	12	180	Nil	42	80	12	24	40	14
Whey	–	–	14	–	–	Nil	–	–	12	–	–	12
Chakka	12	80	Nil	4	120	Nil	36	80	Nil	22	20	Nil
Shrikhand	8	6	Nil	$\geq 10^2$	$\geq 10^2$	Nil	.2	.4	Nil	.40	.32	Nil
pH of whey		4.5			4.7			4.6			4.5	
						S. aureus/Str. cremoris						
Curd	360	320	18	20	320	Nil	170	220	16	164	150	12
Whey	–	–	16	–	–	Nil	1	1	16	–	–	12
Chakka	320	280	Nil	18	320	Nil	166	218	8	158	136	8
Shrikhand	60	56	Nil	.24	.22	Nil	4.6	7.6	Nil	.14	.22	Nil
pH of whey		4.9			5.2			5.0			4.9	

[a]Staphylococcal count expressed as 1×10^6 CFU/ ml/g
[b]Lactic count expressed as 1×10^6 CFU/ml/g
[c]TDNase expressed as zone diameters in mm, includes an initial 5 mm diameter of the agar well in TD-DNA agar plate.
An initial inoculum of 1×10^5 CFU/ml of milk of staphylococcal and lactic cultures was used.

TABLE 2. GROWTH AND PRODUCTION OF TDNASE BY S. AUREUS IN *SHRIKHAND* PREPARED WITH *STR. THERMOPHILUS* AND *STR. LACTIS* SUBSP. *DIACETYLACTIS*

	K–283			A_{100}			K–192			E_{326}		
	STPC[a]	LC[b]	TDNase[c]	STPC	LC	TD Nase	STPC	LC	TD Nase	STPC	LC	TDNase
						S. aureus/Str. thermophilus						
Curd	340	320	16	8.6	300	Nil	4500	280	20	148	320	14
Whey	–	–	16	–	–	Nil	–	–	18	–	–	14
Chakka	228	318	Nil	7.8	282	Nil	3000	272	12	142	280	Nil
Shrikhand	3.4	3.2	Nil	.22	.54	Nil	12	18	Nil	.32	.48	Nil
pH of whey		4.9			5.0			5.0				
						S. aureus/Str. lactis subsp. diacetylactis						
Curd	400	120	14	180	140	10	7200	650	20	5200	640	18
Whey	–	–	14	–	–	10	–	–	20	–	–	16
Chakka	1800	80	8	18	138	10	9200	140	16	4800	180	10
Shrikhand	2.2	4.2	8	.16	6.2	Nil	66	6.8	10	3.2	5.2	8
pH of whey		4.7			5.0							

[a]Staphylococcal count expressed as 1×10^6 CFU/ ml/g
[b]Lactic count expressed as 1×10^6 CFU/ml/g
[c]TDNase expressed as zone diameters in mm, includes an initial 5 mm diameter of the agar well in TB–DNA agar plate.
An initial inoculum of 1×10^5 CFU/ml of milk of staphylococcal and lactic cultures was used.

subsp. *diacetylactis*.

The levels of staphylococcal cell populations recorded in the present study should be considered significant, particularly when they are present in association with the lactic cultures. Earlier studies[7–10] have revealed, that for the most part, *staphylococci* are generally inhibited in a mixed microflora particularly by lactic acid bacteria. This ability of *S. aureus* to produce different levels of cell populations is dependent to a great extent on the type of *S. aureus* strain and *Streptococcus* species used in the preparation of *shrikhand*. A similar view[18] has been expressed in relation to the degree of inhibition of *S. aureus* growing in presence of a lactic culture as well as in relation to variations among different strains of *S. aureus* when grown in combination with lactic cultures during manufacture of cheddar and colby cheeses[12].

It has been observed, that in the studies[10,12,19] pertaining to cheese, a higher initial inoculum of *S. aureus* in cheese milk enabled them to grow well. In the present study also, the initial inoculum of 1×10^5 CFU/ml of *S. aureus* introduced in the milk for preparing *Shrikhand* resulted in good growth.

Thus, simultaneous introduction of both *staphylococci* and lactic culture in milk enabled the former to grow well before the medium was rendered acidic on account of the growth of the lactic acid bacteria, a condition not favourable for the growth of *staphylococci*. This point could be well seen from the different pH values obtained according to the culture combinations (Tables 1 and 2). The pH varied between 4.8 and 5.4 in the curd obtained from the combinations of *S. aureus* and *Str. cremoris*, *S. aureus* and *Str. lactis* subsp. *diacetylactis* and *S. aureus* and *Str. thermophilus* and between 4.5 and 4.7 in the combination of *S. aureus* and *Str. lactis*.

Cell numbers of both staphylococci and lactic cultures in *chakka* was almost the same as those recorded in curd. However, an increase in the numbers of *S.aureus* K–283 and K–192 was noticed in *chakka* prepared using *Str. lactis* subsp. *diacetylactis* (Table 2). In one day samples of *shrikhand* a decline in the numbers of *S.* aureus and lactic culture was observed. This decline may be due to the incorporation of 40 percent sugar.

A situation similar to that of staphylococcal growth was observed in relation to that of TDNase production. From the zone diameters formed (Tables 1 and 2), it can be seen that *S. aureus* strains K-283, K-192 and E_{326} produced TDNase better than *S. aureus* A_{100}. In the culture combinations of *S. aureus* and *Str. thermophilus*, S. aureus and *Str. lactis* subsp. *diacetylactis* and *S. aureus* and *Str. cremoris*, a higher TDNase activity (zone diameter 18-20 mm) was observed as against a zone diameter of 14 mm in the culture combination of *S. aureus* and *Str. lactis*. In the preparation of *chakka*, preformed TDNase was almost completely drained out from the curd into whey. Although *S. aureus* cells were present in *chakka* and *shrikhand*, TDNase was not detected in the same, except in the case of fresh *chakka* prepared from milk

inoculated with the culture combinations of *S. aureus* and *Str. thermophilus* and *S. aureus* and *Str. lactis* subsp. *diacetylactis*. On the other hand, *S. aureus* A_{100} failed to produce TDNase when present in association with the lactic cultures, except for a zone diameter of 10–14 mm when used in combination with *Str. lactis* subsp. *diacetylactis* (Table 2).

The test for TDNase production by *S. aureus* strains has been considered as an important indicator of enterotoxigenicity. All the four strains of *S. aureus* used in the present study were known to produce TDNase and enterotoxins[15]. Further, TDNase production is known to correlate well with enterotoxigenicity[5,6] and it acts as a rapid and a very reliable test in detecting enterotoxigenicity. As compared to enterotoxin production, TDNase has always been detected in products where the *S. aureus* counts have been less than those required for enterotoxin production[20,21] Studies[20,22] have shown that a cell population of *S. aureus* in the range of 1×10^5 to 1×10^7/g or ml was necessary to produce TDNase. Such levels have been achieved by *S. aureus* strains when present in association with *Streptococcus* species.

Among the four species of *Streptococcus* used in the culture combination, it was in the presence of *Str. lactis*, that growth and TDNase production by *S. aureus*, strains K-283, K-192 and E_{326} was comparatively less. On the other hand, growth and TDNase production by *S. aureus* A_{100} was not appreciable in the presence of all the four species of *Streptococcus*.

It is evident from the present study that use of an active lactic culture in the preparation of cultured milk products may not prove very effective in ensuring the safety of final products, if the milk used in the preparation contains a high initial load of enterotoxigenic *staphylococci*.

Acknowledgement

The senior author expresses his sincere thanks to the Indian Council of Medical Research for providing the financial assistance. The authors are thankful to Dr. M.S. Bergdoll, FRI, Wisconsin, USA, for providing the standard strains.

References

1. Krishna Mohan and Misra, S.K., *Staphylococcus aureus* in milk supplied to Patna milk supply scheme. *Indian J. Dairy Sci.*, 1967, **20,** 178
2. Gehlot, R.G. and Saraswat, D.S., Incidence of coagulase positive staphylococci in Indian market milk. *Aust. J. Dairy Technol.*, 1969, **24,** 120
3. Garg, D.N., Bhargava, D.N. and Narayan, K.G., Pathogenic bacterial flora of raw market milk. *Indian J. Dairy Sci.*, 1977, **30,** 36
4. Panduranga Rao, C.C., Enterotoxigenic staphylococci in milk. *Indian J. Microbiol.*, 1977, **17,** 105
5. Lachica, R.V.F., Weiss, K.F. and Deibel, R.H., Relationships among coagulase, enterotoxin and heat-stable deoxyribonuclease production by *Staphylococcus aureus*, *Appl. Microbiol.*, 1969, **18,** 126
6. Tatini, S.R., Soo, H.M., Cords, B.R. and Bennett, R.W., Heat-stable nuclease for assessment of staphylococcal growth and likely presence of enterotoxin in foods. *J. Fd. Sci.*, 1975. **40,** 352
7. Oberhofer, T.R. and Frazier, W.G., Competition of *Staphylococcus aureus* with other organisms. *J. Milk Fd. Technol.*, 1961, **24,** 172
8. Graves, R.R. and Frazier, W.C., Food microorganisms influencing the growth of *Staphylococcus aureus*, *Appl. Microbiol.*, 1963, **11,** 513
9. Iandolo, J.J., Clark, C.W., Bluhm, L. and Ordal, Z.J., Repression of *Staphylococcus aureus* in associative culture. *Appl. Microbiol.*, 1965, **13,** 646
10. Haines, W.C., Growth and production of enterotoxin by *Staphylococcus aureus* grown in association with selected lactic acid bacteria. *Diss. Abstr. Int. B.*, 1972, **33,** 2171
11. Tuckey, S.L., Stiles, M.E., Ordal, Z.J. and Witter, L.D., Relation of cheese making operations to survival and growth of *Staphylococcus aureus* in different varieties of cheese. *J. Dairy Sci.*, 1964, **47,** 604
12. Tatini, S.R., Jezeski, J.J., Morris, H.A., Olson J.C. Jr., and Casman, E.P., Production of staphylococcal enterotoxin A in Cheddar and Colby cheeses. *J. Dairy Sci.* 1971, **54,** 815
13. Lam, N.C.S.I., Growth and enterotoxin production of *Staphylococcus aureus* (isolated from heat treated milk in a ten-month survey) in the manufacture of Cheddar cheese made from heat-treated milk. *Diss. Abstr. Int. B.*, 1981, **42,** 551
14. Santos, E.C. dos, and Genigeorgis, C., Survival and growth of *Staphylococcus aureus* in commercially manufactured Brazilian Minas cheese. *J. Fd. Prot.*, 1981, **44,** 177
15. Varadaraj, M.C. and Nambudripad, V.K.N., Staphylococcal incidence in market *khoa* and their enterotoxins production. *J. Fd. Sci. Technol.*, 1982, **19,** 53
16. Baird-Parker, A.C., An improved diagnostic and selective medium for isolating coagulase positive staphylococci. *J. appl. Bacteriol.*, 1962, **25,** 12
17. Lachica, R.V.F., Genigeorgis, C. and Hoeprich, P.D., Metachromatic agar diffusion methods for detecting staphylococcal nuclease activity. *Appl. Microbiol.*, 1971, **21,** 585
18. Richardson, G.H. and Diviatia, M.A., Lactic culture inocula required to inhibit staphylococci in sterile milk. *J. Dairy Sci.*, 1973, **56,** 706
19. Stadhouders, J., Cordes, M.M. and Schouwenburg Van Foeken, A.W.J., The effect of manufacturing conditions on the development of staphylococci in cheese. Their inhibition by starter bacteria. *Neth. Milk Dairy J.*, 1978, **32,** 193.
20. Schouwenburg Van Foeken, A.W.J., Stadhouders, J. and

Jans, J.A. The thermonuclease test for assessment of the growth of coagulase positive staphylococci in Gouda cheese with a normal acidity development. *Neth. Milk Dairy J.*, 1978, **32,** 217

21. Ibrahim, G.F. and Baldock, A.K., Thermostable deoxyribonuclease content and enterotoxigenicity of Cheddar cheese made with sub-normal starter activity. *J. Fd Prot.*, 1981 **44,** 655

22. Tatini, S.R., Soo, H.M., Cords, B.R. and Bennett, R.W., Heat-stable nuclease for assessment of staphylococcal growth and likely presence of enterotoxins in foods. *J. Fd. Sci.*, 1975, **40,** 352

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 28–30

The Binding Strength of Fish Muscle Rolls

S.V. GHADI AND V. NINJOOR
Food Technology and Enzyme Engineering Division, Bhabha Atomic Research Centre, Bombay–400 085, India

Received 5 *January* 1987, *revised* 31 *March* 1987

Six varieties of low priced fish in the form of cooked fish muscle rolls were examined for their binding strength. The binding capacity was ascertained in terms of tensile and breaking strength, water holding capacity, cooking loss and sensory evaluation. These parameters were found to correlate closely with each other in determining the texture of the cooked minced muscle rolls. Addition of NaCl and sodium tripolyphosphate (NaTPP) improved the binding properties. Among the fish varieties examined, shark and cat fish muscle rolls yielded maximum binding strength.

Underutilized fish harvested along with commercial species are normally channelled to the factories producing fish manure or animal feeds. This practice deprives the malnourished population in dire need of quality proteins from the highly nutritious source of muscle proteins. One way of encountering this problem is to use such fishes in the production of products based on comminuted or minced muscle. Interest in comminuted fish products has been stimulated in recent years by the successful use of mechanical deboning equipment and techniques for fabricating minced fish muscle into commercially acceptable forms[1]. Minced fish flesh offers diverse scope for the development of new fishery products tailored to the requirements of a wide range of consumer interests as demonstrated by the spurt in new products devised in developed countries[2]. Binding of mechanically deboned muscle and the resulting texture have been the major concerns in formulating such fishery products. Blending of minced muscle of fish with various binders such as sugar, salt and tripolyphosphates (TPP) has often been used for improving the textural quality of comminuted minced muscle products[3].

This communication reports on the binding capacity and textural quality of fish muscle rolls prepared from six species of low cost Indian fishes and the influence of sodium chloride (NaCl) and sodium tripolyphosphate (NaTPP) on improving the binding strength of cooked muscle rolls.

Materials and Methods

Source of fish muscle: Six varieties of fish viz. shark (*Caracharhinus* spp.), ray (*Dasyatis* spp.), anchovies (*Thrissocles* spp.), croaker (*Johnius dissumieri*), ribbon (*Tricharus* spp.) and cat fish (*Tachysurus* spp.) were brought in iced condition from a landing site in Bombay. The fishes were beheaded,gutted, washed and cleaned. The minced muscle was collected after mechanically deboning the fish in a deboning machine[4].

Preparation of minced muscle rolls: Minced muscle (100g) of each fish was homogenized for 2 min in a Sorvall omni-mixer with minimum speed. When treatment with NaCl and NaTPP was required, they were added at 2.5 and 0.2 per cent concentrations respectively during homogenization. The proportion of additives was selected based on earlier reports[3]. Two batches of the muscle (control and treated) were weighed again and pressed into an aluminium pan of cylindrical shape to prepare rolls. Fig. 1 shows the shape of fish rolls and the aluminium pans (two sizes 20 × 10 and 20 × 5 cm) used in the preparation. The fish rolls packed in aluminium pans were steam cooked in a pressure cooker till the internal temperature was raised to 85°C. This temperature was maintained for 20 min. The muscle rolls prepared this way were removed from the pans and allowed to attain ambient temperature. They were assessed for cooking loss, binding strength (breaking strength and tensile strength), water holding capacity (WHC) and sensory quality.

Cooking loss: The weight of fish rolls before and after cooking was recorded in six samples of the same fish. The average difference in six sets of readings was used for calculating the per cent loss. The technique has earlier been used for poultry meat loaves[5].

Binding strength: An Instron universal texturometer was employed to measure breaking strength and tensile strength of the cooked rolls. The method of Pepper and Schmidt[6] was followed for the assessment of breaking strength in an apparatus which is

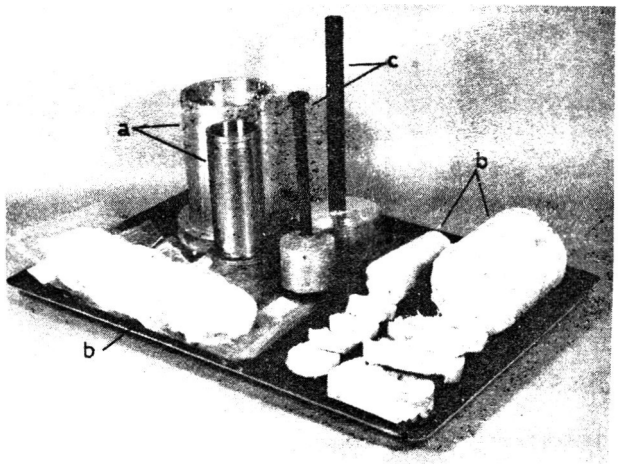


Fig. 1. Cooked fish rolls from the minced muscle of shark and the aluminium cylinders.

a. Cylindrical aluminium pans (20 x 10 cm and 20 x 5 cm)
b. Rolls and slices prepared from minced muscle.
c. Pressure piston.

composed of a base and a breaking bar assembly. Both the base and the fish roll were placed on the pressure sensitive platform of the Instron and zeroed. The breaking bar was bolted with the arm of the instrument. The circumference of the fish roll was measured at the point where the breaking bar would apply the pressure required to break the roll. The break bar was set to travel at a rate of 2 cm/min and the chart speed at a rate of 1 cm/min. The breaking strength was expressed as maximum pressure in grams per square centimetres of the cross sectional area.

For measuring the tensile strength, 8 mm thick slices were prepared in triplicate. One slice at a time was fitted in the jaw width of the pneumatic jaws and the pressure was set to pull the jaws till the point of breakage. The tensile force required to tear the sample apart when the upper jaw was raised at a constant speed of 2 cm/min was recorded[7]. The tensile strength was expressed as maximum force required in grams per square centimetres.

Water holding capacity (WHC): Ten grams of cooked muscle from the rolls in triplicate were placed in centrifuge tubes and spun at 28,000 × g for 30 min in a Sorvall RC-2 refrigerated centrifuge using SS-4 rotor. The released fluid was decanted out and the sediment collected carefully. The weight of the muscle after centrifugation was recorded. The difference in the weights calculated on per cent basis provided WHC[8]. Apparently, the index is based on the extent of fluid loss; lower the WHC index better is the product.

Organoleptic evaluation: Fish roll samples were assessed for textural quality by five experienced panelists, where the texture was rated according to Kudo *et al*[3] on the following 10-point scale: 9–10, excellent; 7–8, good; 5–6, standard; 3–4, substandard; 1–2 poor.

Results and Discussion

Myofibrillar proteins undergo denaturation following cooking, and the extent of denaturation is dependent upon the temperature at the end[5]. Extensive changes in the solubility of proteins, water holding capacity, alterations in the molecular architecture and the bonding (hydrogen and ionic) are reported to occur at the range of 40–65°C for red meats[9]. The binding of pieces or particles of muscle to form composite products is a heat mediated process.

Data on cooking loss, WHC and organoleptic gradings of fish muscle rolls are shown in Table 1. Addition of NaCl and NaTPP decreased cooking loss, increased WHC and promoted the sensory rating in fish rolls of all species. Rolls prepared from shark and cat fish were rated as good even in the absence of NaCl and NaTPP. This is because the fibrillar proteins of these fishes yielded firmer gels after cooking. Incorporation of salt and phosphate improved the texture further, resulting in higher scores.

The data in Table 1 reveal that incorporation of salt and NaTPP reduced the water loss by 50 per cent in fish rolls of all species indicating that these additives

TABLE 1. ORGANOLEPTIC SCORE, WATER HOLDING CAPACITY AND COOKING LOSS IN COOKED FISH MUSCLE ROLLS

Fish	Water holding capacity (%)		Cooking loss (%)		Sensory score	
	Untreated	Treated*	Untreated	Treated*	Untreated	Treated*
Shark	15.5	8.0	20.0	15.0	7.0	8.0
Anchovies	16.5	9.8	20.0	15.0	5.0	6.0
Croaker	20.0	10.0	28.0	14.5	5.0	7.0
Ribbon	18.5	11.0	20.5	12.0	5.5	7.0
Ray	25.2	12.0	30.0	10.0	5.0	7.0
Cat fish	16.5	8.0	18.0	10.0	7.0	8.0

*Treated with 2.5% NaCl and 0.2% NaTPP.

support the retention of water in fish muscle as reported in many studies[10–12]. Shark and cat fish seem to be ideal sources for the preparation of comminuted products since even without the treatment of NaCl and NaTPP they provided rolls with less cooking loss and better WHC. Also, the sensory scores for these fish species were higher than that obtained with other species were higher than those obtained with other enhanced their quality.

In general, salt-phosphate treated rolls demonstrated higher tensile strength and breaking strength than the corresponding controls (Fig. 2). The data also support the results of other physical parameters such as cooking loss and WHC. Among the various fish muscle rolls tested, shark and cat fish provided products with higher binding properties.

Efficacy of NaCl and NaTPP in preventing water loss and retention of textural quality in comminuted fish products have been reported extensively[13,14] Mechanical agitation and mixing in the presence of salt solution bring the salt soluble proteins to the meat surface and facilitate protein-protein interaction. Cooking results in the coagulation of proteins and provides binding on the meat surfaces. Salt not ony enables the muscle particles to bind together but also reduces fluid losses during cooking. Pepper and Schmidt[6] have reported that higher cooking yield and binding strength were achieved in beef rolls prepared with the 2 per cent salt and 0.5 per cent NaTPP. The foregoing results therefore indicate that the fish species chosen in the present study differed significantly in their individual binding properties.

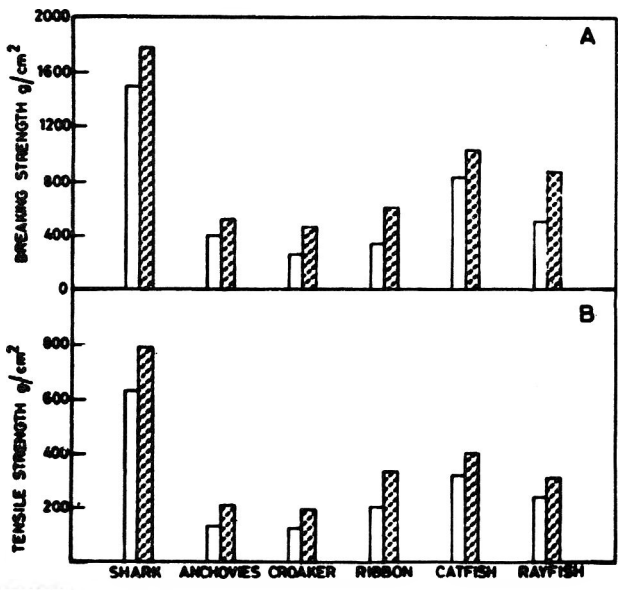


Fig. 2. Breaking (A) and tensile (B) strengths of fish muscle rolls Untreated control; ▨ 2.5% NaCl and 0.25% NaTPP treated.

Further, the binding properties could be improved by the addition of salt and NaTPP. In the absence of non-meat binders however, the natural proteins alone impart a suitable texture to the comminuted products. Because of rapid advances in flesh separation technology, formulations of diverse classes of communited meat products are expected in the future. It is hoped that minced meat from various fish species offer high protential for the preparation of products like fish muscle rolls and patties.

References

1. Regenstein, J.M., The potential for minced fish. *Fd Technol.*, 1986, **40(3),** 101
2. Mandigo, R.W., Restructuring of muscle foods. *Fd Technol.*, 1986, **40(3),** 85
3. Kudo, G., Okada, M. and Miyauchi, D., Gel forming capacity of washed and unwashed flesh of some pacific coast species of fish., *Marine Fish Rev.*, 1973, **12,** 10.
4. Ghadi, S.V., Madhavan, V.N. and Kumta, U.S., Expansion in utility of trash fish for preparation of comminuted meat products. *Proc. Symp. 'Fish Processing in India'*, CFTRI, Mysore, 1976, 111
5. Acton, J.C., Effect of heat processing on extractability of salt soluble protein, tissue binding strength and cooking loss in poultry meat loaves., *J. Fd Sci.*, 1972. **37,** 244.
6. Pepper, F.H. and Schmidt, G.R., Effect of binding time, salt, phosphate and hot-boned beef on binding strength and cook yield of beef rolls *J. Fd Sci.*, 1975, **40,** 227.
7. Gillet, T.A., Brown, C.L., Leutzinger, R.L., Cassidy, R.D. and Simon, S., Tensile strength of processed meats determined by an objective Instron technique. *J. Fd. Sci.*, 1978, **43,** 1121
8. Cheng, C.S., Hamann, D.D., Webb, N.B. and Sidwell, V., Effect of species and storage time on minced fish gel texture. *J. Fd. Sci.*, 1979, **44,** 1087.
9. Hamm, R. and Deatherage, F.E., Change in hydration, solubility and charge in muscle proteins during heating of meat. *Fd. Res.*, 1960, **25,** 587.
10. Weinberg, Z.G., A comparison of the binding properties of fish flesh. *J.Fd Technol.*, 1983, **18,** 441.
11. Cheng, M.L. and Toledo, R.T., Factors affecting textural characteristics of cooked comminuted fish muscle. *J. Fd. Sci.* 1976, **41,** 391.
12. Trout, G.R. and Schmidt, G.R., Effect of phosphate type and concentration, salt level and method of preparation on binding in restructured beef rolls. *J. Fd Sci.*, 1984, **44,** 687.
13. King. F.J., Heiligman, F. and Wierbicki, E., Solubilized fish muscle as food binding material. *Marine Fish Rev.*, 1974, **36,** 18
14. Ahmed, E.M., Cornell, J.A., Tomaszewski, F.B. and Deng, J.C., Effect of salt, tripolyphosphate and sodium alginate on the texture and flavour of fish patties prepared from minced sheepshead *J. Fd. Sci.*, 1983, **48,** 1078.

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 31–34

Use of Unconventional Protein Sources in High Protein Biscuits

L. P. RAJPUT
Department of Food Science, J. N. Krishi Vishwa Vidyalaya, Jabalpur–482 004, India
and
P. HARIDAS RAO AND S.R. SHURPALEKAR
Central Food Technological Research Institute, Mysore– 570 013, India

Received 9 *December* 1986; *revised* 26 *June* 1987

Laboratory studies on the preparation of high protein biscuits indicated that some of the unconventional protein sources such as Mustard Protein Concentrate (MPC), Cottonseed Flour (CSF) or Cottonseed Protein Isolate (CSPI) could be conveniently used to increase the protein content in biscuits from 5.9 to 11.3–18.1%. In general, incorporation of different protein concentrates affected the crispness, taste and overall acceptability, as indicated by the sensory scores but increased the spread ratio and spread factor, which depended on the level of incorporation. The optimum level of incorporation was found to be 15% in case of MPC or CSPI and 10% in case of CSF. The quality of high protein biscuits could be improved by incorporation of 0.5% sodium stearoyl-2-lactylate in the formulation.

In India, biscuits are now gaining increasing popularity and are no more limited in usage to higher income groups of the population only. The total production of biscuits for 1985–86 is estimated at 425,000 tonnes with an annual turnover of over 4250 million rupees. Among different bakery products, biscuits are more amenable to variation in the formulation to meet a wide spectrum of consumer demands with respect to taste and nutritional requirement. Protein-enriched and vitaminised biscuits form a popular carrier of nutrition to vulnerable groups like pregnant and nursing mothers and young children and can be used with advantage in school feeding programmes. Use of unconventional protein source like cottonseed flour, besides improving the nutritional status of different bakery products has also been reported to reduce dough stickiness and thereby improving its machining properties and also the shelf life of biscuits[1–5]. Tsen *et al*[6]. have successfully used soya flour and soya protein isolate with the sodium-stearoyl-2-lactylate in the preparation of acceptable grade protein cookies.

In recent years, processes for preparation of edible grade protein isolates and oilseed meals from cottonseed and mustard have been developed at CFTRI, Mysore[7,8]. The results of studies on the utilisation of cottonseed flour and protein isolate and mustard protein concentrate for the preparation of high protein biscuits are presented in this paper.

Materials and Methods

Materials: Refined wheat flour (*Maida*) was obtained by milling commercially available soft wheat in Buhler Laboratory Mill (Model-MLU 202) after conditioning wheat overnight at 14.5 per cent moisture. Mustard Protein Concentrate (MPC), Cottonseed Flour (CSF) and Cottonseed Protein Isolate (CSPI) were obtained from the Protein Technology Discipline of CFTRI, Mysore[7,8].

Sieve analysis: Sieve analysis was carried out in triplicates by sieving 200 g samples of MPC, CSF and CSPI and *Maida* for 5 min in a Buhler nylon sifter and weighing the overtailings on each sieve.

Chemical analysis: Moisture, total ash, crude fibre, and ether extractives were determined according to standard AACC methods[9]. Protein was determined by micro-Kjeldahl method using factor N × 5.7 for *maida* and N × 6.25 for other protein concentrates.

Preparation and evaluation of biscuits: Biscuits were prepared according to the following recipe:

Ingredients	*Parts*
Maida/Maida-Protein conc. blend	64
Powdered sugar	18
Shortening	16
Milk solids	1
Liquid glucose	1
Sodium chloride	1
Ammonium bicarbonate	0.5
Sodium bicarbonate	0.5
Baking powder	0.25
Vanilla	0.02
Water	Optimum (10–15% depending on ingredients)

The ingredients were mixed in a Hobart mixer for 3 min. The dough was then sheeted to a thickness of 2 mm using a specially designed platform and cut into circular discs of 5 cm diameter and baked on a

wire mesh for 10 min at 205°C in a laboratory baking oven (Model: Despatch, Despatch Oven Co., Minneapolis, Minn. U.S.A.), with a rotating base. The evaluation of biscuits for their physical characteristics viz., thickness, spread, spread factor and spread ratio was carried out according to the method described by Haridas Rao and Shurpalekar[10]. Sensory evaluation of biscuits was carried out for colour, crispness, taste, and overall acceptability by a panel of six judges with a maximum score of 10 for each of the parameters. Statistical analysis of the data was carried out by the New Duncan's multiple range test[11].

Results and Discussion

Proximate composition: Data on the proximate composition of *maida* and mustard and cottonseed protein concentrates are given in Table 1. Low protein value of 7.9 per cent indicated that the *maida* was suitable for the preparation of biscuits. The protein content in MPC and CSF were 51.8 and 48.1 per cent respectively, while that of CSPI was 80.4 per cent. The fat content of protein concentrates was very low (0.57–3.30 per cent). However, the ash content which is indicative of mineral matter was markedly higher (3.87–7.60 per cent) in all the protein concentrates as compared to 0.58 per cent of *maida*.

Sieve analysis: The particle size of flour is known to influence the textural profile of biscuits, particularly the grittiness. Sieve analysis presented in Table 2 shows that *maida* and CSF were comparatively finer than MPC and CSPI. Nearly 98 per cent of *maida* and CSF passed through 10 XX sieve as compared to 75–80 per cent in case of MPC and CSPI.

Effect of incorporation of protein concentrates on dough characteristics: The biscuit dough containing MPC or CSPI was fairly soft and easy to handle upto 15 per cent level of incorporation; however dough containing MPC was somewhat easier to handle than that containing CSPI. Biscuit dough characteristics were not adversely affected upto 20 per cent level of incorporation of MPC and compared favourably with that containing 15 per cent CSPI.

TABLE 1. PROXIMATE COMPOSITION* OF REFINED WHEAT FLOUR (*MAIDA*) AND PROTEIN CONCENTRATES FROM MUSTARD AND COTTONSEED

Constituents	Maida* (%)	Mustard protein conc (%)	Cottonseed Flour (%)	Cottonseed Protein isolate (%)
Moisture	10.1	7.3	7.0	9.0
Protein	7.9	51.8	48.1	80.4
Fat	1.45	2.23	3.30	0.57
Total ash	0.58	7.60	6.04	3.87
Crude fibre	0.29	1.95	3.33	0.71
Carbohydrate (by diff.)	79.68	29.12	32.23	5.45

All values (except moisture) expressed on 14% moisture basis.
*Protein calculated as N × 5.7 for *maida* and N × 6.25 for mustard and cottonseed products.

TABLE 2. SIEVE ANALYSIS* OF MAIDA AND PROTEIN CONCENTRATES FROM MUSTARD AND COTTONSEED

Sieve used	Mesh opening (μ)	Overtailings (%)			
		Maida	Mustard protein conc	Cotton-seed flour	Cotton-seed protein isolate
45 mesh	480	–	–	–	–
60 mesh	355	0.6	–	–	–
6 XX	210	0.4	0.6	–	18.5
10 XX	129	1.2	24.2	2.8	5.5
15 XX	85	8.8	19.4	11.1	20.0
Pan	–	89.0	55.8	86.1	56.0

*Values are average of three trials

Effect of incorporating mustard protein concentrate on the quality of biscuits: The data in Table 3 indicate that the raise in biscuits decreased with increase in the level of MPC. The maximum decrease in thickness was observed, when 5 per cent MPC was included in the recipe and thereafter the decrease was gradual and less marked. However, biscuit diameter indicated that the spread was not affected except in case of biscuits containing 25 per cent of MPC. Gradual reduction in the thickness of biscuits was reflected in a significant increase in the spread ratio as well as spread factor. The surface colour of biscuits changed from golden brown to dull brown, when MPC was incorporated at 25 per cent level. This adverse effect on the colour was also reflected in the sensory scores, which decreased markedly from 8.1 to 1.8. The crispness as well as taste were also affected adversely with increase in the level of incorporation of MPC. Biscuits containing 25 per cent of MPC were hard and gritty, and had the typical taste of mustard. However, biscuits containing 15 per cent MPC were acceptable, as they had the desired crispness and taste. The protein content of these biscuits (13.9 per cent) was more than twice that of *maida* based biscuits (5.9 per cent).

Effect of incorporating cottonseed flour on the quality of biscuits: As in the case of biscuits containing MPC, incorporation of CSF also decreased the thickness, spread ratio and spread factor and increased the spread of biscuits (Table 4). However, the extent of increase in the spread observed was more in CSF enriched biscuits than those with MPC. The colour of

TABLE 3. EFFECT OF INCORPORATION OF MUSTARD PROTEIN CONCENTRATE (MPC) ON THE QUALITY* OF BISCUITS

MPC level (%)	Colour*	Crispness*	Taste*	Overall acceptability*	Thickness (T) (cm)	Diameter (D) (cm)	Spread ratio (D/T)	Spread factor (%)
0	8.1^{a}	8.8^{a}	8.5^{a}	8.5^{a}	0.34^{a}	4.8^{a}	14.1^{a}	100.0^{a}
5	7.5^{a}	7.0^{b}	8.3^{a}	7.3^{b}	0.25^{b}	4.9^{a}	19.6^{b}	139.0^{b}
10	6.0^{b}	6.0^{b}	6.5^{b}	6.8^{b}	0.24^{b}	4.9^{a}	20.4^{b}	144.7^{b}
15	4.8^{c}	4.8^{c}	5.0^{c}	5.5^{c}	0.23^{c}	4.9^{a}	21.3^{c}	151.1^{c}
20	3.0^{d}	3.3^{d}	3.8^{d}	4.0^{d}	0.21^{d}	4.9^{a}	23.3^{d}	165.2^{d}
25	1.8^{e}	1.5^{e}	2.3^{e}	2.3^{e}	0.19^{e}	5.0^{b}	26.3^{e}	186.5^{e}
S. EM (18 df)	±0.39	±0.36	±0.34	±0.38	±0.03	±0.05	±2.7	±11.8

* Max score is 10.
Any two means in the same column not followed by a common superscript differ significantly ($P < 0.05$).

TABLE 4. EFFECT OF INCORPORATION OF COTTONSEED FLOUR (CSF) ON THE QUALITY* OF BISCUITS

CSF level (%)	Colour*	Crispness*	Taste*	Overall acceptablity*	Thickness (T) (cm)	Diameter (D) (cm)	Spread ratio (D/T)	Spread factor (%)
0	8.3^{a}	8.8^{a}	8.5^{a}	8.5^{a}	0.34^{a}	4.8^{a}	14.1^{a}	100.0^{a}
5	6.8^{b}	7.5^{b}	7.3^{b}	7.3^{b}	0.26^{b}	5.0^{b}	19.2^{b}	136.2^{b}
10	5.5^{c}	5.8^{c}	5.5^{c}	6.3^{c}	0.25^{b}	5.0^{b}	20.0^{b}	141.8^{b}
15	4.8^{d}	4.8^{d}	3.8^{d}	4.8^{d}	0.24^{c}	5.1^{b}	21.2^{b}	150.4^{c}
20	3.5^{de}	4.5^{d}	3.3^{d}	4.0^{de}	0.23^{cd}	5.2^{c}	22.6^{c}	160.3^{d}
25	2.8^{e}	3.3^{e}	2.8^{d}	3.3^{e}	0.20^{e}	5.2^{c}	26.0^{d}	184.4^{e}
S.EM (18 df)	±0.31	±0.26	±0.31	±0.29	±0.04	±0.03	±2.5	±9.7

*Max. score is 10.
Any two means in the same column not followed by a common superscript differ significantly ($P < 0.05$)

biscuits changed from golden brown to undesirable greenish brown with 25 per cent level of incorporation of CSF; they were comparatively less crisp and crispness score decreased from 8.8 for control to 3.3. The biscuits also tended to have typical taste of CSF at higher level of incorporation. However, biscuits containing upto 10 per cent of CSF had 11.3 per cent protein and were quite acceptable.

Effect of incorporating cottonseed protein isolate on the biscuit quality: The thickness of biscuits decreased considerably with increase in the level of incorporation of CSPI (Table 5). Though the spread increased gradually, the values for spread ratio and the spread factor increased significantly with increasing levels of CSPI. The surface colour of biscuits changed from golden brown to somewhat dark

TABLE 5. EFFECT OF INCORPORATION OF COTTONSEED PROTEIN ISOLATE (CSPI) ON THE QUALITY* OF BISCUITS

CSPI level (%)	Colour*	Crispness*	Taste*	Overall acceptability*	Thickness (T) (cm)	Diameter (D) (cm)	Spread ratio (D/T)	Spread factor (%)
0	8.8^{a}	8.5^{a}	8.5^{a}	8.5^{a}	0.34^{a}	4.8^{a}	14.1^{a}	100.0^{a}
5	7.8^{b}	7.8^{ab}	8.3^{a}	7.0^{b}	0.26^{b}	5.0^{b}	19.2^{b}	136.2^{b}
10	6.3^{c}	7.0^{b}	7.3^{b}	6.0^{bc}	0.25^{b}	5.0^{b}	20.0^{b}	141.8^{b}
15	5.8^{cd}	5.8^{c}	5.5^{c}	5.5^{c}	0.24^{c}	5.0^{b}	20.8^{b}	147.5^{bc}
20	5.0^{d}	5.5^{c}	4.3^{d}	4.3^{d}	0.23^{c}	5.1^{c}	22.1^{c}	156.7^{a}
25	3.5^{e}	5.3^{c}	3.0^{e}	3.3^{d}	0.22^{d}	5.1^{c}	23.1^{c}	163.8^{c}
S. EM (18df)	±0.29	±0.34	±0.29	±0.32	±0.04	±0.05	±2.8	±10.9

*Max. score is 10.
Any two means in the same column not followed by a common superscript differ significantly ($P < 0.05$)

TABLE 6. EFFECT OF SODIUM STEAROYL-2-LACTYLATE (SSL) ON THE QUALITY* OF HIGH PROTEIN BISCUITS

Protein source	Level used (%)	SSL (%)	Colour*	Crispness*	Taste*	Overall acceptability*	Thickness (T) (cm)	Diameter (D) (cm)	Spread ratio (D/T)	Spread factor (%)
MPC	15	0	4.5^{a}	4.8^{ab}	5.3^{ab}	5.5^{a}	0.23^{a}	4.9^{a}	21.3^{a}	100.0^{a}
MPC	15	0.5	4.8^{ab}	5.8^{bc}	5.5^{b}	6.3^{b}	0.25^{b}	5.0^{a}	20.0^{b}	94.0^{b}
CSF	10	0	5.2^{c}	5.5^{bc}	5.8^{b}	6.5^{b}	0.24^{a}	5.0^{a}	20.8^{a}	100.0^{a}
CSF	10	0.5	5.5^{cd}	6.5^{c}	5.8^{b}	7.5^{c}	0.26^{b}	5.2^{b}	20.0^{b}	96.1^{a}
CSPI	15	0	6.3^{de}	5.3^{b}	5.5^{b}	6.0^{ab}	0.24^{a}	5.0^{a}	20.8^{a}	100.0^{a}
CSPI	15	0.5	6.5^{e}	6.5^{c}	5.3^{ab}	6.8^{b}	0.26^{b}	5.1^{b}	19.6^{b}	94.2^{b}
S. EM	(27df)		±0.27	±0.33	±0.28	±0.26	±0.02	±0.06	±2.1	±9.1

*Max. score is 10.

Any two means in the same column not followed by a common superscript differ significantly ($P < 0.05$)

brown at higher levels of incorporation. Though slight off-flavour was observed in biscuits containing more than 15 per cent CSPI; the effect on crispness was negligible. Considering the various parameters, it was observed that acceptable quality biscuits can be prepared by including 15 per cent CSPI in the recipe. Protein content of these biscuits was 18 per cent and was about three times that of common biscuits based on *maida* only.

Effect of sodium stearoyl-2-lactylate (SSL) on the quality of biscuits containing different protein concentrates: SSL at 0.5 per cent level improved significantly the quality of high protein biscuits, particularly in respect of crispness (Table 6). This was also indicated by the significant increase in the thickness as well as overall acceptability. However, as expected SSL had no significant effect on colour and taste of biscuits containing different protein concentrates. The improving effect of SSL was also observed in biscuits, irrespective of the type of protein source used. Similar improvement in quality of high protein cookies, as a result of SSL addition has also been reported by Tsen *et al*[6].

References

1. Altschul, A.M., The Agricultural, Scientific and Economic Basis for Low Cost Protein Foods. *Presented at International Conference on Single-cell Protein*, MIT, USA, Oct. 1967.
2. Bacigalupo, A., Protein-rich cereal foods in Peru. In: *Protein-enriched Cereal Foods for World Needs.*, by Milner, M. Amer. Assoc. Cereal Chemists, St. Paul, Minnessota, 1969, 288.
3. Bressani, R., Formulated vegetable mixtures. in *Proc. Western Hemisphere Nutrition Congress.*, Amer. Med. Asoc. Chicago, 1965, 86
4. Bressani, R., Elias, L.G. and Graham, E., Cottonseed protein in human foods. In *World Protein Resources*, by Gould, R., Amer. Chem. Soc. Publ. Washington, D.C. 1966, 75.
5. Overman, A., Antioxidant effect of soy and cottonseed flours in raw pastry mixes and baked pastry. *Fd Res.*, 1951, **16,** 39.
6. Tsen, C.C., Peters, E.M., Schaffer, T. and Hoover, W.D., High-protein cookies. I. Effect of soy fortification and surfactants. *Bakers Dig.*, 1973, **47,** 43.
7. Hanumantha Rao, K., *Studies on Cottonseed Proteins*. 1977, M.Sc. Thesis, University of Mysore.
8. Kantharaj Urs. M., and Kowsalya, S.M., Integrated processing of mustard seed for food and feed purposes. *Proceedings of the Symposium on Rapeseed and Mustard*, Department of Science and Technology, and CFTRI, Mysore, Nov. 1976, 162.
9. *Cereal Laboratory Methods*, American Association of Cereal Chemists, St. Paul, Minnessota, 1962. 7th Edn.
10. Haridas Rao, P. and Shurpalekar, S.R., Use of milo in bakery products. *J. Fd Sci. Technol.*, 1976, **13,** 293.
11. Duncan, D.B., The multiple range test. *Biometrics*, 1955, **11,** 1.

J. Fd. Sci. Technol., 1988. Vol. 25, No. 1, 35–38

Nutritional Quality of Extruded Rice, Ragi and Defatted Soy Flour Blends

R.K. DUBLISH, G.S. CHAUHAN, AND G.S. BAINS*
Dept. of Food Science and Technology
G.B. Pant University of Agriculture and Technology, Pantnagar, Nainital, India

Received 3 *December* 1986; *revised* 18 *April* 1987

Nutritional quality of rice, ragi and defatted soy flour blends as affected by extrusion cooking was evaluated. The blends were processed in the Wenger X-5 extruder using a feed rate of 27.2 kg/hr, water flow of 6.8 kg/hr, screw speed of 300 rpm, extruder exit temperatures (EET) of 65 and 95 ±2°C through a die of 1/8 inch. The extrusion cooking resulted in the inactivation of trypsin inhibitor activity and at 65 and 95 ± 2°C the inactivation was 42.6–71.4 and 72.3–100%, respectively. The losses in available lysine content ranged from 3.6–12.8% in various blends depending on the temperature of extruder barrel. The proportion of phosphorus as phytic P decreased (11.1–29.1%) during extrusion cooking. A discernible loss in tannin like compounds was observed in extruded blends. The PERs of processed rice-defatted soy flour (85:15), rice-ragi-defatted soy flour (42.5:42.5:15) and ragi-defatted soy flour (85:15) blends were 2.61, 2.41 and 2.23, respectively as compared to 2.21, 1.92 and 1.81 of their unprocessed counterparts.

Extrusion cooking is a recent innovation in the field of food processing. Nutrient retention is one of the criteria used for evaluating any newly developed product[1]. Lu and Whitaker[2] used nutrient retention as the basis for assessing the thermal processes applied to foods. It has been reported that well established processes such as canning, dehydration and roller drying cause higher nutrient losses in the products. De-Muelenaere and Buzzard[3] reported a meagre loss in available lysine content of extruded full fat soy flour while it was about 35 per cent in roller dried product. Since the literature on the nutritional quality of extruded rice, ragi and defatted soy flour blends is scanty, the present investigation was undertaken to study the effect of extrusion cooking on such blends. Defatted soy flour was used to enhance protein level of rice whereas ragi was blended to combat the deficiency of S-containing amino acids of defatted soy flour.

Materials and Methods

Samples of raw milled *Jaya* rice and defatted soy grits were ground separately to about 80 mesh using Fitz mill whereas the sample of finger millet (ragi) conditioned to 16.5 per cent moisture for 30 min was milled in a Buhler mill (Model MLU 202) according to AACC[4] procedure. Blends of rice, ragi and defatted soy flour were prepared as presented in Table 1. The blends were sifted through 60 mesh sieve and were extruded in the Wenger X-5 extruder using blend feed rate, 27.2 kg/hr; water flow, 6.8 kg/hr; extruder speed, 300 rpm; extruder exit temperature, 65 and 95 ± 2°C; die size, 1/8 in. and steam supply, 40 psi. The extrudates were dried at about 45°C to a moisture content of about 6 per cent.

Analytical methods: Moisture, protein, fat and ash contents were determined using AACC methods[4] and nitrogen free extract was arrived at by difference. Method of Kakade *et al*[5] was used to estimate trypsin inhibitor activity (TIA). Benzoyl-DL-arginine-p-nitroanilide (BAPANA, Sigma chemicals) was used as substrate. The TIA was expressed as per cent trypsin units inhibited (TUI). Water soluble proteins were determined by AACC method[6]. Available lysine was determined by the method of Carpenter[7]. Total

TABLE 1. PROPORTIONS OF RICE, RAGI AND DEFATTED SOY FLOUR IN DIFFERENT BLENDS

Blend code	Rice (%)	Ragi (%)	Defatted soy flour (%)
A	100	–	–
B	85	–	15
C	70	–	30
D	42.5	42.5	15
E	50	50	–
F	–	100	–
G	–	85	15

Research paper No. 4635 through the experiment station, G.B. Pant University of Agriculture & Tech. Pantnagar, Nainital, U.P.

* Dept. of Food Sci. & Tech. P.A.U. Ludhiana

phosphorus was estimated by the method of Fiske and Subbarow[8]. Phytic phosphorus was determined by the method of McCance and Widdowson as described by Tara *et al*[10]. Tannin like compounds were determined according to Ranganna[11].

Protein efficiency ratio (PER): Rat growth method as described by Campbell[12] was followed to determine the protein efficiency ratio (PER) of the products and the unprocessed blends. Raw and processed blends containing defatted soy flour (B,D & G) were incorporated with the experimental diets so as to provide 10 per cent protein. All the diets contained in addition (for every 100 g) 4 g of salt mixture; 1 g vitamin mixture; 9.5 g groundnut oil; and 2.5 g cellulose. Sufficient quantity of corn starch was added to make up the diets to 100 g. Salt mixture was prepared according to formula of Oser[13] whereas the vitamin mixture was prepared as prescribed by Chapman *et al*[14].

Weanling albino rats (21–24 days old) weighing between 28 and 30 g were grouped randomly to six groups, 8 in each group, by taking into account the initial weights. The animals were housed in individual cages and fed *ad lib*. Records of food intake and gain in weight of the rats were maintained for a period of 4 weeks. The PER values were calculated from the data on the gains in body weight (g) and the protein intake (g).

Results and Discussion

Samples of rice, ragi and defatted soy flour contained 8.6, 5.8 and 56.9 per cent protein, respectively where as the corresponding values for ash content were 0.68, 2.67 and 6.90 per cent. Thus higher protein content of defatted soy flour made it admirably suitable for blending.

Trypsin inhibitor activity (TIA): The effects of extrusion processing on TIA of rice, ragi and defatted soy flour blends are shown in Table 2. Rice did not exhibit any trypsin inhibitor activity (TIA) whereas the blend of rice with 30 per cent defatted soy flour showed maximum TIA. Degree of trypsin inhibition observed in the case of raw blends ranged from 2.0 to 26.0 per cent TUI, the values being lowest for rice-ragi and highest for rice-defatted soy flour (70:30) blends. Extrusion cooking resulted in the inactivation of TIA; the inhibition at 65 and 95 ±2°C was 42.6–71.4 and 72.3–100 percent respectively. The greater inactivation observed at 95°C extrusion exit temperature (EET) might be attributed to increased expansion of the product allowing more heat penetration. De-Muelenaere and Buzzard[3] reported 93 per cent inactivation of TIA in extruded mixture of defatted soy flour, corn and sorghum. Similarly Chauhan *et al*[15]. reported 86 per cent inactivation of TIA in rice-full fat soy flour extruded products.

TABLE 2. EFFECT OF EXTRUDER TEMPERATURES ON THE TRYPSIN INHIBITOR ACTIVITY (PER ML OF EXTRACT) OF EXTRUDED RICE-RAGI-DEFATTED SOY FLOUR BLENDS

Blend	Raw TUI*	65°C		95°C	
		TUI*	%inacti-vation	TUI*	%inacti-vation
A	–	–	–	–	–
B	1.9	0.8	58.3	0.1	94.5
C	3.9	1.4	64.2	0.4	89.6
D	2.1	0.6	71.4	0.1	95.0
E	0.3	0.1	65.0	0.0	100.0
F	0.7	0.4	42.6	0.2	72.3
G	2.4	1.0	58.1	0.3	87.5

[1]One gram extracted with 50 ml of 0.01N NaOH, 1 ml made to 50 ml with distilled water and 1 ml taken for TIA.

*out of 15 units, TUI – Trypsin Units Inhibited

Water soluble proteins (WSP): The per cent decrease in WSP ranged from 28.6 to 57.1 and 42.9 to 71.4, for the products extruded at an EET of 65 and 95°C, respectively; the decrease being of a greater magnitude at higher EET (Table 3). Kim and Rottier[16] reported a similar effect of extruder temperatures on WSP in extruded *aestivum* semolina. Peri *et al*[17] reported that the NSI of extruded corn germ flour and milk proteins decreased from 50 to 20 per cent as the extruder temperature was increased from 150 to 170°C. Chauhan and Bains[18,19] reported a decrease of about 32.7 to 50 per cent in WSP of extruded rice-defatted soy flour product.

Loss in available lysine content ranged from 3.6 to 7.7 per cent in the product extruded at an EET of 65°C and from 5.7 to 12.8 per cent in those extruded at an EET of 95°C. The maximum and minimum losses were observed in the extruded rice flour and ragi flour, respectively. The differences in the per cent decrease in available lysine content of various blends may be due to the differences in the extent of cooking as reflected by expansion ratio (Table 3). A similar effect of extrusion cooking on the available lysine content was reported by De-Muelenaere and Buzzard[3], Chauhan[20] and Peri *et al.*[17]

Comparative values for phytic phosphorus content of unprocessed and of their processed counterparts are presented in Table 3. The decrease in phytic phosphorus content ranged from 11.1 to 29.1 per cent the lowest value being in extruded ragi product and highest in rice-ragi-defatted soy flour product (42.5: 42.5 : 15). Anderson[21] reported 19.1 to 26.5 per cent decrease in phytate content of cereals as a result of

TABLE 3. EFFECT OF EXTRUDER EXIT TEMPERATURE ON EXPANSION, AND PER CENT DECREASE OF WATER SOLUBLE PROTEINS (WSP), AVAILABLE LYSINE, PHYTIC P AND TANNIN-LIKE SUBSTANCES IN EXTRUDED RICE - RAGI - DEFATTED SOY FLOUR BLENDS

Blend	Expansion ratio at °C		WSP (% decrease)		Available lysine (% decrease)		Phytic P (% decrease)	Tannin-like substances (% decrease)	
	65	95	65	95	65	95	95	65	95
A	2.24	2.49	57.1	71.4	7.7	12.8	26.4	–	–
B	1.92	2.17	49.0	63.3	3.6	7.1	23.2	7.7	42.3
C	1.44	2.04	48.9	72.2	4.4	5.9	28.2	31.8	47.7
D	1.70	2.17	42.2	66.6	4.8	7.1	29.1	25.0	37.5
E	2.30	2.60	42.9	71.4	3.6	7.1	18.1	23.1	30.1
F	1.37	2.01	28.6	42.9	4.5	5.1	11.1	17.4	21.7
G	1.60	1.92	35.7	52.4	5.1	5.7	14.1	31.4	45.7

TABLE 4. AVERAGE FOOD INTAKE AND PER OF DIFFERENT DIETS FED TO ALBINO RATS FOR FOUR WEEKS

Diet blend	Protein source in diet	Food intake (g)	Protein intake (g)	Body wt. gain (g)	PER	
					Mean	± S.D.
B	Unprocessed	227.8	24.5	54.2	2.21	0.09
	Extruded	247.8	26.2	68.5	2.61	0.06
D	Unprocessed	193.5	21.3	41.0	1.92	0.10
	Extruded	262.8	27.2	65.5	2.41	0.06
G	Unprocessed	202.5	23.2	42.0	1.81	0.07
	Extruded	242.5	25.2	56.2	2.23	0.06

extrusion cooking.

The effects of extrusion cooking on the content of tannin-like compounds are presented in Table 3. Tannin-like compounds were not detected in rice. Rice-defatted soy flour (70:30) blend contained 0.44 per cent of tannin-like compounds. The degradation of tannin-like compounds ranged from 21.7 to 47.3 per cent. The degradation was greater at higher temperatures as compared to lower extruder temperatures.

Protein efficiency ratio: The mean PER values of extruded products were significantly higher than those of unprocessed counterparts (Table 4). The PER values of experimental diets containing unprocessed blends of rice-defatted soy flour (85:15), rice-ragi-defatted soy flour (42.5:42.5:15) and ragi-defatted soy flour were 2.21, 1.92 and 1.81, respectively whereas the corresponding values for extruded blends were 2.62, 2.41 and 2.23, respectively. The intake of diets containing extruded products was higher than those of the unprocessed blends. This may be attributed to the improved palatability of the extruded products. Mustakas *et al*[22]., and Jansen *et al*[23] reported an improvement in the PER of full-fat soy flour when extrusion cooked. Similarly, Chauhan *et al*[15] reported that the PER of rice-legume blends improved significantly after extrusion cooking.

The results of this investigation indicated that extrusion cooking improved significantly the nutritional quality of rice, ragi and defatted soy flour blends as reflected by PER values. Inactivation of trypsin inhibitor and degradation of phytic phosphorus and tannin-like compounds are added to nutritional advantages of extrusion cooking. Some of the disadvantages of the process of extrusion cooking are loss of water-soluble proteins with slight loss in available lysine.

References

1. Chauhan, G.S. Verma N.S. and Bains, G.S. Effect of extrusion processing on the nutritional quality of protein in rice-legume blends. *Nahrung.*, 1986 (In press).
2. Lu, A.T. and Whitaker, J.R., Some factors affecting rates of heat inactivation and reactivation of Horseradish peroxide. *J. Fd. Sci.*, 1974, **39**, 1173.
3. De-Muelenaere, H.J.H. and Buzzard, J.L., Cooker extruders in service of world feeding. *Fd Technol.*, 1969, **23**, 345.
4. *Approved Methods.* American Association of Cereal Chemists, St. Paul, Minn, U.S.A., 1976.
5. Kakade, M.L., Rackis, J.J., Mc-Ghee, J.E. and Puski, G.A., Determination of trypsin inhibitor activity of soy products. *Cereal Chem.*, 1974, **51**, 376.
6. AACC, Approved methods of AACC, American Association of Cereal Chemists, Inc. St. Paul. Minn., U.S.A. 1968.

7. Carpenter, K.J., The estimation of available lysine in animal protein foods. *Biochem. J.*, 1960, **77,** 604.

8. Fiske, C.H. and Subbarow, Y., The colorimetric determination of phosphorus, *J. biol. Chem.*, 1925, **66,** 375.

9. Mc Cance, R.A. and Widdowson, E.M. Phytin in human nutrition. *Biochem. J.*, 1935, **29,** 2694.

10. Tara, K.A., Usha, M.S., Prabhavati, C. and Bains, G.S., Vitamin, mineral, colour grade and limiting amino acid contents of Indian commercial flours. *Res. Ind.*, 1971, **16(3),** 196.

11. Ranganna, S., *Manual of Analysis of Fruit and Vegetable Products.* Tata Mc-Graw Hill Publ. Co. Ltd., New Delhi., 1977.

12. Campbell J.A. Methodology of protein evaluation. Nutrition Document R. 10/Add. 37, WHO/FAO/UNICEF-PAG. International Nutrition Conference, 1961.

13. Oser, B.L., *Hawk's Physiological Chemistry.* Tata Mc-Graw Hill Publ. Co. Ltd., New Delhi., 1979. 14th Edn.

14. Chapman, D.G., Castillo, R. and Campbell, J.A. Evaluation of protein in foods. 1. A method for the determination of protein efficiency ratios. *Can. J. Biochem. Physiol.*, 1959, **37,** 679.

15. Chauhan, G.S., Verma, N.S. and Bains, G.S., Effect of extrusion cooking on the nutritional quality of extruded rice-legume blends. *Paper presented in Vth Indian Convention of Food Scientists and Technologists.* 1985.

16. Kim, J.C. and Rottier, W., Modification of Aestivum wheat semolina by extrusion. *Cereal Fd. Wld.*, 1980, **24 (2),** 62.

17. Peri, C., Barbieri, R. and Casiraghi, E.M., Physical, chemical and nutritional quality of extruded corn germ flour and milk protein blends. *J. Fd Technol.*, 1983, **18,** 43.

18. Chauhan, G.S. and Bains, G.S. Effect of defatted soy flour on the physico-chemical characteristics of extruded rice products. *J. Fd. Sci Technol.*, 1985, **22,** 115.

19. Chauhan G.S. and Bains G.S. Effect of granularity on the characteristics of extruded rice snack. *J. Fd Technol.*, 1985, **20,** 305.

20. Chauhan G.S. *Studies on the Procssing of Rice-Legume Blends Using Extrusion Technology* , 1982, Ph.D. Thesis, Punjab Agric. University.

21. Anderson, R.A., Conway, H.F., Pfeifer, V.F. and Griffin, E.L., Roll and extrusion cooking of grain sorghum grits. *Cereal Sci. Today.*, 1969, **14,** 372.

22. Mustakas, G.Ç. Albrecht, W.J., Bookwalter, G.H., Mc Ghee, J.E., Kwolek, W.F. and Griffin, E.L. Jr, Extruder processing to improve nutritional quality, flavour and keeping quality of full fat soy flour. *Fd Technol.*, 1970, **24,** 1290.

23. Jarsen, G.R., Harper, J.M. and O'Dean, L., Nutritional evaluation of blended foods made with low cost extruder cooker. *J. Fd Sci.*, 1978, **43,** 912.

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 39–41

SEPARATION OF CAROTENOIDS AND ESTIMATION OF BETA-CAROTENE CONTENT OF SELECTED INDIAN FOOD AND FOOD PREPARATIONS BY HPLC

S.N. DIKSHIT, S.A. UDIPI
Department of Food Science and Nutrition,
S.V.T. College of Home Science, S.N.D.T. Women's University,
Juhu, Bombay–400 049.
and
A. RAO, V. MANOHAR
R & D Centre, Searle (India) Limited,
Thane–400 601, India

Received 27 *January* 1987; *revised* 18 *May* 1987

The beta-carotene content of 15 vegetables and 9 cooked preparations were estimated by HPLC. The HPL chromotograms revealed the absence of alpha-carotene in all 15 vegetables, presence of significant amounts of lycopene, and presence of beta-cryptoxanthin in carrots and tomato. The HPLC method was sensitive in estimating losses in beta-carotene due to cooking. Beta-carotene losses occurred in almost all cooked preparations and were greater when the vegetables were either cooked for a prolonged period or were highly macerated or were deep-fried in oil.

Carotenoids derived from plant foods are the chief sources of Vitamin A in the diet of many population groups in India[1] and are thus important for the prophylactic treatment of Xerophthalmia[2]. Recently a wider, non-vitamin A role of the carotenes in cancer prevention has been suggested[3,4], although foods high in beta-carotene were shown to have no specific association[5].

Carotenoids are extremely unstable compounds, susceptible to oxidation and cis/trans isomerization, the latter process being catalysed by light, heat and acid[2,6]. Carotene values reported for Indian foods have been derived using the AOAC method which relies on open-column chromotography wherein the carotenes are exposed to oxidation and adsorbents for a prolonged period of time[7,8]. Moreover, this method does not separate the individual carotenoids, their cis/trans isomers or esters and thus may result in significant error in estimating the biopotency of the carotenoids in foods[2].

Although HPLC analysis is relatively new, it is considered to be rapid, reproducible, accurate and creates fewer artifacts. Though several reports[9-12] are available regarding HPLC estimation of beta-carotene in foods, little information is available on the separation of carotenoids in Indian foods by HPLC.

The aim of the present investigation therefore was to estimate the beta-carotene content of some vegetables and its losses in 9 cooked preparations by HPLC. Amaranth green and red (*Amaranthus gangeticus*), coriander (*Coriandrum sativum*), *Chavli (Vigina catjang)*, Colacasia (*Colacasia antiquosum*) black and green, Knol-khol leaves, (*Brassica oleracea* var. caulorapa), Mayalu (*Bassella rubra*), Methi (*Trigonella foenum graecum*), Radish leaves (*Raphanus sativus*), Shepu (*Peucedanum graveolens*), Spinach (*Spinacia oleracea*), Pumpkin (*Curcurbita maxima*) red, Carrot (*Daucus carota*) and tomato (*Lycopersicon esculentum*) were purchased from the local market. Carrot salad and *halwa*, *chavli bhaji*, coriander *chutney*, *mayalu pakoda*, *methi thepla*, *shepu bhaji*, spinach *bhaji* and *alu palak* were prepared.

Samples were extracted with an acetone-petroleum ether mixture (50:50) until the solvent layer was colourless. The extract was washed with 100 ml saturated NaCl solution. It was subsequently washed with 100 ml water and evaporated to dryness in a rotary vacuum evaporator. The pigments were dissolved in 10 ml of ether and 10 ml of 10 per cent methanolic KOH. The flask was then filled with N_2, stoppered tightly and allowed to stand for 2 hr for saponification. The flasks were shaken intermittently to ensure thorough mixing of the contents. The pigments were then transferred to the ethyl ether layer (50 ml) and washed free of the alcoholic alkali. They were then evaporated to dryness and subsequently made upto 10 ml in hexane containing 1 per cent BHT. All analyses were carried out in duplicates.

A Varian 5000 HPLC unit was used for analysis. Carotenoids were separated on a reversed phase Micropak MCH-10 (30 cm × 4 mm S.S.) column using an isocratic solvent system of acetonitrile : methanol (80 : 20) at a flow rate of 1.2 ml/min. The UV detector of the HPLC unit was set at 400-500 nm.

Ten µl of standard alpha, beta-carotene, beta-cryptoxanthin and lycopene solutions were injected and the retention times and peak areas were recorded. Ten µl of the test material was then injected and the chromatogram was developed under identical conditions. Peaks were identified on the basis of retention time and quanfitied by peak area. This system gave complete resolution of alpha-and beta-carotene, beta-cryptoxanthin and lycopene in 15 min and recovery was 95 per cent.

Lycopene, beta-cryptoxanthin, alpha-carotene and beta-carotene were clearly separable with retention times of 8.2, 11.6, 13.2 and 14.7 min respectively. In the present investigation, none of the vegetables analysed, including carrots was found to contain any alpha-carotene.

This may be due to the conversion of alpha-carotene to lutein[12]. Beta-cryptoxanthin was detected only in carrots and tomato. A trailing of the beta-carotene peak was found in most raw vegetables studied. This may be attributed to the presence of trace amounts of some cis isomer. Although lycopene was present in significant quantities in all vegetables, it is of little consequence in terms of biological potency. Two unidentified peaks were present in carrot and tomato and all leafy vegetables. The beta-carotene content of the raw vegetables analyzed by HPLC is shown in Table 1. Many studies have demonstrated that beta-carotene analyzed by the AOAC method lead to an overestimation[9-12]. In the present study, the beta-carotene content obtained by HPLC and AOAC methods were similar when interfering carotenoids were absent as is seen in the case of most of the leafy vegetables analysed. A discrepancy in the beta-carotene content obtained by the HPLC and AOAC method was observed in vegetables, when other interfering carotenoids were present.

Slightly higher beta-carotene values for spinach and tomato have been reported by Yang, Tsou and Simpson[2] and Hsieh and Karel[9]. (Spinach 60.54 μg/g; tomato: 9.1 μg/g); significantly less amount of beta-carotene was estimated to be present in Indian carrots (16.11 μg/g) as compared to those reported by Hsieh and Karel (421.4 μ g/g)[9]. These disparities may be attributed to differences in geographic location, genetic variations and post harvest losses.

TABLE 1: CAROTENE CONTENT OF SOME VEGETABLES

Vegetables	HPLC* Carotene (μ g/100g)	Spectrophotometry published (μ g/100g)
Amaranth (Green)	6688	5520
Amaranth (red)	7173	–
Carrot	1611	1890
Chavli	6448	6072
Colacasia (black)	9111	12000
Colacasia (green)	9081	10278
Coriander	5699	6918
Knol-Khol Leaves	3526	4146
Mayalu	3004	7740
Methi	6421	2340
Pumpkin (red)	121	50
Radish Leaves	3668	5295
Shepu	3026	7182
Spinach	5531	5580
Tomato	619	351

Values represent average of duplicate samples analyzed in triplicate.

TABLE 2: CAROTENE CONTENT OF PREPARATIONS

Food Preparations	Wt of recipe/ serving (g)	HPLC[$] β-Carotene (μg/serving)	% loss
Carrot *Halwa*	25 (50) +	181	78
Carrot Salad	75 (50) +	688	15
Chavli Bhaji	75 (50) +	796	75
Coriander *Chutney*	10 (10) +	148*	74
Mayalu Pakoda	40 (10) +	94	69
Methi Thepla	75 (15) +	1120	5
Shepu Bhaji	75 (50) +	1300	15
Spinach *Bhaji*	100 (75) +	2014	51
Alu Palak	75 (50) +	482	83

$ – Values represent average of duplicate samples analysed in triplicate

* – Value is for 2 servings

+ – Values in parentheses represent amount (g) of raw vegetables used to prepare 1 serving of recipe.

Losses in carotene content due to cooking for some Indian recipes are presented in Table 2. Losses in beta-carotene were calculated using values obtained per 100 g of the cooked food. This value was then used to represent the beta-carotene content in the amount of vegetable used per 100 g of the cooked recipe. This was compared to the same amount of unprocessed vegetable that had been previously analysed for beta-carotene content and the loss in beta-carotene content was calculated. Generally, losses of the provitamin tended to be higher when vegetables were macerated finely such as for coriander *chutney*, or cooked for long periods eg. carrot *halwa*, *chavli bhaji*, and *alu palak*, or were deep-fried as in the case of *pakoda*. The extent of loss was less when the processing treatment and exposure to light, heat and oxidation were kept to a minimum eg. carrot salad and *methi thepla*. Variation in carotene losses may be attributed to a combination of reasons i.e. chopping the vegetable finely, increased time and temperature of cooking, exposure to air and light and remaceration of the vegetable after cooking.

In this study, variables such as maturity of vegetable, variety, region of growing, length of storage after harvest and in market have not been taken into consideration. Therefore, further investigations are necessary.

The authors express their gratitude to Dr. K. Nagarajan, Director, R & D Centre, Searle (India) Limited, for allowing them to use the HPLC system, and M/s. Hoffmann La-Roche, Basel, Switzerland for supplying the carotenoid standards.

References

1. Murthy, N.K., Joseph, A. and Saroja, S., Availability of beta-carotene from carrots in a human feeding trial. *Ind. J. Nutr. Diet.*, 1973, **10,** 65.
2. Simpson, K.L., Relative value of carotenoids as precursors of vitamin A. *Proc. Nutr. Soc.*, 1983, **42,** 7.
3. Peto, R., Doll, R., Buckley, J.D. and Sporn, M.B., Can dietary beta-carotene materially reduce human cancer rates? *Nature, Lond.* 1981, **290,** 201.
4. Wolf, G., Is dietary beta-carotene an anti-cancer agent? *Nutr. Rev.*, 1982, **40,** 257.
5. Colditz, G.A., Branch, L.G. Lipnick, R.J., Willet, E.C., Rosner, B., Posner, B.M. and Hennekens, C.H., Increased green and yellow vegetable intake and lowered cancer deaths in an elderly population., *Am. J. Clin. Nutr.*, 1985, **41,** 32.
6. Isler, O. (Ed), *Carotenoids*, Birkhauser Verlog, Basel. 1971.
7. Gopalan, C., Rama Sastri, B. V. and Balasubramanian, S.C., *Nutritive Value of Indian Foods*, National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, 1982.
8. *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, D.C., 1980.
9. Hsieh, Y. and Karel, M., Rapid extraction and determination of beta-carotene in foods. *J. Chromatogr.*, 1983, **259,** 515.
10. Simpson, K.L., Tsou, C.S., Chicester, C.O., in *Methods of Vitamin Assay* by Augustin, J., Klein, B.P., Becker, D.B.S., Venugopal, P.B., (Eds) Wiley Inter-science Publication, John Wiley and Sons, New York, 1985.
11. Braumann, T. and Grimme, L.H., Reversed phase high performance liquid chromatography of chlorophylls and carotenoids. *Biochem. Biophys. Acta*, 1981, **8,** 637.
12. Khachik, F., Beecher G.R. and Whittaker, N.F., Separation, identification and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography. *J. agric. Fd. Chem.*, 1986, **34,** 603.

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 42–43

SPECTROPHOTOMETRIC DETERMINATION OF DI-T-BUTYLHYDROQUINONE (TBHQ) IN OILS

U. VIPLAVA PRASAD, T.E. DIVAKAR, R.V. RAMI REDDY AND C.S.P. SASTRY
Food and Drug Laboratories, School of Chemistry, Andhra University, Waltair 530 003, India

Received 25 *November* 1986; *revised* 6 *April* 1987

Two simple spectrophotometric methods were developed for the determination of di-t-butylhydroquinone in commercial samples and oils based on the formation of coloured species with 4-aminophenazone – IO_4 – or sodium nitroprusside – hydroxylamine under alkaline conditions.

Di-t-butylhydroquinone (TBHQ) is an antioxidant permitted for use in foods. The methods available for the determination of TBHQ are based on HPLC[1-3], GLC[4,5] and pulse polarography[6]. Since sophisticated instruments are required in the above methods, attempts were made to develop a simple spectrophotometric method for its determination. Vinod *et al*[7]. reported a procedure for determining microgram amounts of p-cresol by oxidative coupling with 4-aminophenazone (AP) in the presence of potassium ferricyanide. Prasad *et al*[8] developed a procedure for determining phenolic compounds by complex formation with sodium nitroprusside (SNP) in the presence of hydroxylamine. We have adopted these two sensitive methods for the estimation of TBHQ in commercial samples and in oils.

All the chemicals and reagents were GR grade. TBHQ was a gift from Eastman Kodak, USA.

Recommended procedures

Method A (4-aminophenazone – IO_4 reagent). Into a series of 10 ml standard graduated test tubes containing aliquots of aqueous methanolic (1: 1 v/v) TBHQ (80 – 400 μg), ammonium hydroxide (3N, 2 ml), sodium metaperiodate (0.2%, 0.5 ml) and 4-aminophenazone (0.1%, 2 ml) were added and diluted up to the mark with distilled water. The absorbance was measured at 490 nm against the corresponding reagent blank after 5 min and before 50 min. The amount of TBHQ was deduced from a standard calibration curve obtained through linear least squares treatment of the results.

Method B (sodium nitroprusside – hydroxylamine reagent): Aliquots of TBHQ (0.25 – 3 ml, 1 ml~ 200 μg) were delivered into a series of 10 ml graduated test tubes. Sodium nitroprusside (2%, 1 ml), hydroxylamine hydrochloride (5%, 0.5 ml) and sodium hydroxide (IN, 0.5 ml) were successively added to each of the tubes and allowed to stand till the effervescence ceased. The volume was then made upto the mark with distilled water and the absorbances were measured at 650 nm after 1 min and before 60 min against a reagent blank. The amount of TBHQ was computed from the standard calibration graph obtained through linear least squares treatment of the results.

Recovery experiments with oils: Ten grams of oil containing TBHQ were initially dissolved in 100 ml of petroleum ether and extracted with four 20 ml portions of acetonitrile. The combined acetonitrile extract was evaporated to dryness and the residue was then dissolved in an appropriate volume of aqueous methanol (1:1). The above procedures were then followed for estimating TBHQ.

A study of the effect of the concentrations of the different reagents, alkali and its strength, time, solvent and order of addition of reagents in both the procedures with respect to maximum sensitivity, minimum blank, obedience to Beer's law led to methods A and B described above. The optical characteristics such as λ max, Beer's law limits, molar absorptivity, Sandell's sensitivity, etc. for each method are given in Table 1.

In experiments using groundnut, gingelly, sunflower and coconut oils, recovery of added TBHQ amounted to 96.3 – 98.4 per cent. Other natural and synthetic (α-tocopherol, BHT, BHA) phenolic antioxidants, and synergists (citric acid, tartaric acid, methyl silicone) do not interfere even when they are 5 fold excess (i.e. 1 mg for 200 μg of TBHQ) in combination. However, the other phenolic antioxidants such as propyl gallate (PG) or gallic acid (GA) interfere in both the methods. To avoid the

TABLE 1. OPTICAL CHARACTERS FOR THE ESTIMATION OF TBHQ WITH AP AND SNP

Parameter	Method A	Method B
Beer's law limits (μg/ml)	8 – 40	5 – 40
Stability period (min)	5 – 50	1 – 60
Molar absorptivity (lit/mol/cm)	1.942×10^3	2.22×10^3
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.114	0.1
Slope	0.0085	0.0096
Intercept	0.002	0.0127
Correlation coefficient	0.9991	0.9988
Relative standard deviation (%)	1.955	1.58
Range of error (%) (95% confid. limits)	2.057	1.66

The reproducibility of each method was found by measuring absorbances of six replicate samples containing 300 μ g of TBHQ.

interference, thin layer chromatographic procedure is necessary for separating them initially. The combination of silica gel G - Kieselguhr (2: 1) impregnated with EDTA disodium salt (2.4%), benzene – n-butanol (12:1) and reagent A or B as stationary phase, mobile phase and spraying agent respectively appears to be good among the several tried for the separation (Rf values: GA, 0.05; PG, 0.35; and TBHQ, 0.7).

In conclusion, the proposed methods are simple, sensitive, selective and can be used for the routine determination of TBHQ in commercial samples and in oils with reasonable precision and accuracy.

References

1. Page,B.D., HPLC determination of 7 antioxidants in oil and lard. *J. ass. off. analyt. chem.*, 1983, **66,** 727.
2. Pujol, F.M. and Lopez Sabater, M.C., Separation and determination of gallates, BHT, BHA, TBHQ, PA and ethoxyquin by HPLC. *Circ. Farm.*, 1984, **42,** 3.
3. Kitada, Y., Tamase, K., Mizobuchi, M., Sasaki, M., Tanigawa, K., Komiyama, S. and Nakazawa, H., Determination of TBHQ in oily foods and dried fish by HPLC. *Shokuhin Eiseigaku Zasshi*, 1984, **25**, 209 (*Chem. Abst.*, 1985, **101,** 109061a)
4. Min, D.B. and Schweizer, D., G.C. determination of BHA, BHT, and TBHQ in soybean oil *J. Fd. Sci.*, 1983, **48,** 73.
5. Min, D.B., Ticknov, D. and Schweizer, D., Analysis of antioxidants in oil. *J. Am. Oil Chem. Soc.*, 1982, **59,** 378.
6. Thunyaudom Tonmanee, N and Archer, V.S., Determination of ter. butyl hydroquinone in edible oils by differential pulse polarography. *Talanta*, 1982, **29,** 905 (*Chem. Abst.*, 1983, **98,** 105782h)
7. Vinod, D., Stothers, J.B. and Warnhoft, E.W., On the reaction of p-cresol with 4-aminophenazone. *Can. J. Chem.*, 1974, **52,** 2932.
8. Prasad, M.R., Şugumaran, M. and Vidyanadhan, C.S., A new colorimetric method for the estimation of resorcinol. *Analyt. Biochem.*, 1977, **80,** 483.

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 44–45

ANTIOXIDANT PRINCIPLES OF KOKUM RIND

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

Received 17 *December* 1986; *revised* 25 *May* 1987

The antioxidant principles of dried kokum (*Garcinia indica*) rind were soluble in hexane, ether, acetone, ethylacetate or ethanol. The hexane extract incorporated at 0.2% level in groundnut oil showed a peroxide value of 23 meq/kg oil as against 14 and 600 for TBHQ (0.02%) and control respectively after 164 hr incubation. Garcinol, an yellow coloured fat – soluble pigment isolated from the hexane extract did not possess any antioxidant activity at 0.02% level. Iso-garcinol an isomer of garcinol showed weak activity. The hexane extract on fractionation with sodium hydroxide/sodium bicarbonate solutions or by column chromatography on silica gel or sephadex gave fractions with reduced activity.

The dried rind of kokum (*Garcinia indica*) fruit contains three important constituents viz. anthocyanin pigments, hydroxycitric acid and garcinol. Their isolation and identification were reported earlier[1,2]. Garcinol is a polyisoprenyl phenolic pigment present to the extent of 2 – 3 per cent in the dried rind of kokum. In the present study, the antioxidant efficacy of kokum powder, its extractives as such and after fractionation, garcinol and isogarcinol have been evaluated, based on their ability to supress the development of rancidity (peroxide value) in refined groundnut oil. Tertiary butyl hydroxyquinone (TBHQ, Tenox Codex) was used as reference at 0.02 per cent level. Peroxide value (PV) was determined according to the AOAC method[3].

Peroxide Value (PV) determination: Six to seven ml portions of experimental/control samples were introduced into petri plates of 7.5 cm diameter and kept in an incubator maintained at 75 ± 2°C. Samples were periodically analysed for PV. The samples which had developed rancidity were withdrawn from further analysis.

a) *Antioxidant activity of kokum rind directly extracted into oil:* Thirty grams of kokum powder dispersed in 50 ml oil was ground thoroughly using a triple roller. The slurry was left overnight at room temperature and centrifuged at 2000 rpm; 35 ml clear oil could be decanted. Two ml of this was diluted to 100 ml with oil and used for PV estimation. There was clear indication that kokum rind contained antioxidant principles that could be extracted into oil as seen from the reduced P.V. i.e. 24.0 as against 163.0 for control and 7.7 for TBHQ after 120 hr incubation. It was observed that when soaked in oil, kokum imparted a light yellow colour to oil but there was no noticeable change in taste.

b) *Antioxidant activity of kokum extracts obtained by successive extraction of kokum powder with various solvents:* One hundred and eighty grams of kokum powder was successively extracted with hexane, ether and acetone in a soxhlet extraction unit for 20 hr each. The extracts were concentrated individually.

The hexane extract (1 litre) was concentrated to about 400 ml and left in the refrigerator for 48 hr to facilitate garcinol separation and crystallisation. The mother liquor was decanted and desolventised. The viscous brown mass obtained was tested for antioxidant activity at 0.2 per cent level.

The ether and acetone extracts were desolventised to get a brown viscous liquid and antioxidant efficacy tested at 0.2 per cent level. The residue left after extraction with the above solvents was soaked in oil (at 5 per cent level) for 3 days with occasional stirring, decanted and tested for antioxidant activity.

The results are presented in Table 1. The initial PV was 10.9 while after 204 hr it was 30.0 and 15.5 for the hexane extract and TBHQ incorporated oil respectively. The control sample had an initial PV of 10.5 and at 116 hr it was 163.0 and the sample had turned rancid. The ether and acetone extract samples were very similar to control. These data show that antioxidant constituents are extracted completely by hexane. Preliminary extraction studies indicated that the antioxidant constituents of kokum rind could be extracted individually into various solvents like hexane, acetone, ether, ethyl acetate or ethanol. The spent residue (left after soxhlet extraction) when

TABLE 1. ANTIOXIDANT ACTIVITY OF FRACTIONS OBTAINED BY SOXHLET EXTRACTION.

Sample	PV after indicated periods of incubation (meq/kg)					
	Initial	48 hr	116 hr	140 hr	164 hr	204 hr
Hexane extract	10.9	12.8	18.0	19.0	23.0	30.0
Ether extract	11.0	33.5	112.0	300.0	550.0	–
Acetone extract	10.6	37.5	119.0	500.0	530.0	–
Spent residue, oil extracted	11.1	28.0	113.0	300.0	600.0	–
Control	10.5	40.0	163.0	500.0	600.0	–
TBHQ	10.9	10.5	11.5	12.2	14.0	15.5

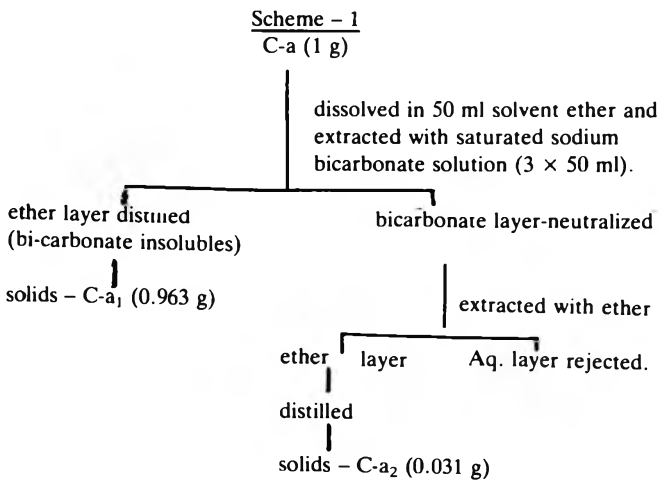
Results are average of duplicate determinations.
(–) indicates sample withdrawn.

extracted into oil did not possess noticeable antioxidant activity. Since only the hexane extract was found effective, further studies were confined to this.

c) *Antioxidant activity of fractions of hexane extract:* Sixty three grams of fine kokum powder was soxhleted for 20 hr using hexane to get 150 ml extract. This was extracted with 2 per cent sodium hydroxide (3 × 50 ml). The alkaline extracts were pooled, neutralised with HCl and extracted with solvent ether (3 × 60 ml). The ether extracts were pooled dried over anhydrous sodium sulphate, filtered and concentrated to get 3.66 g of a powdery material (C-a). The hexane layer was also dried over anhydrous sodium sulphate and concentrated to get 0.83 g. of a dark brown mass (C-b). C-a and C-b were separately dispersed in the oil at 0.2 per cent level, and tested for antioxidant activity.

The antioxidant efficacy of alkali soluble (C-a) and alkali insoluble (C-b) fractions of hexane extract is presented in Table 2. Fraction C-a possessed strong activity (P.V. 37) similar to TBHQ (P.V. 20) upto 168 hr. C-b was only slightly superior to control and very much poorer than C-a. This indicated that the antioxidant principles were alkali soluble.

Since C-a possessed good antioxidant activity it was subjected to further fractionation according to scheme-1. The fractions C-a_1 and C-a_2 so obtained were tested for PV.



C-a_1 possessed a better antioxidant activity compared to C-a_2 (Table 3). However C-a_1 itself was inferior to C-a (Table 2), the corresponding PV being 150.0 (144 hr) and 37.0 (168 hr). This indicated that the antioxidant activity got lowered on partition.

d) *Antioxidant activity of column chromatographic fractions from C-a_1:* C-a_1 was subjected to column chromatography using silica-gel and Sephadex LH-20 as adsorbents. When fractionated over a silica column after elution with benzene containing progressively more and more methanol to increase the polarity, it was seen that most of the fractions were devoid of antioxidant property except the first where slight activity was indicated. Similar fractionation on Sephadex column indicated only some mild activity in the middle fractions.

TABLE 2. ANTIOXIDANT ACTIVITY OF HEXANE EXTRACT FRACTIONS.

Sample	PV after indicated periods of incubation (meq/kg) Initial	55 hr	96 hr	125 hr	168 hr	288 hr
Fraction C-a	13.8	12.2	15.5	17.9	37.0	550.0
Fraction C-b	14.3	41.0	73.0	122.0	640.0	–
Control	13.7	44.0	103.8	650.0	–	–
TBHQ	14.1	13.8	15.3	15.6	20.0	82.0

Results are average of duplicate determinations.
(–) indicates sample withdrawn.

TABLE 3. ANTIOXIDANT ACTIVITY OF FRACTIONS C-a_1 and C-a_2.

Sample	PV after indicated periods of incubation (meq/kg) Initial	72 hr	144 hr
C – a_1	4.5	19.6	150.0
C – a_2	7.9	64.0	700.0
Control	6.3	46.0	600.0
TBHQ	6.1	7.7	21.0

Results are average of duplicate determinations.

e) *Antioxidant activity of garcinol and isogarcinol:* Garcinol and iso-garcinol were prepared in the laboratory according to the method of Krishnamurthy et al[2], and were used at 0.02 per cent level for PV estimation. Garcinol did not possess any antioxidant activity and on some occasions it behaved like a pro-oxidant. The PV value for garcinol, control and TBHQ were 280, 150 and 7 respectively after 72 hr incubation. Iso-garcinol, the colourless isomer of garcinol possessed weak antioxidant activity.

The authors are thankful to Dr. Y.S. Lewis, (Retd) C.F.T.R.I., Mysore and to Dr. B. Ravindranath, United Breweries, Bangalore for useful discussions.

References

1. Krishnamurthy, N., Lewis, Y.S. and Ravindranath, B., Chemical constituents of kokum fruit rind. *J. Fd Sci. Technol.*, 1982,**19**, 97.

2. Krishnamurthy, N., Lewis, Y.S. and Ravindranath, B., On the structures of garcinol, iso-garcinol and camboginol. *Tetrahedron Lett.*, 1981, **22**, 793.

3. *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington. D.C. 1980, 13th Edn., 440.

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 46–48

TRYPSIN INHIBITOR AND HEMAGGLUTININ ACTIVITIES IN CHICKPEA (*CKER ARIETINUM L.*): EFFECTS OF HEAT AND GERMINATION

K.K. BANSAL, K.S. DHINDSA AND V.I.P. BATRA*
Department of Chemistry and Biochemistry,
Haryana Agricultural University, Hissar – 125 004, Haryana, India.

Received 29 November 1986; *revised* 3 *June* 1987

Chickpea (*Cicer arietinum* L.) seeds of five different cultivars were analyzed for trypsin inhibitor activity and hemagglutinin activity and the effects of heat treatment and germination on the activities of these antinutritional factors were investigated. Activities of these factors were at the same level among the cultivars studied. Heat treatment and germination decreased the activities of both trypsin inhibitor and hemagglutinin. Trypsin inhibitor activity was eliminated completely by heating chickpea seeds in boiling water for 20 min. Complete elimination of the low level of hemagglutinin activity could be achieved by dry heating for 1 hr, heating in boiling water for 10 min, and on germination of seeds for 8 days. Overnight soaking of seeds in water removed as much as 23% of trypsin inhibitor and 50% of hemagglutinin activity.

Pulses constitute a cheap source of protein for human nutrition. The antinutritional factors present in raw pulses are partly removed during domestic and industrial processing, resulting in improved nutritive quality[1,2]. Dry and moist heating at normal or increased pressure are major steps in processing procedures. Pulses are also consumed after soaking in water or as sprouts by a section of the population. Effects of heat and germination on antinutritional factors in pulses have been reported[3-5]. However, the major emphasis so far, has been on soybean and the *Phaseolus* species. Little information is available on this aspect in chickpea (Bengal gram, *Cicer arietinum* L.) – one of the more important pulse crops of India. We report in this communication, the effects of several heat treatments, soaking and germination on trypsin inhibitor activity and hemagglutinin activity in several cultivars of chickpea.

Chickpea seeds of four local (*Desi*) cultivars viz. 'C-235,' 'G-130', 'H-82-19' and 'S-208', and one *kabuli* cultivar viz. 'HK-81-69' were obtained from pulses section, Department of Plant Breeding, Haryana Agricultural University, Hissar. The seeds were freed of husk, stones, etc. A portion of the seeds of each cultivar was ground to fine flour. The seeds or the flour were subjected to further treatment and analysis as required.

Trypsin inhibitor and hemagglutinin activities were determined according to the methods described elsewhere[6,7].

Procedures followed for different heat treatments and germination have been detailed earlier[6]. The flour samples were subjected to dry heating (121°C, 1 and 2 hr) and autoclaving (120°C, 15 psi, 10 and 20 min). The seeds were heated in boiling water for 10 and 20 min. Prior to germination, the seeds were soaked overnight (24 hr) in distilled water. The soaked seeds were germinated in a BOD incubator at 30°C upto 8 days.

Different cultivars of chickpea had almost the same level (358–376 units) of trypsin inhibitor activity (TIA) (Table 1). Singh *et al*[2] have, however, observed that chickpea seeds of local (*Desi*) cultivars have higher TIA, as compared to *Kabuli* cultivars. Dry heating of chickpea flour for one hour caused substantial decrease in TIA (Table 1). Different cultivars were affected to different extent by dry heating. The loss of activity was maximum (90 per cent) for 'HK-81-69' (*Kabuli*) and minimum (55 per cent) for 'G-130'. Further loss of TIA occurred when the time of heating was extended to 2 hr. The resultant activity was only 3 to 10 per cent of that in untreated flour, and the *Kabuli* cultivar ('HK-81-69') recorded maximum loss of TIA.

Autoclaving chickpea seeds for 10 min decreased TIA in different cultivars to different extents (Table 1). The cv 'G-130' was most resistant and cv 'HK-81-69' (*Kabuli*) was most susceptible. Prolonging the time of autoclaving to 20 min caused marked decrease in TIA and the loss in TIA was similar among the cultivars. Sharp decline (73-90 per cent) in TIA occurred, when chickpea seeds were heated in boiling water for 10 min, and the activity was completely lost after 20 min of heating (Table 1).

Soaking of chickpea seeds in water, resulted in substantial decrease in TIA (Table 2). Germination of soaked seeds caused only a slight further decline in activity. At 6 days after germination, maximum loss in TIA occurred in cv 'H-82-19' (37 per cent) and minimum in 'C-235' (24 per cent).

Hemagglutinin activity (HA) was at a very low level in all the cultivars of chickpea analysed in the present investigation (Table 3). The activity decreased further to 50 per cent of that in untreated samples, on soaking the seeds for 24 hr in water. The activity of soaked seeds remained unchanged on germination upto 4 days and disappeared completely in 8 days old seedlings. HA was lost completely also on dry heating of seeds for one hour and heating in boiling water for 10 min

*To whom all correspondence should be addressed

TABLE 1. EFFECT OF HEAT TREATMENT ON TRYPSIN INHIBITOR ACTIVITY* OF CHICKPEA SEEDS

Cultivar	Control (untreated flour)	Dry heating		Autoclaving		Boiling	
		1 hr	2 hr	10 min	20 min	10 min	20 min
C-235	370 ± 21	121 ± 6 (67.3)	35 ±3 (90.5)	313 ± 23 (15.4)	23 ± 2 (93.8)	66 ± 5 (82.2)	Nil (100.0)
G-130	364 ± 12	165 ±8 (54.7)	23 ± 2 (93.7)	340 ± 30 (6.6)	23 ±2 (93.7)	44 ± 4 (88.0)	Nil (100.0)
H-82-19	370 ± 25	55 ± 5 (85.1)	12 ±1 (96.8)	223 ± 10 (39.7)	12 ± 1 (96.8)	80 ± 6 (78.4)	Nil (100.0)
S-208	358 ±10	151 ± 10 (57.8)	35 ± 2 (90.2)	226 ±21 (36.9)	12 ± 2 (96.6)	35 ±4 (90.2)	Nil (100.0)
HK-81-69 (*Kabuli*)	376 ± 24	37 ±3 (90.2)	12 ±1 (96.8)	114 ±13 (69.7)	36 ±3 (90.4)	101 ±8 (73.1)	Nil (100.0)

Values in parentheses indicate % loss of activity
Mean ± SD of four determinations.
* μ mol tyrosin released/g of material.

TABLE 2. EFFECT OF PRESOAKING AND GERMINATION ON TRYPSIN INHIBITOR ACTIVITY* OF CHICKPEA SEEDS

Cultivar	Control (untreated flour)	Soaking	Germination					
			1 day	2 days	3 days	4 days	5 days	6 days
C-235	370±21	286±24 (22.7)	238±22 (35.7)	247±23 (33.3)	275±10 (25.7)	298±25 (19.5)	234±21 (36.8)	282±30 (23.8)
G-130	364±12	273±21 (25.0)	239±22 (34.4)	268±25 (26.4)	211±15 (42.0)	281±29 (22.8)	271±25 (25.6)	241±24 (33.8)
H-82-19	370±25	287±26 (22.5)	304±28 (17.8)	285±28 (23.0)	278±30 (25.0)	282±26 (23.8)	254±15 (31.4)	232±20 (37.3)
S-208	358±10	264±25 (26.3)	256±26 (40.0)	215±20 (40.0)	264±17 (26.3)	256±20 (28.5)	269±17 (25.0)	239±24 (33.3)
HK-81-69 (*Kabuli*))	376±24	314±28 (16.5)	256±24 (32.0)	256±15 (32.0)	250±14 (33.5)	234±22 (37.8)	225±14 (40.0)	236±21 (37.2)

Values in parentheses indicate % loss of activity
Mean ± SD of four determinations.
* μ mol tyrosin released/g of material.

TABLE 3. EFFECT OF HEAT TREATMENTS, PRESOAKING AND GERMINATION ON HEMAGGLUTININ ACTIVITY (DILUTION FACTOR FOR OBSERVABLE AGGLUTINATION) IN CHICKPEA

Cultivar	Control (untreated) flour	Boiling	Heating	Presoaking	Germination (days)			
		10 min	1 hr	24 hr	2	4	6	8
C-235	4	Nil	Nil	2	2	2	Nil	Nil
G-130	4	Nil	Nil	2	2	2	Nil	Nil
H-82-19	4	Nil	Nil	2	2	2	Nil	Nil
S-208	4	Nil	Nil	2	2	2	2	Nil
S-208	4	Nil	Nil	2	2	2	2	Nil
HK-81-69 (Kabuli)	4	Nil	Nil	2	2	2	2	Nil

Each value is mean of duplicates.

(Table 3).

TIA in pulses may be resistant[8] or susceptible[4,9] to heat. Purified trypsin inhibitors are, in general, resistant to heat[10,11]. Since trypsin inhibitor is a biologically active protein molecule, heat may change its native conformation, depending on micro-environment of the molecule. Much information is not available on the effects of heat and germination on HA of pulses in general, and chickpea in particular. de Muelenaere[12] found about 30 per cent decrease in HA on dry heting of soybean and *Phaseolus vulgaris* spp. Chen *et al*[13] observed consistant decrease in HA of peas and several beans with progress of germination. However, little is known about the biochemical basis of these changes.

In conclusion, it may be stated that trypsin inhibitor activity and hemagglutinin activity in chickpea seeds are decreased by heat treatment and germination. The TIA and HA activity are eliminated completely on heating the seeds in boiling water for 20 and 10 min, respectively.

The authors gratefully thank the Professor and Head, Pulses Section, Department of Plant Breeding, for supplying seeds; Professor and Head, Department of Chemistry and Biochemistry for providing research facilities, and the Indian Council of Agricultural Research for financial assistance.

References

1. Kakade, M.L. and Evans, R.J., Nutritive value of navy beans (*Phaseolus vulgaris*). *Br. J. Nutr.*, 1965, **19**, 269.
2. Singh, U. and Jambunathan, R., Studies on *desi* and *kabuli* chickpea (*Cicer arietinum* L.) cultivars: Levels of protease inhibitors, levels of polyphenolic compounds and *in vitro* protein digestibility. *J. Fd. Sci.*, 1981, **46**, 1364.
3. Liener, I.E., *Toxic Constituents of Plant Foodstuffs*. Academic Press, New York, 1980.
4. Gupta, K. and Wagle, D.S., Changes in antinutritional factors during germination in *Phaseolus mungoreous*, A cross between *Phaseolus mungo* (M_{1-1}) and *Phaseolus aureus* (T_1). *J. Fd. Sci.*, 1980, **45**, 394.
5. Khokher, S. and Chauhan, B.M., Antinutritional factors in mothbean (*Vigna aconitifolia*): varietal differences and effects of methods of domestic processing and cooking. *J. Fd. Sci.*, 1986, **51**, 591.
6. Batra, V.I.P., Vasishta, R. and Dhindsa, K.S., Effect of heat and germination on trypsin inhibitor activity in lentil and pigeon pea. *J. Fd. Sci. Technol.*, 1986, **23**, 260.
7. Vasishta, R., Dhindsa, K.S. and Batra, V.I.P., Pigeon pea strains deficient in phytohemagglutinin activity. *Curr. Sci.*, 1986, **55**, 1236.
8. Ghorpade, V.M., Kadam, S.S. and Salunkhe, D.K., Thermal stability and changes in trypsin inhibitor during germination and cooking of horse gram. *J. Fd. Sci. Technol.*, 1986, **23**, 164.
9. Tan, N., Wong, K. and delumen, B.O., Relationship of tannin level and trypsin inhibitor activity with the *in vitro* protein digestibility of raw and heat treated winged beans (*Psophocarpus tetragonolobus*). *J. agric. Fd. Chem.*, 1984, **32**, 819.
10. di Martino-Ferrer, M. and Ferrer, A., Trypsin inhibitor from bambara pea. *Phytochemistry*, 1983, **22**, 813.
11. Tsukamoto, I., Miyoshi, M. and Hamaguchi, Y., Purification and characterization of three trypsin inhibitors from beans, *Phaseolus vulgaris* "Kinotiki". *Cereal Chem.*, 1983, **60**, 281.
12. de Muelenaere, H.J.H., Effect of heat treatment on the hemagglutinating activity of legumes. *Nature, Lond.*, 1964, **201**, 1029.
13. Chen, L.N., Thacker, R.R. and Pan, S.H., Effect of germination on hemagglutinin activity of pea and bean seeds. *J. Fd Sci.*, 1977, **42**, 1666.

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 49–50

WATER VAPOUR LOSSES FROM DIFFERENT REGIONS OF ONION *(ALLIUM CEPA L.)* BULB DURING STORAGE

V. THAMIZHARASI AND P. NARASIMHAM
Central Food Technological Research Institute,
Mysore-570 013. India

Received 11 *February* 1987; *revised* 15 *May 1987*

Water vapour loss from different regions of onion bulb during storage for 45 days at (i) 21-35°C, RH 2%, and (ii) 21-35°C (ambient RH 50-80) were determined by blocking the neck and base regions. Onions stored at (i) showed maximum loss of water vapour when both neck and base were blocked, whereas in onions stored at (ii), the losses showed the reversed trend. Further, contrary to the established belief that loss of water vapour occurs mainly through the neck region of the bulb, it was found that it is largely through the sides although losses do occur through neck and base regions. Of the total loss that occurred in the first three days, 41% was from the side, which increased to over 90% during the 45 days of storage under both conditions.

During the storage of onions at ambient temperature (above 20°C) and humidity, the extent of sprouting is low because of high temperature that maintains dormancy[1,2]. However, the losses due to desiccation are high reaching up to 30 per cent during storage for five months[3]. When the outer scale of the bulb dries up due to water loss, the water from the inner scale evaporates. Woodman and Barnel[4] reported that loss of water vapour occurs mainly through the neck region of the bulb. Further, Karmarkar and Joshi[2] observed that immature bulbs lose more weight and therefore, concluded that bulbs with thicker neck allowed greater evaporation of water from the interior. These reports suggest that, although the surface area of the bulb is quite large, the loss of water occurs through a relatively smaller area covered by the neck which is quite surprising. The present study was, therefore, undertaken to find out the role of the outer dry scale as well as the exposed portion of the stem (base) of the onion bulb in the water vapour loss.

Fresh onions (cv 'Pune Red') of medium size (50–70 g) free from visible infections, with the outer dry scale intact, were obtained from the local market and used. For one batch of 20 onion bulbs, the neck was trimmed and sealing wax (mixture of 60 g of microcrystalline and 40 g of paraffin waxes) was applied to prevent the water vapour loss through the neck. For another lot of 20 onions, both the neck and base were similarly blocked. Yet another lot of 20 onion bulbs without any blocking served as controls. Ten bulbs from each of these three lots were kept stored: (i) at ambient temperature (21–35°C) and 2 per cent RH (anhydrous $CaCl_2$) in a desiccator or (ii) at ambient temperature (21–35°C) at 50 to 80 per cent RH in a basket for 45 days.

Weight loss, rooting and intactness of the dry scale of the bulbs were observed at intervals. From the data for weight losses, the loss through different parts of the bulb was calculated taking the loss in weight of bulb without blocking as W_1, loss in weight of bulb with neck alone blocked as W_2 and loss in weight of bulb with neck and base blocked as W_3. Then, W_3 gives the loss through side, $W_1 - W_2$ the loss through neck and $W_2 - W_3$ the loss through base.

As indicated in Table 1, loss in weight of bulbs not blocked (whole) was more at 2 per cent RH than those held at fluctuating ambient RH of 50 – 80 per cent. Whereas at 2 per cent RH, blocking the neck alone or blocking both neck and base, resulted in progressively lesser loss compared to controls, at RH of 50-80 per cent progressively higher losses were observed. This

TABLE 1. CUMULATIVE PHYSIOLOGICAL LOSS IN WEIGHT (PER CENT) OF ONION BULBS DURING STORAGE

Storage period (days)	Stored at 2% RH			Stored at 50-80% RH		
	Whole bulb	Neck blocked	Neck & base blocked	Whole bulb	Neck blocked	Neck & base blocked
3	1.06±0.04	0.72±0.04	0.44±0.02	0.67±0.07	0.72±0.06	1.15±0.08
5	1.51±0.07	1.12±0.07	0.81±0.07	1.11±0.11	1.40±0.06	1.80±0.08
7	1.96±0.11	1.48±0.07	1.15±0.10	1.57±0.16	1.61±0.11	2.60±0.21
10	2.65±0.18	2.07±0.12	1.60±0.15	1.82±0.17	1.94±0.15	2.96±0.20
15	3.35±0.21	2.57 ±0.22	1.76±0.18	2.16±0.22	2.18±0.24	3.23±0.27
30	5.30±0.27	4.59±0.32	4.24±0.27	3.12±0.17	4.42±0.30	8.72±0.75
45	7.19±0.33	7.14±0.41	6.57±0.33	4.8±0.15	6.53±0.38	10.0±1.75

Values are mean ± SE of 10 bulbs

TABLE 2. CUMULATIVE PHYSIOLOGICAL LOSSES IN WEIGHT (PER CENT) FROM DIFFERENT REGIONS OF ONION BULBS DURING STORAGE

Storage period (days)	Neck	Base	Side
3	0.36 (34)	0.26 (24)	0.44 (42)
5	0.39 (26)	0.31 (20)	0.81 (54)
7	0.48 (22)	0.33 (18)	1.15 (60)
10	0.58 (22)	0.47 (18)	1.60 (60)
15	0.78 (23)	0.81 (24)	1.76 (53)
30	0.71 (13)	0.35 (15)	4.24 (72)
45	0.05 (1)	0.57 (8)	6.57 (91)

Bulbs stored at 21-35°C and RH 2%
Figures in parentheses indicate per cent loss

could be attributed to the differences in vapour pressure deficits and also difference in the total pressure of air on the two sides of the outer scales of the bulb.

The actual weight losses from the different regions of the onion bulbs kept at RH of 2 per cent indicate that the major contribution to the total loss is by the sides during storage (Table 2). Further, the loss in weight through sides, when expressed as cumulative per cent of total loss was around 40 per cent on the third day of storage which reached upto 91 per cent in 45 days. The loss of water vapour through the base was about the same as through the neck region of the bulb (Table 2).

It may also be noted that in onion bulbs in which the base was blocked, slight rooting was noticed in some bulbs (43 per cent at fluctuating RH and 21 per cent of bulbs at constant RH) at 10th day ($\leq$ 0.5 cm length) and then suppressed. It may not be sufficient enough to account for differences in the losses attributed to blocking of bulbs observed in this study. Breakage of the outer dry scale of onions during storage was noticed but there was no definite trend to draw inferences.

Hence, the results represented in Tables 1 and 2 on the relative losses of water vapour through the neck, base and side regions of the bulb were further confirmed in a separate experiment using onions in which the outer dry scale was removed in order to prevent the breakage of the dry scale during storage. Even here, through the 40 days storage period, the per cent loss in weight from the sides of the bulbs was more than that from the neck (Table 3). The loss through the base region of the bulb was higher in the first ten days due to more exposure of the stem caused by removal of the outer scale. Further in these bulbs, the weight losses were also higher than those in which the outer scale was removed, which is due to the exposure of the fleshy scale.

Therefore, it can be concluded that the loss in water vapour from the onion bulbs during storage occurs largely through the sides.

The first author (VT), is grateful to CSIR for the award of a fellowship. The authors are grateful to K.V.R. Ramana, for going through the manuscript. Thanks are due to M.V. Patwardhan and B. L. Amla, Director, CFTRI for their keen interest in this work.

References

1. Abdalla, A.A. and Mann, L.K., Bulb development in the onion (*Allium cepa* L.) and the effect of storage temperature on the bulb rest. *Hilgardia*, 1963, **35**, 85.

2. Karmarkar, D. V. and Joshi, M.M., Investigations on the storage of onions. *Indian J. Agric. Sci.*, 1941, **11**, 82.

3. Paul Thomas, Padwal-Desai, S. R. Srirangarajan, A.N., Joshi, M.R. and Janave, M.T., Pilot scale storage tests on the efficacy of gamma irradiation for sprout inhibition of onions under commercial storage conditions. *J. Fd Sci. Technol.*, 1985, **23**, 79.

4. Woodman, R.M. and Barnell, H.R., The connection between the keeping qualities of commercial varieties of onions and the rates of water loss during storage. *Ann. appl. Biol.*, 1937, **24**, 219.

TABLE 3. CUMULATIVE PHYSIOLOGICAL LOSS IN WEIGHT (PER CENT) OF PEELED ONION BULBS DURING STORAGE AT 2 PER CENT RH AND 21-35°C

Storage period (days)	Whole bulb	Neck blocked	Both neck & base blocked	Neck	Base	Side
2	1.27±0.08	1.18±0.13	0.47±0.07	0.09 (7)	0.71 (56)	0.47 (37)
5	3.10±0.15	2.46±0.20	1.28±0.13	0.64 (21)	1.18 (37)	1.28 (42)
10	4.41±0.21	3.76±0.56	1.31±0.27	0.65 (15)	2.45 (55)	1.31 (30)
25	9.30±0.59	7.91±0.79	6.85±0.67	1.39 (15)	1.06 (11)	6.85 (74)
40	11.07±0.82	11.73±1.10	12.51±1.96	–0.66 (–6)	–0.78 (–7)	12.51 (113.0)

The outer dry scale which was prone to breakage was removed exposing the more fleshy inner scale. Values are mean ± SE of 10 bulbs.
Figures in parentheses indicate per cent loss from different regions.

J. Fd. Sci. Technol., 1988, Vol. 25, No. 1, 51–52

DISCRIMINATION OF ANIMAL MEATS BY ENZYME ASSAY

A. BHATTACHARYYA, M. MUKHOPADHYAY AND D.R. CHAUDHURI
Department of Food Technology and Biochemical Engineering, Jadavpur University, Calcutta–700 032, India

Received 8 *December* 1986; *revised* 1 *June* 1987

Fresh meat samples from the caprine group can be discriminated from those of the bovine group by assaying Mg^{++} ATPase and peroxidase, the activities of which are significantly higher in the latter group. The individual species of the bovine group can be identified by phosphatase and catalase activities while those of the caprine group can be identified by succinic dehydrogenase and alkaline phosphatase activities.

Identification of fresh meat from different species is desirable either from the view point of misbranding and/or on religious grounds. Several attempts have been made to identify meats using serological methods[1], electrophoresis,[2,3] immunological methods[4-6] and Rf value of the myoglobin band[7]. In the present study, an attempt has been made to detect specific fresh meat samples by assaying activities of different enzymes.

Meat samples obtained from a nearby slaughter house were brought to the laboratory keeping in ice. The samples were homogenised in 10 mM phosphate buffer, pH 7.4, with sea sand. The volume of the extract was adjusted to make it 10 per cent with isotonic saline (pH 7.0). It was subsequently centrifuged at 10,000 × G for 40 min at 4°C. The supernatant termed as 'meat extract' was used for enzyme assays.

Bovine serum albumin (BSA), adenosine triphosphate (ATP), 0 – dianisidine and dichlorophenolindophenol were purchased from the Sigma Chemical Co., USA and p – nitrophenyl phosphate (PNPP) was procured from E. Merck, West Germany.

The protein content of the meat extracts was estimated according to the method of Lowry *et al*[8] using BSA as a standard.

Acid and alkaline phosphatases were estimated in the different meat extracts following the method of Michell *et al*[9]. The specific activities were expressed as the μ g of p – nitrophenol released/mg of protein/hr. Peroxidase activity was estimated according to the method of Mahely and Chance.[10] The specific activity of the enzyme was expressed as the change in O.D/mg protein/hr. Activity of catalase was assayed following the method of Beer and Sizer[11]; the specific activity was defined as the change in O.D/mg protein/hr. The activity of Mg^{++} ATPase was estimated according to the method of Lowry[12]. The specific activity of the enzyme was expressed as the μ mole of Pi liberated/mg protein/hr. Succinic dehydrogenase was assayed following the method of Earl;[13] the specific activity was defined as the μ mole of indophenol reduced/mg protein/hr.

Table 1 represents the different enzyme activities in goat, sheep, cattle and buffalo meat extracts. The activities of phosphatases (both acid and alkaline) were found to be lowest in buffalo muscle while the activities of alkaline phosphatase were significantly higher in goat ($P \leq 0.05$) and cattle meat ($P \leq 0.001$) in comparison to those in sheep and buffalo meat extracts. Though catalase activities in goat and sheep muscle showed no appreciable differences, it was significantly ($P \leq 0.05$) higher in buffalo muscle than that in cattle muscle.

It is evident that the activities of Mg^{++} ATPase and

TABLE 1. PHOSPHATASE, CATALASE ATPASE, SUCCINIC DEHYDROGENASE AND PEROXIDASE ACTIVITIES IN DIFFERENT FRESH MEAT MUSCLE EXTRACTS

Enzymes	Goat	Sheep	Cattle	Buffalo
Acid phosphatase*	0.91 ± 0.04 (6)	0.63 ± 0.30 (6)	0.83 ± 0.40 (6)	0.38 ± 0.01 (6)
Alkaline phosphatase*	0.82 ± 0.04 (6)	0.49 ± 0.01 (6)	0.77 ± 0.02 (6)	0.39 ± 0.01 (6)
Peroxidase**	11.10 ± 2.30 (7)	7.53 ± 1.76 (7)	14.04 ± 3.11 (6)	14.75 ± 2.95 (7)
Catalase**	28.98 ± 5.38 (7)	29.76 ± 3.45 (5)	23.35 ± 4.38 (7)	37.26 ± 2.44 (6)
Mg^{++} ATPase***	8.54 ± 1.01 (7)	13.01 ± 0.95 (6)	21.67 ± 1.14 (6)	32.14 ± 2.14 (6)
Succinic dehydrogenase‡	0.19 ± 0.04 (4)	0.23 ± 0.09 (6)	0.10 ± 0.01 (5)	0.15 ± 0.06 (6)

Values are means ± SEM of the number of observations as indicated in parentheses.
Units: * μg PNP released/mg of protein/hr.;
**Change in O.D/mg of protein/hr.:
*** μ moles Pi released/mg of protein/hr,
‡ μg indophenol reduced/mg protein/hr

peroxidase are higher in the bovine muscle group (cattle and buffalo) by about two times that in the caprine group (goat and sheep). On this basis, meat samples can easily be categorised into two groups. Once this has been done, identification of individual species within the groups can be accomplished as indicated below:

Catalase and phosphatases (both acid and alkaline) activities are respectively higher and lower in fresh buffalo meat than that in cattle meat. Thus buffalo and cattle meat, both belonging to bovine group can be discriminated from one another. Again the activity of alkaline phosphatase is almost double in fresh goat muscle compared to that in sheep muscle, while succinic dehydrogenase activity is significantly higher in sheep muscle than that in goat. So, the individual species in the caprine group can also be identified.

The authors are grateful to the ICAR for financing the scheme.

References

1. Hashimoti, Y. and Yasmi, T., Researches on the detection of meat by serological test *J. Fac. Agric. Hokkaido.*, 1957, **50**, 171.
2. Lee, Y.B., Rickansurd, D.A., Hagberg, E.C. and Briskey, E.J., Application of SDS – polyacryl -amide gel electrophoresis for determination of the maximum temperature to which bovine muscles have been cooked. *J. Fd Sci.*, 1974, **39**, 428.
3. Bandyopadhyay, S.K., Sanyl, A. and Chaudhuri. D.R., Identification of different species of meat by gel electrophoresis of mitochondria. *J. Fd Sci. Technol.*, 1985, **22**, 373.
4. Hayden, A.R., Immunological detection of bovine, porcine and equine-flesh in beef products with antisera to species myoglobin. *J. Fd Sci.*, 1979, **44**, 494.
5. Helm, M.B., Warnecke, M.O. and Saffle, R.L., Gamma globulin isolated from rabbit antisera for rapid detection of meat adulteration. *J. Fd. Sci.*, 1971, **36**, 998.
6. Ozawa, S., Yano, S., Abe, T. and Mogi, K., Discrimination of animal species by immunological method II. Experiments on cooked meats. *Jap. J. Zootechn. Sci.*, 1972, **43**, 395.
7. Chin-sheng, C. and Parrish, F.C. Jr., Heat induced changes in myofibrillar proteins of bovine longissimus muscle. *J. Fd. Sci.*, 1979, **44**, 22.
8. Lowry, O.H., Rosebrough, N.J. Farr, A.L. and Randall, R.J., Protein measurement with Folin-phenol reagent. *J. biol. Chem.*, 1951, **193**, 265.
9. Michell, R.H., Karnovsky, M.J. and Karnovsky M.L., The distributions of some granule-associated enzymes in guinea pig polymorphonuclear leucocyte. *Biochem. J.*, 1970, **116**, 207.
10. Mahely, A.C. and Chance, B., In *Methods of Biochemical Analysis*. Vol. 1 by Glick, D.J. (Ed) Interscience Publ. Inc. New York, 1954, Vol. 1, 357.
11. Beers, R.F. Jr., and Sizer, I.W., A spectrophotometric method for measuring the breakdown of hydrogen peroxide *by catalase*. *J. biol. Chem.*, 1952, **195**, 33.
12. Lowry, O.H. in *Methods in Enzymology* by S.P. Colowick and N.O. Kaplan (Eds) . Academic press, New York, 1957. Vol. V, 372
13. Earl, D.C.N., The isolation and properties of cardiac ribosomes and polysomes. *Biochem. J.*, 1965. **94**, 721.

BOOK REVIEWS

Encyclopedia of Food Engineering: by Carl W. Hall, A.W. Farral and A. L. Rippen; M/S AVI Publishers 1986; pp: 880; Price: $ 135.

The first edition of the encyclopedia of food engineering was published in 1971. It fulfilled the longfelt need of food engineers and technologists to have a collection of information of day to day use from a single source. The second edition of the book, brought out by the same publishers in 1986, has greatly enhanced the usefulness of the book.

Besides the subjects covered in the first edition, the new edition includes a number of new topics; also, several topics have been rewritten and enlarged to include additional information. Some of the more important topics included in the new edition are: absorption spectroscopy, breakfast cereals, brewing of beer, candy and confectionery, computer systems in food processing plants, unit operations like dehydration, distillation, fermentation, fruit processing, potato processing, malting, meat processing, flour milling, ultrasonics, radiation sterilization, energy management, extrusion cooking, food process plant design and lay-out for vegetable processing and wine processing.

The chapter on computer systems for food processing plants introduces application of computer techniques for process control, process optimization, hardware structures, interfaces, microcomputers and their applications. The authors also discuss certain system softwares and their applications in routing and sequence control, inventory control and selected operations like blending. The chapter provides a good introduction to the area.

The chapter on dehydration discusses in brief the principles of drying foods by various drying techniques; several drying equipments like tunnel, spray, pneumatic fluidized bed, foam mat, freeze and drum dryers, besides the recent developments like microwave dryers.

There are four different chapters on energy; viz energy conservation, utilisation, energy from new techniques and energy content of foods. Under the topics on conventional energy sources, some strategies on optimal use of fossil and nuclear energies are also discussed. Several energy management systems like heat recovery and recirculation, insulation and its impact on energy consumption, heat generation from wind, solar energy, geothermal, gasohal and ocean energy are described. Also, an analysis of various processing operations and energy requirement for these operations is presented.

The chapter on plant design and lay-out discusses, besides general principles of good design, many constructional details like process plant floors, drains and electric installations.

Several features of potato processing and plants for processing of various other vegetables is described in fair detail in two separate chapters. The products covered include French fries, chips, flakes, dehydrated and canned vegetables.

S.I. units are used in the majority of the topics. However, convertions to F.P.S. units are provided in most cases within brackets. The encyclopedia is certainly a very useful compilation.

A. RAMESH
C.F.T.R.I., MYSORE

Detergency Theory and Technology; Volume 20, Surfactant Science Series: by W. Gale Cutler and Erik Kissa (Ed) Marcel Dekker Inc. New York; 1987; pp 550 Price: Bound Illustrated $99,75 (US & Canada): $119,50 (All other countries).

Volume 20 of the Surfactant Science Series pertains to the area of the largest industrial application of surfactants, viz. detergents, and covers many features of detergency. This is an update of the earlier volumes by Cutler & Davies and together with them will form a useful collection.

There are nine chapters: The first on Evaluation of Detergency by Kissa discusses relevant details like types of soils, test cloths, soiling methods, washing and evaluation methods and the pitfalls in using artificial soils and test cloths for drawing inferences about real article wash.

The next, on New Physical and Analytical Techniques in Detergency by R.A. Llenado is a brief recapture of techniques for analysis of detergents and is likely to be of limited use. The third on Radioisotope Techniques in Detergency by W.T. Shebs gives a concise but very informative description of the theory and experimental details of the tracer method in detergency evaluation.

The fourth and fifth chapters are by Kissa on the Kinetics and Mechanisms of Soiling and Detergency

and Soil Release. The former, a resume of contemporary theories and controversies on the subject, is likely to be more useful to researchers in selecting the right actives for each situation. The chapter on soil release captures the concepts involved in the manufacture of fabrics with soil releasing finishes.

Inorganic Builders by M.J. Schwuger and E.L. Smulders is a description of zeolite A and is followed by a chapter by W.M. Linfield on the various types of LSDAs and their LSDRs.

Detergency in Nonaqueous Systems by M. Wentz is likely to interest most in view of the scarcity of published literature in the area.

The final chapter, Detergency Advances in Japan by J. Mino is a summary of the rapid strides made in theoretical considerations of detergency by Japanese Detergent Chemists.

Many important areas like anti redeposition agents, bleaches and other novel concepts currently under intense research, however, find scant attention. On the whole, the volume is a useful review of current concepts with well over 900 references and will be a worthy addition to the existing literature on detergency in every library.

E.V. SRISANKAR
HINDUSTAN LEVER LIMITED
BOMBAY

11th International Scientific Colloquium on Coffee: Association Scientifique Internationale du Cafe (AIC), 42, rue scheffer, 75116 – Paris, 1986, pp: 696; Price: FF. 550.

The publication contains the lectures presented at the 11th International Scientific Colloquium on Coffee held at Lome (Togo), from 11-15 February, 1985, sponsored by ASIC.

There are 57 papers covering four categories: Physiology, Chemistry, Technology and Agronomy. The plenary lectures covered the potentials of modern biotechnology with special reference to its applications for the coffee plant; modern developments in gas chromatography; progress and development of coffee technology in the past twentyfive years; genetic improvement of coffee plants and cyto-embryological studies of coffee hybrids.

The subject matter covered in the various sessions was as follows: *Physiology* – pancreas, mutagenicity, effects on fruit fly and human caffeine consumption; *Chemistry* – flavour analysis, benzopyrene residues, organic acids, caffeine and chlorogenic acids, etc; *Technology* – survey and development of coffee technology, quick roasts and soluble yields, free and bound amino acids, influence of fermentation methods, influence of roasting temperatures on the chemical composition, comparison of the different methods of drying, etc., *Agronomy* – genetics, hybrids, cultural methods, fertilization trials, soil and leaf analysis, insect pests, etc.

There is also an exhaustive review dealing with chemistry, technology of coffee and its products covering recent patents and publications. This compilation would serve as a useful guide reflecting the trends of research in coffee.

M.L. SHANKARANARAYANA
C.F.T.R.I., MYSORE

Food Borne Micro-organisms and Their Toxins: Developing Methodology: Ed by Merle D. Pierson and Norman J. Stern, Marcel Dekker, Inc, 270, Madison Avenue, New York-10016; 1986; pp: 496; Price; $59.75 (US and Canada) $71.50 (All other countries).

Unlike in the past, today, food industry is one of the highly developed and organised industries which in coordination with the government agencies assures the quality and safety of food. These agencies, therefore, continually strive for economic but rapid, reproducible and accurate techniques for detection of micro-organisms and microbial toxins in food. As a result, the regulations, referenced methodologies, assessment of the potential health risk associated with food and industrial competition, form an integral part in the development of food microbiology. Of late, food microbiology has undergone substantial changes in the most important areas like methods of analysis of foods for detection of micro-organisms and their metabolic products as well as in interpretation of data. In this volume, the authors provide up-to-date knowledge and share their thoughts regarding methodology to detect microbial quality of food and specific pathogens and their toxic metabolites which might be present in food.

The newer non-cultural rapid or very rapid systems for detection of micro-organisms in food are impedimetric and bioluminescence ATP techniques. They have been successfully used in the laboratory and are vying for microbiologists' approval to be employed in the field or market place. These

techniques are described and discussed in detail with the future outlook. The other non-cultural techniques like Direct Epifluorescent Microscopy technique is also presented with due emphasis. The DNA probe system, a biotechnology, in vogue in contemporary biological sciences, is an exciting new approach for detection of virtually any micro-organism in food or environment. It also provides a means for discrimination between virulent and avirulent bacterial varieties. This technique is reviewed in the present volume with an idea to give basic introduction to the subject and as a guide to the investigator who is new in the field. The other exciting technique which finds its due place in this volume is ELISA. ELISA and other immuno assays can identify target organisms or their toxins. These tests are extremely precise when monoclonal antibodies are used, and can be employed to identify a specific virulent determinant to differentiate between pathogenic and innocuous microbes. The use of such highly specific techniques can help the processor to prevent the access of adulterated foods to the market place. In addition to discussing developments in instrumental methods, biotechnology and immunological techniques, advances in the knowledge of specific micro-organisms and their toxins are also elaborated.

Thought provoking and up-to-date information is also provided regarding regulatory criteria and means for predicting shelf life for food products. Regulating aspects both positive and negative of the microbiological methodology, various factors affecting microbiological criteria and the predictive modelling of food deterioration and safety are discussed at length. The very fundamentals of analytical techniques and the points to be pondered over while developing new methodology are brought out emphatically. In short, the book precisely brings out the perspectives and prospectives in food microbiology particularly from food safety and deterioration points of view. The book is highly recommended to food microbiologists, food scientists and students working in the concerned field.

USHA MANDOKHOT
H.A.U., HISSAR

Fundamentals and Applications of Freeze-Drying to Biological Materials, Drugs and Foodstuffs: Published by the International Institute of Refrigeration, 177, Boulevard Malesherbes, F-75017 Paris, (France 1985-1 Pages: 318; Price: 150 FF).

This book is the result of the meeting organised by the International Institute of Refrigeration at Tokyo Garden Palace from May 20th to 22nd, 1985, in which 129 participants from 11 countries participated. In this meeting, two plenary lectures were delivered and 40 papers on freeze-drying and freezing in the following 6 fields were presented: Basic aspects, Pharmaceuticals, Foodstuffs, Engineering, Micro-organisms and Medicine.

In Section 1, four papers dealing with the basic aspects of freezing and freeze-drying have been presented. Section 2 deals with freeze-drying of pharmaceuticals in which 3 papers were presented.

Freezing of foods which is an area of significance to food scientists and technologists is covered in Section 3 with 8 papers on different aspects in the following order:

1) Effect of lyophilization on sulfhydryl content in soybean protein and its application to gelation
2) Factors affecting the biological activity of lyophilized myofibrils – protective substances and collapse temperatures
3) Water sorption and retention of volatile components in dehydrated soy sauce and B-cyclodextrin
4) Two types of frozen and dried products; waxy rice and soybean products by the improved processes of freezing and drying
5) Studies on freezing and freeze-drying of food products for consumer marketing
6) Food container with closed cooling system operated by means of dry-ice
7) Present situation of freeze-dried foods in Japan
8) Study on storage and transportation of food products using controlled/modified atmosphere

Section 4 deals with engineering in freeze-drying and 10 papers on different aspects of freeze-drying engineering have been presented. Preservation of micro-organisms has been dealt with in Section 5; 10 papers on different aspects of preservation. The last Section with 6 papers deals with application of freezing in the medical field.

As such, freeze drying has not received as much attention as it deserves in India due to its exorbitant cost. So far, this has only been confined to foods and their preparations with delicate flavour and texture. In future, freeze-drying is going to receive importance in view of preserving some of the food products meant for export.

This book will be very useful to the scientists, academicians, industrialists and students engaged in research and applications of freeze-drying of various foodstuffs.

P.G. ADSULE
SMALL INDUSTRIES SERVICE INSTITUTE
MADRAS

AFST (1) News

Nagpur Chapter

The Annual General Body Meeting was held on 15th June 1987. The following office bearers were elected for 1987-88.

President	:	Shri A.K. Bhiwapurkar
Vice-President	:	Shri A.M. Pande
Hon. Secretary	:	Shri Dinesh Garg
Jt. Secretary	:	Mr. I.H. Ali
Hon. Treasurer	:	Dr. S.D. Bhalerao.

Palayamkottai Chapter

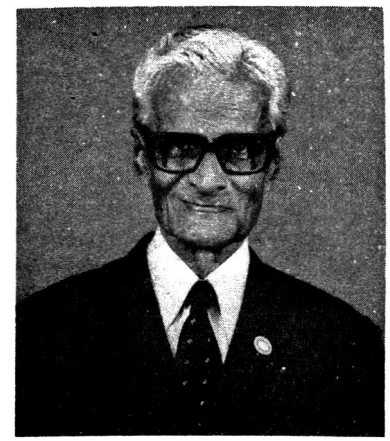
The Annual General Body Meeting of the above chapter was held on 27th June 1987. The following office bearers were elected for 1987-88:

President	:	Dr. T.K. Kandaswamy
Vice-President	:	Sri. Muthukumaraswamy
Hon. Secretary	:	Sri V. Theetharappan

The Annual General Body Meeting was held on 14th May 1987 and the following office bearers were elected for 1987-88.

President	:	Sri S.V Ramakrishna
Vice-President	:	Sri K.C.M. Raja
Hon. Secretary	:	Sri K. Muraleedharan
Hon. Jt. Secretary	:	Sri. P. Mohan,
Hon. Treasurer	:	Sri M. Sreekumar

OBITUARY



Dr. G. S. Siddappa retired Asst. Director, CFTRI and well a known authority in fruit and vegetable technology in this country passed away after a brief illness on the night of Sunday the 11th October '87.

Dr. Siddappa was born on 1st November 1906 at Singanallur of Karnataka State. After completion of Master's degree from Presidency College, Madras, he joined agricultural services of erstwhile Madras Province and was deputed to Bristol University in U.K. where he obtained his Doctorate Degree in Biochemistry in 1936. After his return to India, he served in various positions in the agricultural services division of pre-independent India in Madras as well as in Punjab (Layalpur) and Northwest Frontier Provinces (Quetta).

In the post-independence era, Dr. Siddappa joined the fruit and vegetable technology discipline of Central Food Technological Research Institute, Mysore in 1950 and worked on its staff till he attained superannuation in September 1967. Dr. Siddappa was the doyen of the Fruit and Vegetable Technology Course in India and retired as Chairman of International Food Technology Training Centre which was established in August 1965. He laid a sound foundation for the training programme in food technology and allied areas in CFTRI and was a successful teacher. He is one of the authors of the only Indian Text Book on Fruit and Vegetable Preservation, which has run into Second Edition, and is followed extensively by the food technology, horticulture and agricultural Institutions in India as a standard Text Book.

His research interest was essentially in the field of fruit and vegetable preservation including the areas like canning, fruit juice concentration and dehydration. He has published over 100 research and review papers in reputed journals both in India and abroad. After retiring from the Institute, he continued to work and give advice to the fruit and vegetable industry in various parts of India and was quite active even at the age of eighty.

Dr. Siddappa was the recipient of some of the highest awards in India, like Kashalkar Memorial Award of All India Food Preservers' Association and Prof. V. Subrahmanyan Industrial Achievement Award of the Association of Food Scientists and Technologists of India.

In his death, the Food Technology fraternity and the Fruit and Vegetable Preservation Industry in this country has suffered a severe loss which may not be easy to replace for many years to come.

ADDITIONAL INSTRUCTIONS FOR REPORTING RESULTS OF SENSORY ANALYSIS

1. *Objectives:* The objective of the study should be stated clearly.

2. *Sensory test methods:* The methods are classified under two major categories; Analytical and Affective. Laboratory analysis with trained or semi-trained panels must use analytical methods. Affective methods can be used in consumer studies. Adequate details and references should be provided regarding methods used for pattern of collection, analyses and interpretation of data.
Analytical (Trained Panel): The Major types of tests which can be made use of are:
Discriminative. Difference or similarity testing and sensitivity assessments using difference tests, ranking, thresholds, dilutions, etc.,
Descriptive/Quantitative: Flavour profiles, Texture profiles, Interval Scaling, Ratio Scaling, Descriptive Quality Scoring etc.,
Affective (Untrained/consumer Panels): Difference/Preference, Hedonic rating, FACT ratings-Preference rankings etc.,

3. *Experimental designs*: The designs used are to be clearly stated. e.g. Randomized block, Latin squares, Factorials, Fractional factorials, incomplete blocks and so on.

4. *Panel:* For analytical tests, the source of panel, whether inhouse or outside organisation to be indicated. The number of panelists should be stated, which should be normally not less than 15. Also whether the same panelists or different panelists have participated in testing the samples has to be indicated. Information on the composition (age, sex, etc) of the panel to be provided. The panel should be trained to function as a human analytical instrument, with periodic re-orientation and at required sensitivity.

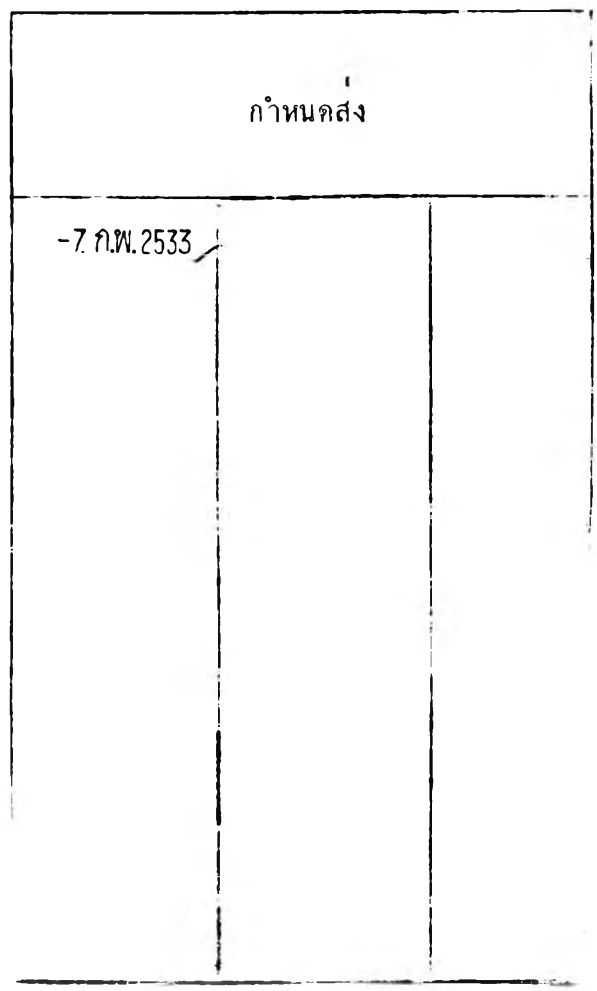
For affective test, the panel (sample of population) should be representative of target population selected on the basis of defined sampling procedures. The number should not be less than 200. The composition (age, sex,income group, etc) of the panel should be indicated.

5. *Physical requirements:* For the analytical tests, the laboratory set up should be reported e.g. conducted in a booth with soft neutral shade walls or separators, without distraction from external sound or odour, with comfortable room temperature (22° - 25°C) and relative humidity conditions (35-40%) and suitably illuminated.

The equipment and methods of sample preparation, testing temperature conditions, sample size and number of samples evaluated per panelist and per session should be reported.

The time of evaluating and sequence of testing and data entry cariers,if any, and nature of palate-clearing agents used should be indicated.

6. *Statistical analysis:* The data handling procedure should be appropriate to the design, and should be clearly indicated including any transformations or derivations that are carried out, e.g. assignment of numbers to intervals, categories and the like. The type of analysis carried out, categories, the level of significance and the decisions made are to be provided with appropriate tables and graphs. Appropriate and adequate data should be provided to justify conclusions and enable repeatability. For e.g. while reporting results of tests of significance, the relevant tests like F, x^2, t, r, Rank sum, Mann-whitney, Rank correlations and so on. The probability levels, degrees of freedom, the observed value of the test criterion, the direction of the effect and the decision based on these are to be indicated.


กำหนดส่ง
-7 ก.พ. 2533

INSTRUCTIONS TO AUTHORS

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

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

3. Names of chemical compounds and not their formulae should be used in the text. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Superscripts and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.

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

5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. They should be typed on separate sheets. Nil results should be indicated and distinguished clearly from absence of data, which is indicated by '—' sign. Tables should not have more than nine columns.

6. **Illustrations:** Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper not bigger than 20 cm (oy axis) $\times$ 16 cm (ox axis). The lettering should be such that they are legible after reduction to column width. Photographs must be on glossy paper and must have good contrast; *three copies* should be sent.

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

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

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

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

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

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

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

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

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

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

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

REG. NO. 24918/64

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Vol. 25. No. 2 Contents of forthcoming issue March / April 1988

Research Papers

Research Notes

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