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Volume 13 Number 6 Nov.-Dec. 1976

CONTENTS

Review

Virus Transmission through Foods 287
V. Chalapati Rao

Research Papers

Utilization of Milo in Bakery Products 293
P. Haridas Rao and S. R. Shurpalekar

Comparison of Lactose Synthetase Activity of alpha-lactalbumin and Galactosyl-transferase from Cow, Buffalo and Goat Milk 300
M. P. Mathur

Use of Milk Clotting Enzyme of *Absidia ramosa* in Cheddar Cheese Preparation 305
S. S. Sannabhadti and R. A. Srinivasan

Studies on the Preparation of Wort from Barley-malt and Degermed Maize Using Microbial Enzymes 310
B. A. Satyanarayana Rao, M. S. Prasad and S. Venkatanarayana

Bread and Chapati Making Quality of Indian Durum Wheats 313
C. Prabhavathi, P. Haridasa Rao and S. R. Shurpalekar

Physico-chemical, Rheological and Milling Characteristics of Indian Durum Wheats 317
P. Haridasa Rao, A. Rahim, C. Prabhavathi and S. R. Shurpalekar

Chemical Composition of Sunflower Seed 322
K. S. Sekhon and S. K. Gupta

Development of Dehydrated Omelette Mix Based on Spray, Foam-mat and Accelerated Freeze Dried Egg Powder 325
K. S. Jayaraman, L. A. Ramanathan, P. Pitchamuthu and B. S. Bhatia

Effect of Amylase Supplements on the Rheological and Baking Quality of Indian Wheats 328
Maninder Kaur and G. S. Bains

ห้องสมุด กรมวิทยาศาสตร์
26 พ.ค. 2520

Research Notes

A Thurstone—Mosteller Model for Evaluating the Palatability of Chapaties of Some Improved Bread Wheats 333
G. Sadasivan, S. S. Sundaram and A. Austin

Heat Induced Changes in Buffalo Milk Serum Proteins 334
Jose Stephen and N. C. Ganguli

Proteolysis of Casein by Rennet from Fistulated Cow, Buffalo and Goat—A Note 337
I. A. Angelo

Colorimetric Determination of Chloral Hydrate in Toddy 339
M. S. Prasad and M. S. Subba Rao

Book Reviews 341

Association News 345

REVIEW

Virus Transmission through Foods

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Besides water, food constitutes an important but the least explored vehicle in the transmission of human enteric and other viruses. Association of food with transmission of overt viral diseases is not common, but food transport of viruses to humans and animals can be very frequent. Viruses in foods can be derived from sources like sewage contaminated water, food handler and virus intrinsically present in animals. Among the reported food borne outbreaks of virus disease, infectious hepatitis predominates.

Methods available for isolation of enteroviruses from foods are presented. The need for examination of large quantities of food to detect the small number of viruses is stressed and a few approaches for concentration of viruses from different foods are outlined.

The World Health Organization developed a food virology programme to get more scientists and institutions interested in studies on the extent and type of viral contaminants present in various foods so as to determine the role that viruses play in food borne diseases.

Introduction

Besides water, food constitutes an important but the least explored vehicle in the transmission of human enteric and other viruses. Food borne health hazards have resulted mainly from centralisation of food production, increase in communal eating and the expansion of international trade and tourism. Public health officials, in general, did not evince much interest in the problem of exposure of humans to animals infected with viruses and the consequences of consumption of virus containing foodstuffs by humans. Available epidemiological data do not indicate that many people are being infected through the consumption of such contaminated foods. However, the probability exists that a variety of virus diseases may be traced to the food supply. This review highlights some of the problems related to virus transmission through foods such as milk and milk products, vegetables, shellfish, and meat and meat products.

Occurrence of Viruses in Foods and Food Animals

A variety of viruses that infect humans have been demonstrated in food animals like calves, cows, goats and swine[1-6]. These viruses and the animals in which they occurred are as follows: Poliovirus 1 (cattle and goats) coxsackie virus A_5, A_{10} and A_{20} (Pigs), Echovirus 8 (Pig) and Echo 10 (Cattle) Reovirus 1, 2, 3 (Cattle) and influenza virus A_2 (Pigs).

The same viruses were sometimes isolated from human contacts living in close association with the animals[5]. The presence of the above human viruses in animals indicates that animal viruses may be agents of subclinical infections or disease in man.

Human and animal viruses have been isolated from food and food animals. They are listed in Table 1.

The above data clearly demonstrate that viruses of both human and animal origin are present in foods. In addition, the isolation of known human enteroviruses from foods is of obvious public health importance.

Source of Contamination

An analysis of the epidemiological record of transmission of viruses by food was presented[8]. Ten outbreaks of poliomyelitis reported during 1914–1949 have been traced to contaminated raw milk[8]. Twenty two food

TABLE 1. VIRUSES ISOLATED FROM FOODS[7]

Virus	Food
Encephalitis (tick borne)	Milk, butter (goat)
,, ,,	Milk (cow)
,, ,,	Milk (sheep)
Poliovirus 1 and 3	Raw milk (cow)
Para influenza 3	,, ,,
Bovine syncitial virus	,, ,,
Foot and mouth disease virus	Beef, milk and butter
Poliovirus 1, 2, 3	Oysters
Echovirus 4, 6, 9	,,
Coxsackie virus B2, 3, 4	,,
Reovirus 1	,,
Adenovirus 5	,,
Hepatitis candidate virus AR-17	,,
Poliovirus, 1, 2, 3	Ground beef
Echovirus 6	,,
Poliovirus, 3	Mussels
Echovirus 3, 5, 6, 8, 9, 12, 13	,,
Coxsackie virus A-18	,,
Avian leukosis complex	Eggs
Infectious bronchitis virus	,,
New castle disease virus	,,

associated outbreaks of infectious hepatitis between 1943 and 1964 have been reviewed[9]. The majority of foods implicated had been cooked very little or not at all. A variety of reasons are brought out to indicate how contamination took place. They fall under the following categories:

(*a*) Cafetaria trays of the compartmental type in which food is served in direct contact with the trays was indicated in the outbreaks in Missouri City, Tex. in 1959[10].

(*b*) There was an instance of cooked food like the roast pork being incriminated in the Bushy Hall, England outbreak in 1963. But this was handled by a cook who was jaundiced. Explosive outbreaks of poliomyelitis[11] and infectious hepatitis[12,13,14] have been reported in association with direct human contamination with clear indications of gross negligence in food handling.

(*c*) In some of the investigations of outbreaks, secondary or contact cases were cited. The index person in the Missouri City, Tex., outbreak[13] infected 5 persons by contact and 21 persons in his role as a kitchen employee. Thus an infected human, who works as a food handler or a kitchen worker in the final preparation constitutes a potentially significant source of virus in foods.

(*d*) The possibility of mechanical vectoring of viruses into foods is indicated by the fact that polio virus was recovered from blow flies 15 days after experimental contamination[15] and cockroaches harboured the virus for 51 days[16]. An explosive outbreak of poliomyelitis at a west coast naval training school in U.S.A.[17] indicated that milk contaminated by flies was the common source of infection. Milk used was mostly raw, regularly contained flies which had access to human and domestic animal feces. In another instance, food was shown to be contaminated with virus by flies[18].

(*e*) Numerous cases of infectious hepatitis in humans resulting from consumption of shell fish harvested from polluted waters have also been reported[19-22]. The species of shell fish which have been implicated in these outbreaks have differed and were mostly eaten raw. In only two of these infections, they were reported to have been cooked. One person in the Mississipi and Alabama outbreak[20] of 1961 had eaten oysters prepared by bringing milk and seasoning to a boil, adding the oysters, and cooking them just until the edges curled. Another study[23] suggests a significant association with consumption of both raw and steamed shellfish among hepatitis patients surveyed in Boston.

(*f*) Yet another likely source could be vegetables raised in fields irrigated with sewage. Poliovirus and echo viruses have been isolated from the soil of fields irrigated with sewage and some of the vegetables grown in these fields were contaminated with cytopathic agents[24]. No outbreaks from the above sources were recorded. But a possibility of their occurrence exists. Role of vegetables raised on sewage contaminated soils in the dissemination of food associated viral diseases can be understood better if an effort is made while conducting epidemiological investigations.

Persistence of Viruses in Foods

A number of factors can influence the survival of viruses in foods. They are (*i*) extent of virus adsorption to a particular food, (*ii*) its ability to survive and remain infectious under conditions of handling and storage, (*iii*) the effect of ingredients such as flavourings and preservatives and (*iv*) the decomposition products from bacteria in the food samples.

There is evidence that viruses can survive for long periods in raw meat[25,26]. Foot and mouth disease virus survived in meat for 73 days[27]. Echo and coxsackie viruses survived on vegetables stored under household conditions and polioviruses survived on radishes for over 2 months[28]. Experimental work by Lynt[29] indicates that polio 1, coxsackie B_1 and B_6 viruses added to eight different commercial frozen foods before storage at room temperature, 10°C and -20°C were still viable after various intervals of time upto 1 week, 1 month and 5 months respectively. Only in the case of coleslaw, a rapid significant reduction of coxsackie B_6 virus at the three temperatures was noticed and this was believed to be due to the additive, sodium bisulphite. Studies carried out by Herrmann and Cliver[30] in which ground beef was seeded with coxsackie virus A_9 at a concentration of 9.3×10^4 plaque forming units (PFU)/g and stored either at 4 or 23°C indicate that on the 8th day of storage, the virus content was 7.8×10^4 at 4 C and 3×10^4 at 23C and 2 weeks were necessary before significant (90.8 per cent) virus reduction could be noticed.

The above study also shows that even in the presence of extensive bacterial growth ($2.8–6 \times 10^9$ colonies/g) in the ground beef during periods of upto 8 days at 23 C or 4°C respectively, no marked loss of virus occurred. The observation that the decomposition products from bacteria in foods did not inactivate the viruses present in these samples confirmed a previous report[29].

Virus Inactivation

Viruses in large concentrations are not likely to occur in contaminated foods since only small amounts of virus is excreted by infected food handlers and only small amounts of feces can be introduced into foods by these people. Coupled with this, viruses are unable to grow and multiply outside living cells. These facts make it unlikely that food route could be responsible for widespread dissemination of virus. Nevertheless, the small amounts of virus contamination of foods which may occur can be considered as one means of seeding infec-

tions in communities after which such infections may be spread by more rapid routes in epidemic fashion[31]. As such, there is need to explore for inexpensive methods to inactivate viruses in foods.

A number of approaches have been studied to determine their efficacy of inactivating viruses in different foods. Possible inactivating agents are (*i*) heat, (*ii*) drying, and (*iii*) ionizing radiation.

Heat

Pasteurisation times and temperatures recommended by U.S. Public Health Service are (*i*) 62.8°C for 30 min or 71.7°C for 15 sec for milk, (*ii*) 65.6°C for 30 min or 74.4°C for 15 sec for milk products with added milk fat or sweeteners, and (*iii*) 86.3°C for 30 min or 79.4°C for 25 sec for ice cream mix.

Effect of pasteurisation temperatures on the destruction of a variety of viruses like adenovirus 12, reovirus 1 and herpes simplex virus and two leukemia viruses suspended in raw milk, raw chacolate milk, raw ice cream mix and sterile milk were studied[32]. At 65°C, which is near the pasteurisation temperature, the destruction curves for adenovirus 12, herpes simplex virus and reovirus 1 approached a first order reaction. But in respect of oncogenic viruses, maloney and rauscher leukemia viruses and maloney and rous sarcoma viruses it was indicated that rous sarcoma virus was the most resistant and survived at 55°C for 20 min and at 65°C for 2 sec.

Drying

In the case of many drying processes applied to foods, one could consider the heat employed as the principal source of inactivation of the virus. In one study[33] frozen salmon salad inoculated with poliovirus type 1 was freeze dried at 50-150 mm of g pressure, condencer temperature –51°C and a platen temperature of 21.8°C. Virus concentration before and after dehydration and storage was 2.3×10^6 PFU and 6.9×10^2 PFU/ml of sample and this indicates that freeze drying cannot be depended upon for efficient destruction of virus in foods.

Ionizing Radiation

The use of gamma radiation has been advocated as a means of obtaining sterile, organoleptically acceptable raw and cooked foods that require no refrigeration storage and have a long shelf life[34]. Present systems utilize low temperature treatment followed by irradiation of the frozen (–20 to –40°C) food product.

An irradiation dose of 0.6 Mrads brought 99% reduction of poliovirus in fish fillets[33]. Coxsackie B_2 virus suspended in raw and cooked ground beef was irradiated at temperatures ranging from 16 to –90°C in a cobalt 60 gamma radiation source[33]. The D value (dose in megarads required to reduce the viral numbers by 1 $\log_{10}$ or 90% reduction) for raw ground beef were 0.75 (–30°C) 0.71 (–60°C) and 0.68 (–90 C) and for cooked ground beef, the values were 0.70 (16°C), 0.76 (0.5°C), 0.68 (–30°C), 0.78 (–60°C) and 0.81 (–90°C). It may be of interest to note that the D values were higher in frozen material in contrast to water held at 0.5°C, the value being 0.14 Mrads[37] and this was attributed to inhibition of free radical formation or to impeding of free radical travel in frozen foods. The authors feel that the D values for other foods and other viruses may be different and require investigation.

A recent report[38] indicates that 7 to 13% of poliovirus survived when 400 Krads of gamma radiation from cobalt source was applied to whole and shucked oysters.

Detection of Viruses in Foods

The various foods for which simple and sensitive methods are available or have got to be developed are considered under three categories viz. vegetables and fruits grown on sewage/effluent irrigated farms; shell fish (oysters, mussels and clams); meat and meat products. Relevant background information and approaches for study are presented here.

Vegetables and Fruits Grown on Sewagee / Effluent Irrigated Farms

No documented outbreaks of human virus disease attributed to the consumption of crops contaminated by sewage polluted irrigation water are available. Possible contamination of food crops irrigated by wastes like sewage, sludge and effluent may be of serious public health significance and a hazard too.

Most States in U.S.A. generally prohibit the use of raw or settled sewage for irrigation of crops grown for human consumption. Some states, however do allow the use of completely treated, oxidised and disinfected sewage for production of vegetables and fruits that may be eaten raw. Such a practice cannot be considered safe in terms of virus hazard because two recent reports[39,40] indicate that the quantity of virus discharged in the effluent of an activated sludge sewage treatment plant (which incidentally is the best biological treatment method) amounts to 9.7×10^6 and 2×10^8 PFU per million gallons respectively. Data are also available from several field studies indicating the presence of virus even in chlorinated secondary effluents[41–44]. It is evident that complete treatment of sewage followed by chlorination as is presently practised with low chlorine residue will not yield virus free effluents and as such their utilisation in raising food crops is likely to result in low level virus contamination of such crops.

Limited data is available from the field and laboratory studies. Polioviruses and echoviruses have been isolated from soils of fields irrigated with sewage and some vege-

tables grown therein showed the presence of cytopathogenic agents[24]. Prolonged survival of these viruses on vegetables, for example poliovirus perisistence for over two months was also demonstrated[28]. Larkin *et al*[45] studied viral persistence on crops irrigated with poliovirus I inoculated sludge and effluent. Virus was recovered from the soil after 20 days and from vegetables like radishes and lettuce 36 days after final irrigation.

In India sewage farming is practised by using mostly raw sewage and sometimes partially treated sewage. There are 132 sewage farms covering approximately 30,000 acres and utilising 223 million gallons of sewage per day. Different types of human enteric viruses are reported to occur in the range of 1000–12000 PFU/l of raw sewage. Eighty per cent of the total virus in sewage was seen to belong to poliovirus types. Of this, at least 50 per cent appears to be associated with virulent strains of poliovirus (Rao *et al*—unpublished data). All varieties of vegetables are grown in the sewage farms and sold in many urban areas. The extent of virus dissemination by these vegetables has not been investigated yet.

In order to determine the public health significance and the types and numbers of viruses in or on fruits and vegetables, studies are needed for development of a method for isolation and recovery of viruses. In vegetables like tomato, radish and cucumber where the surface is even, recovery of virus using simple swabbing with eluants like nutrient broth, beef extract or glycine at high pH can be made. The virus from the eluate can be concentrated on any one of the adsorbants like iron oxide, polyelectrolytes, membrane filters, etc. Vegetables like cauliflower, where the surface is rugged, and the virus entrapped, the following steps may be useful: blending of the material in a suitable eluant, stirring it by a magnetic stirrer with pH adjusted to 8.5 to 9; centrifugation at 3000 rpm for 15-30 min; collection of supernatant, adjustment of its pH suitably; concentration of the virus on a chosen adsorbant and elution of the virus from it using a suitable eluant.

Shell Fish (Oysters, Clams and Mussels)

Procedures used in the detection of enteroviruses in oysters have been either laborious or relatively insensitive. This is true because oyster suspensions are toxic to the tissue cultures used for isolation of these viruses. One has had either to treat the suspensions intensively to reduce toxicity[46-50] or to dilute them to minimise toxic effects[51].

Kostenbader and Cliver[52] showed that a polycation sewage flocculant promoted cohesion of oyster solids and thereby aided separation of these from the viruses and it was shown that the suspension or extract obtained could be inoculated directly into tissue cultures. Recovery of 80-100 per cent of experimentally inoculated virus was achieved. The limitations of the method have also been described by the same authors[53]. They are (*i*) of the combined stack of filters used, one of them was not generally available through laboratory supply channels and outside U.S.A., (*ii*) it was not known for certainty that the method would be useful for foods other than oysters.

In further investigations, employing a variety of foods like beef, oysters, clams, lettuce, carrots, Kestenbader and Cliver[53] tried filtration methods for recovering experimentally added virus with about 80 per cent efficiency. Essential steps of the method include (*i*) suspension of 20 g food sample in 100 ml glycine-NaOH buffer, pH 8.8, (*ii*) clarification of sample by flocculation with cat-floc (polycation flocculant), (*iii*) suspension filtered through 11 cm dia. whatman ACG/B filter, (*iv*) clarified food extract made bacteria free by filtering through a 47 mm dia. 0.20 μm porosity GA-8 filter, (*v*) virus in the food extract (filtrate) concentrated on a 62 mm diaflo PM-30 (Amicon) ultrafilter and virus recovered with 5 ml of 3 per cent beef extract, pH 8 from the filter membrane or concentrated by ultra centrifugation.

Konowalchuk and Spiers[54] examined enterovirus recovery from laboratory contaminated samples of shell fish. In order to avoid toxicity of homogenates to cell cultures, pH of homogenate was adjusted to 3.0–3.5 and the acid treated sample was diluted 1:4 in fetal bovine serum and then mixed with cell suspensions to adsorb the virus which were subsequently enumerated as plaques.

Rao *et al* (unpublished) developed a simple method for eliminating toxicity and further concentration of virus using magnetic iron oxide. Virus was added to minced tissue of the clam suspended in nutrient broth/beef extract, pH 8.8 and blended. The homogenate was incubated for 1 hr at 37°C with stirring, and then centrifuged for 30 min at 3000 rpm. Supernatant pH was adjusted to 3, $AlCl_3$. added to a molarity of 0.0005, 1 g of magnetic iron oxide introduced and stirred with glass rod intermittently for 30 min. Supernatant was discarded and the virus retained on iron oxide was eluted with 5 ml beef extract prepared in borate-buffer, pH 8.5. Complete recovery of virus added in the range of 50-1000 PFU per a Pool of 5 clams was obtained.

Meat and Meat Products

Sullivan *et al*[55] described a method for isolating virus from ground beef seeded with known PFU (10–7700/g) coxsackie virus B2 and obtained an average recovery of 75 per cent of the added virus. The meat filtrate from 1 g beef was approximately 10 ml which was assayed in 5 monolayer cultures. No efforts were made to concentrate the sample.

In testing of a few samples from market places, it was reported that the quantity of virus recovered was nil from 9 samples and in two samples the PFU were 1 and 6 per 5 g sample (however, one meat loaf yielded 195 PFU/5 g). The results from majority of the samples clearly indicate the need for examination of large quantities of, say 100 g or so of the sample to make virus detection possible. This creates the problem of handling large volumes of slurries demanding large number of bottle cultures when minimal number of virus occurs in large quantities of meat. It is obvious that concentration of the meat slurries have got to be carried out.

Tierney *et al*[56] examined the efficiency of certain clarification methods like (*i*) glasswool and woven fiber glass method, (*ii*) potato ricer method, and (*iii*) low speed centrifugation. From a 100 g sample of ground beef seeded with virus, a clarified meat slurry of 100 ml was obtained and this was assayed in its entirety in 30 bottles of vero culture. Sample clarification by the above methods allowed a mean recovery of 19-49 per cent of the added virus. In additional experiments, the authors employed different membrane filters of 0.40 to 0.45 μm poresize (pretreated with fetal bovine serum) to clarify the meat slurry. Each filtrate was concentrated by ultrafiltration using a protein enrichment membrane of 0.0075 μm porosity.

Not only the final eluate from the ultrafilter was large (60 ml), but the time needed was 8-16 hr. Virus recoveries of not more than 55.6 per cent were obtained. From the above points, ultra filtration as a method of concentration of meat slurries does not seem to hold much promise for investigating food associated disease outbreaks in which large number of samples may have to be processed in a short time.

Detection of viruses from the meat of a food animal subject to a subclinical infection resulting in the spread of the virus through various organs and tissues require an approach which helps release the virus hidden or incorporated in the tissues. Mincing of the meat samples followed by trypsinisation would be useful. Optimum factors for the process of trypsinisation have to be developed. Once the virus has been extracted, it can be concentrated and eluted using adsorbants like iron oxide, poly electrolytes, activated carbon/bituminous coal or chemical precipitation methods using alum, lime, or ferric chloride.

World Health Organization Program in Food Virology

In order to determine the role that viruses play in food borne disease, the World Health Organization (WHO) initiated a programme to get more scientists interested in studies pertaining to the extent and type of viral contaminants present in various foods.

As a first step, compilation of data regarding current activity in the field of various countries is undertaken. A part of this task has been assigned to Dr. Dean. O. Cliver, of the Food Research Institute of the University of Wisconsin, Madison, U.S.A. which is designated as a WHO collaborating center. The papers being added to the data collection include the following food groups: Animal meat and products, milk and milk products, poultry meat and eggs, shell fish, fish and other sea foods, vegetables and fruits and water and sewage. The last named group is considered only when water or wastewater transmits virus to a food or become a constituent of a food or beverage but not if virus contaminated water is examined or consumed by itself.

The food virology group in Madison tries to summarise all available information of virus transmission and transmissibility through foods. Reports in the collection concern: (*a*) properties of viruses which affect their transmissibility, (*b*) instances of virus occurrence in foods, as evidenced by laboratory detection of the virus or by an outbreak of human disease, (*c*) other evidence of virus occurrence in foods, (*d*) methods for detecting food borne viruses, and (*e*) studies of virus stability or inactivation in foods.

These reports have recently been converted to a new form on edge punch cards for efficient mechanical data retrieval. This system has been designed to provide the scientist working in the field with precisely the information needed (if it exists). A master set of the reports is available with the Food Hygienist, Veterinary Public Health, World Health Organization, Geneva 27, Switzerland. The retrieval system is available chiefly for the use of: (*i*) food control authorities, (*ii*) research and laboratory workers in food virology, and (*iii*) research programme planners.

Future Research

To determine the extent of viral contamination of foods and the public health significance of the viruses present in the food supply additional research is needed in the following areas: (*a*) the incidence of viral disease in food animals and the distribution of virus in the infected animal carcass, (*b*) the ability of virus to persist in animal tissues and organs after slaughter, storage and distribution, (*c*) the extent of cross contamination of foods during handling by the food processor or consumer, (*d*) the infectivity of the virus to the food handler, (*e*) the ability of virus to survive the final food processing in the home or food establishment, (*f*) the infectivity of the virus to the consumer after entry *via* the oral route, (*g*) the minimum infectious dose of "animal" viruses for humans, and (*h*) the development of simple and sensitive methods for detecting virus in foods.

When answers are obtained to the questions posed above, the significance of viruses in food borne disease

will be revealed and this is possible only when a food virology programme is initiated in this country and when more scientists in other countries initiate studies of the extent and type of viral contaminants present in world food supply.

It is pertinent to point out that India's export of meat which was a meagre 980 tonnes in 1974–75 is likely to reach 70,000 tonnes earning a foreign exchange of about $ 62 million or 50 crores in about three years from now. But at the moment, meat of the slaughter houses in India are unhygienic and many animals are known to be diseased. In order to gain the confidence and acceptability of our meat and other foods in advanced countries, studies on food borne viruses and their elimination are essential.

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RESEARCH PAPERS

Utilization of Milo in Bakery Products

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A simple method of pearling and grinding has been described to obtain acceptable grade milo (reddish brown sorghum) flour of 80 per cent extraction. In trials on Buhler roller mill, conditioning of milo decreased the flour yield from 61.6 to 53.6%. The yield could be increased to 74.4% by remilling the shorts fraction.

Upto 10% of milo flour (80% extraction) could be blended with *maida* for bread making without any adverse effect. By using potassium bromate, glyceryl mono-sterate and sodium stearoyl lactylate the blending level for preparing satisfactory bread couldbe increased to 25%. A biscuit preparation based on 80:20 blend of *maida* and milo flour compared well with biscuits based only on *maida*.

Sorghum (*Sorghum vulgare*) with a world production of about 46 million tons occupies a prominent position among cereals[1]. Though mainly used as a feed grain in America, sorghum, known as jowar in India, is used in different forms as a staple food in many developing regions like some countries of Africa, Latin America and India. The presence of husk layers affects the acceptability of sorghum and also restricts its usage in other processed foods. This is all the more so in case of milo-a variety of sorghum with reddish brown husk—as the husk affects the colour of the house-hold preparations or processed food product. Different laboratory as well as pilot scale processing methods have been tried to remove the outer husk of white sorghum and the pearled grain is ground into flour or grits for use in traditional Indian preparations[2,3]. However, less information is available on the processing of milo.

Use of non-wheat flours for extending wheat supplies has been experimented through composite flours for use in bakery products[4-6]. However, only processed flours from white sorghum, casava, maize and other oil-seed flours have been tried along with wheat.

In the recent past, India has been importing considerable quantities of milo. One of the ready avenues for usage of suitably processed milo can be in bakery products, especially in the relatively low cost products which can be used for different feeding programmes. However this area has received little attention of research workers. Further, most of the work reported on the use of composite flour in bakery products is based on hard bread wheats and not soft-to-medium hard wheats which account for the major portion of Indian wheat production. It was, therefore, considered necessary to study the different aspects of processing milo into an acceptable grade flour and its usage along with commercial varieties of Indian wheats for the preparation of bakery products. The results of such studies are presented in this paper.

Materials and Methods

Raw materials: Sharbati sonora, a medium hard Indian aestivum wheat supplied by Indian Agricultural Research Institute, New Delhi was milled in Buhler laboratory mill (model MLU 202) after conditioning overnight at 15.5 per cent moisture level. The white flour (*maida*) obtained was used for bread making trials. Maida milled similarly from soft white Australian wheat conditioned to 14.5 per cent was used for biscuit making trials. Commercial sample of milo procured locally was used for processing experiments.

Barley pearler for milo processing: Two hundred gram milo samples were polished for different periods (0-7 min) in a laboratory barley pearler (Corcoran make, London) to obtain pearled milo of acceptable colour. After removing the husk by sieving through 32 mesh sieve, the pearled grain was weighed to calculate the percentage polish. The amount of brokens were determined by sieving the pearled milo through a 14 mesh sieve. For minimising the percentage of brokens, roller speeds of 280, 335 and 400 rpm were tried for the pearling operation. To study the effect of conditioning on pearling 200-g samples were conditioned for 5 min with addition of 5 per cent water. Milo sample with moisture content reduced to 7.2 per cent by sun drying was also included in these trials. The samples thus conditioned were pearled for 1.5 min at 400 rpm. The percentage polish as well as brokens were determined as described earlier.

Buhler laboratory mill for milo processing: Two milo samples, one containing 8.5 per cent moisture (without any conditioning) and the other conditioned overnight to 15 per cent moisture (as followed for white sorghum[6]) were milled in the Buhler laboratory mill. The yields of different fractions were calculated on the basis of total weight of milled product obtained. Two methods were tried to improve the flour yield.

Firstly, the shorts were sieved through a Buhler plansifter and the fine fraction passing through 6XX sieve was added to the flour fraction. The overtailings of 6XX sieve were recycled through the mill and the weight of the extra flour noted. Alternately, shorts obtained from Buhler mill were ground in a Kamas laboratory hammer mill (Model Slaggy Sweden) using 0.8 mm sieve, and the fraction passing through 6XX sieve was added to the flour fraction.

Milo flour processing for use in bakery products: Conditions for processing milo into flour suitable for use in bakery products were arrived at on the basis of different trials. Milo was pearled for 1.5 min at a speed of 400 rpm. Grains of 20 per cent polish thus obtained were ground either in a Kamas hammer mill or in a disc mill locally known as *Chakki.* This flour was blended at 10-30 per cent levels with *maida* from *Sharbati sonora* for bread making trials and with *maida* from Australian wheat at 10-50 per cent levels for biscuit making trials.

Analysis of flour blends: The colour grade of differently milled milo flours as well as their belnds with maida were recorded in a photovolt reflectance meter. The moisture, total ash, crude fibre and ether extractives in different milo flour and *maida* samples from *Sharbati sonora* and Australian wheats were determined by AACC methods[8]. Crude protein was determined by micro-kjeldahl method using a conversion factor of 6.25.

Dough characteristics: Dough characteristics of *maida* and its blends with milo flour were studied using Brabender's farinograph as well as extensograph according to AACC procedures[8].

Bread making trials: Bread making quality of different *maida*-milo flour blends were tested according to remix procedure[9]. Loaf volume of breads was determined by rapeseed displacement method using loaf volume meter. Breads were evaluated for different parameters like colour and shape of the crust, colour, softness, grain fineness and uniformity of crumb and eating quality by a panel of 6 judges.

Biscuit making trials: The following recipe (100 g basis) was used for the preparation of biscuits based on different blends of milo flour and *maida* from Australian wheat.

Milo flour *maida* blend, 65 g; sugar, 16 g; fat, 18g; non-fat milk, 1.0 g; common salt, 0.4 g; baking powder, 0.5 g; ammonium bicarbonate, 0.5 g; vanillin, 0.05 g; and water, 13-15 ml sugar, fat and vanillin were creamed in a Hobart Mixer for 2 min. To this a well mixed blend of the flour, non-fat milk and baking powder was added along with water containing common salt, ammonium bicarbonate and mixed further for 2 min Using a wooden rolling pin the dough thus obtained was sheeted to a uniform thickness of 2.5 mm. Circular biscuits of 1.5 in. diameter were cut and baked for 9 min. at 250°C. Average diameter, thickness and spread factor (diameter/thickness) of 5 biscuits were noted for assessing the physical characteristics. In addition, the colour, crispness, eating quality and overall acceptability were assessed by a panel of 6 judges.

Results and Discussions

Effect of pearling time on processing of milo in barley pearler: The data presented in Table 1 indicate that a significant increase in per cent polish as well as brokens were observed, as the pearling period increased from 0.5 to 2 min. When the pearling at 400 rpm was extended beyond 2 min only slight increases were observed. This may be probably explained by easy removal of loosely bound husk in the initial stages of pearling. From visual observation, it was inferred that 20-25 per cent polish was necessary for almost complete removal of the reddish brown husk. In this way, white pearled grains could be obtained.

Effect of pearler speed on processing of milo in barley pearler: It is interesting to note that for the same pearling duration of 3 min the per cent polish obtained at 280, 335 and 400 rpm were 4.0, 13.0 and 27.5 per cent respectively (Table 1). Corresponding values for brokens were 0.5, 3.0 and 20.0 per cent respectively. However, as the roller speed decreased, the period of pearling increased considerably to 5 min at 335 rpm as compared

TABLE 1. EFFECT OF THE ROLLER SPEED OF BARLEY PEARLER ON THE DEGREE OF POLISH AND BREAKAGE OF MILO

	Degree of polish (%) at			Broken grains (%) at		
Pearling time (min)	280 rpm	335 rpm	400 rpm	280 rpm	335 rpm	400 rpm
0.5	—	—	6.5	—	—	5.0
1.0	—	4.0	15.5	—	0.5	11.0
1.5	—	—	20.0	—	—	13.0
2.0	—	8.0	24.5	—	1.5	18.0
2.5	—	—	26.5	—	—	19.0
3.0	4.0	13.0	27.5	0.5	3.0	20.0
4.0	9.0	17.0	—	2.0	5.0	—
5.0	14.0	20.0	—	3.5	7.0	—
6.0	17.0	23.5	—	4.0	8.0	—
7.0	18.0	25.0	—	4.5	8.5	—

to 1.5 min at 400 rpm for obtaining the desired 20 per cent polish. From practical considerations of minimum pearling time and per cent brokens however, 1.5 min pearling at 400 rpm was considered optimum for processing of milo into flour and 5 min at 335 rpm for obtaining whole pearled grain.

Effect of conditioning: No difference was observed in respect of per cent polish or brokens, when the moisture content of the milo was reduced from the initial 8.5 to 7.2 per cent. From the different trials carried out using 1.5 min pearling period, it was observed that as the conditioning moisture level increased, the degree of polish decreased (Table 2). In contrast, the percentage of brokens showed a slight increase. Consequently, to obtain the desired degree of 20 per cent polish, the pearling period may have to be increased considerably. The above results indicate that conditioning of milo has no beneficial effect on pearling efficiency. In contrast,

TABLE 2. EFFECT OF CONDITIONING[a] INTO DIFFERENT MOISTURE LEVELS ON THE DEGREE OF POLISH AND BREAKAGE

Grain moisture %	Degree of polish %	Broken grains[c] %
7.2	19.5	13.0
8.5[b]	19.5	13.0
9.5	18.3	13.5
10.5	14.2	15.0
11.5	12.2	15.5
12.5	11.1	16.0
13.5	9.1	16.3

a–Conditioning period 5 min

b–Initial moisture content

c–Through 14 mesh sieve.

Raghavendra Rao *et al*[3]. had observed that for white sorghum the optimum pearling could be carried out by conditioning with 3 per cent extra moisture for a period of 5 min. However, their observation that conditioning period of more than 5 min increased percentage brokens is in conformity with the present findings.

Processing of milo in Buhler laboratory mill: The data presented in Table 3 indicate considerably higher flour yield from unconditioned milo, as compared to that from milo conditioned to 15 per cent moisture. In contrast, the pattern regarding yield of shorts was just the reverse. However, the colour grade value of flour from conditioned milo was better.

By just passing shorts through 6XX sieve, the yield of flour could be increased by 4-6 per cent. Recycling the 6XX overtailings through Buhler mill further improved yields of flour by 9-10 per cent. By this method of sieving and recycling flour yields of 63.7 and 74.4 per cent respectively were obtained from milo with or without conditioning. It is interesting to note that as compared to low flour yield of 53.6 per cent from Buhler mill, acceptable grade milo flour of about 80 per cent extraction rate was obtained by pooling the flour fractions obtained from Buhler mill and that recovered from shorts by grinding in Kamas mill and passing through 6XX sieve.

Proximate composition of processed milo: The data on the analyses of differently processed milo flours given in Table 4 indicate that the flour obtained from (*a*) 20 per cent pearled milo, and (*b*) milo conditioned to 15 per cent and milled in a Buhler mill, compared well in

TABLE 3. MILLING CHARACTERISTICS OF MILO IN A BUHLER LABORATORY MILL

	Without conditioning[a]	Conditioned to 15% moisture[e]
Break flour (%)	14.8	13.1
Reduction flour (%)	46.8(24.5)[c]	34.9(14.4)[c]
Flour yield (%)	61.6	48.0
Flour recovered from shorts[d] (%)	3.9	5.6
Flour recovered from shorts[e] (%)	8.9	10.1
Total flour yield[d] (%)	74.4	63.7
Husk (%)	3.4	6.0
Colour grade value[f]	64.0	68.5

a–Initial moisture content, 8.5%.

b–Conditioned overnight for 16 hr.

c–Values in parentheses indicate yield of flour from reduction roll R_1

d–By passing through 6XX sieve

e–By recycling the overtails of 6XX sieve through the Buhler mill

f–Values for flour including the shorts fraction passing through 6XX sieve.

TABLE 4. PROXIMATE COMPOSITION[a] OF DIFFERENTLY PROCESSED MILO

Constituents	Un-pearled	Pearled[b]	Buhler milled: Without conditioning[c]	Buhler milled: Conditioned to 15% moisture[d]
Crude protein (%) N×6.25	9.60	9.20	7.80	6.30
Ether extractives (%)	2.60	1.62	1.84	1.33
Total ash (%)	1.23	0.76	0.96	0.72
Crude fibre (%)	2.10	0.62	0.79	0.58
Carbohydrates (by diff.) %	70.47	73.80	74.61	77.07

a–On 14% moisture basis

b–20% polish

c–Yield of 65.5% includes flour recovered from shorts through 6XX sieve.

d–Yield of 53.6% includes flour recovered from shorts through 6XX sieve.

respect of ash and fibre contents. However, protein content of the flour from polished milo (9.2 per cent) was only slightly lower than the whole milo flour (9.6 per cent) and significantly higher than that (7.8 per cent) from Buhler mill. Protein, ash and fibre contents of flour were considerably higher in case of unconditioned milo as compared to conditioned milo.

The data (Table 5) on the effect of incorporation of milo flour in *maida* indicate that polished milo flour could be used upto a maximum of 30 per cent without adversely affecting the colour grade value of *maida*.

Rheological characteristics of maida-*milo flour blend:* Based on several preliminary trials carried out on Farinograph it was concluded that a maximum of 25 per cent of milo flour could be used for blending with *maida* without affecting desirable characteristics. As such, Fig. 1 includes farinograms and extensograms of only a 3:1 blend used for bread making trials. The water absorption values (59.5–60.0 per cent) and dough stability (8-9 min) for *maida* as well as *maida*-milo flour blend were practically the same. However, the dough development time decreased significantly from 5.5 to 1.5 min as a result of incorporation of milo. Though the extensibility of both the doughs were comparable, the resistance to extension decreased from more than 1000 EU for the *maida* dough to 760 EU for the dough based on 3:1 blend. Also, the strength of the *maida* dough (110 cm²) as seen from the area under extensograms (Fig. 1) was lowered (85 cm²) due to blending.

TABLE 5. COLOR GRADE VALUES OF DIFFERENT BLENDS OF WHEAT FLOUR WITH PEARLED MILO FLOUR[a]

Wheat flour %	Milo flour %	Colour value
100	0	77.5
90	10	76.5
80	20	76.0
75	25	75.5
70	30	75.0
0	100	68.5

a–Pearled to 20% polish and ground in Kamas hammer mill

Effect of incorporation of milo flour on the bread quality: Data presented in Table 6 show that upto a 10 per cent level milo flour could be used with practically no adverse effect on the crust and crumb characteristics as well as taste of bread. The volume was, however, slightly lower. The maximum level of milo flour that could be used for blending to obtain satisfactory bread was found to be 25 per cent. Though the volume decreased significantly the other quality characteristics were only slightly affected. It was also observed that inclusion of milo flour gave a somewhat better body and chewing quality to the bread, as compared to somewhat bland taste of *maida* bread. This observation may be of practical significance to the consumers in the Indian subcontinent. Bread containing 30 per cent milo flour had a distinct 'milo' flavour and the crumb characteristics were somewhat coarse and unacceptable.

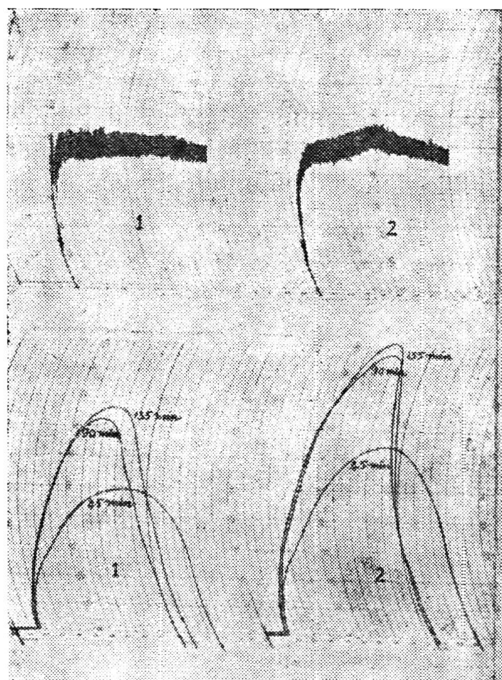

Fig 1. Farinograph and extensograph curves 1. Blend (3:1) of maida and milo flours; 2, Control-*maida*.

TABLE 6. EFFECT OF BLENDING OF MILO FLOUR PEARLED TO 20% POLISH AND GROUND IN KAMAR HAMMER MILL WITH WHEAT FLOUR ON THE QUALITY OF BREAD

Milo used (%)	Loaf volume[a] ml	Crust colour[b]	Crumb				Taste[c]
			Colour	Texture	Grain size	Grain distribution	
0	525	Dark brown	Creamy white	Soft	Fine	Uniform	Normal
10	505	,,	Fairly whitish	,,	,,	,,	,,
20	485	Light brown	Slightly brownish white	Slightly soft	Slightly fine	Fairly uniform	Slightly inferior[d]
25	475	,,	,,	,,	,,	,,	,,
30	450	Greyish dull brown	Brownish white	Slightly tough	Slightly coarse	,,	Inferior[e]

a—Based on 100 g flour
b—Shape was normal in all breads
c—As compared to control wheat bread
d—Due to mild milo flavour
e—Due to perceptible milo flavour.

Improvement of bread based on maida-*milo flour blend:* Results of studies carried out to improve the quality of bread based on 3:1 blend of *maida* and milo flours by using different additives are presented in Tables 7 and 8 From the Farinograph characteristics on water absorption and the dough stability, the dough based on 3:1 blend of wheat and milo flour was found to be somewhat tough. It was observed that addition of 3 per cent extra water (in addition to Farinograph water absorption) improved considerably the loaf volume as well as crumb characteristics. Though loaf volume improved with further increase in water added, the dough became sticky and difficult to handle. Use of 0.5 per cent GMS—(glyceryl mono stearate), in addition to 3 per cent extra water gave a loaf with improved softness and fineness of the crumb.

Among different levels tried, use of 20 ppm potasium bromate showed maximum improvement in the loaf

TABLE 7. EFFECT OF WATER ADDITION ON THE BREAD[a] BASED ON MAIDA-MILO FLOUR[b] (3:1) BLEND

Water added	Loaf volume ml	Crumb			Taste[e]
		Texture	Grain size	Grain distribution	
FWA	475	Slight soft	Slightly fine	Fairly uniform	Slightly inferior[f]
FWA + 1.5 ml	495	,,	,,	,,	,,
FWA + 3.0 ml	505	Soft	Fine	Uniform	,,
FWA + 4.5 ml[c]	510	,,	,,	,,	,,
FWA + 6.0 ml[c]	500	Very soft	,,	,,	,, and gummy
FWA + 3.0 ml[d]	512	,,	Very fine	,,	Normal

FWA—Farinograph water absorption.
a—All the breads had normal shape, light brown crust colour, and somewhat brownish white crumb colour.
b—Of 20% polish and ground in Kamas hammer mill.
c—Doughs were slightly flowy and sticky
d—Included 0.5% of GMS in the formulation.
e—As compared to control wheat bread
f—Slightly inferior due to slight milo flavour

TABLE 8. EFFECT OF POTASSIUM BROMATE AND SODIUM STEAROYL LACTYLATE (SSL) ON BREAD[a] QUALITY BASED ON 3:1 BLEND OF MILO FLOUR[b] AND MAIDA

Potassium bromate ppm	SSL %	Loaf volume ml	Crumb texture	Crumb grain	Taste[c]
0	—	465	Slightly soft	Slightly fine, uniform	Slightly inferior[d]
10	—	480	,,	Fine, uniform	,,
20	—	490	Soft	,,	,,
30	—	490	,,	,,	,,
10	0.3	495	,,	Very fine, uniform	,,
10	0.4	525	Very soft	,,	Normal
10	0.5	540	,,	,,	,,
10	0.6	550	,,	,,	Inferior[e]
10	0.7	560	Very soft, sticky	,,	Inferior[e]
10	1.0	575	,,	,,	Bitter, unacceptable

a—All the breads had normal shape, light brown crust colour and slightly brownish white crumb colour.
b—of 20% polish and ground in Kamas hammer mill
c—As compared to control wheat bread
d—Slightly inferior due to mild milo flavour.
e—Inferior to due to slight off-flavour of lactylate.

volume as well as crumb characteristics. SSL (sodium stearoyl lactylate) at levels ranging from 0.4 to 1.0 per cent had definite improving action on loaf volume and crumb characteristics (Fig. 2). However, beyond 0.5 per cent the dough became sticky and the bread had slight off flavour. As such, it was inferred that 0.4 to 0.5 per cent of SSL was the optimum level. It is interesting to note that bread based on 3:1 blend of *maida* and milo flour with 0.5 per cent SSL gave a bread which compared favourably with the loaf volume and the crumb characteristics of the control bread based only on *maida* (Fig. 2).

Effect of making differently processed milo flour on the bread making quality of maida: Except for the crumb colour, the bread prepared from Buhler milled milo flour (53.6 per cent extraction)—*maida* blend was comparable in different quality characteristics with those made by using 80 per cent extraction flour from polished milo (Table 9). However, bread based on blend of *maida* and milo flour (Buhler milled+fraction from shorts milled in Kamas hammer mill) having a comparable extraction rate (80 per cent) as that of polished and ground milo flour was found to be just acceptable, as the colour and grain characteristics of the crumb were not

TABLE 9. QUALITY OF BREAD[a] BASED ON 3:1 BLENDS OF MAIDA AND DIFFERENTLY PROCESSED MILO FLOUR

Processing of milo	Extraction rate %	Loaf volume ml	Crumb				Taste[b]
			Colour	Texture	Grain size	Grain distribution	
Milled in Buhler laboratory mill	51.0	485	Fairly whitish	Soft	Slight fine	Uniform	Normal
,,	80.0	480	Brownish white	,,	Slightly coarse	Slightly uniform	Slightly inferior[c]
Pearled and ground in disc mill (*chakki*)	80.0	480	Slightly brownish white	,,	Slightly fine	Uniform	,,
Pearled and ground in hammer mill	80.0	480	,,	,,	,,	,,	Slightly inferior
Control-*maida*		520	Creamy white	Very soft	Fine	,,	Normal

a—All the breads had normal shape and light brown crust colour except control which had dark brown crust colour.
b—As compared to control wheat bread
c—Slightly inferior due to slight milo flavour.

TABLE 10. EVALUATION OF BISCUITS BASED ON BLENDS OF MILO FLOUR[a] AND MAIDA[b]

Milo added (%)	Water added ml	Av. thickness[c] (T) cm	Av. dia.[d] (W) cm	Ratio (W/T)	Spread factor	Colour	Crispness[e]	Mouthfeel[e]	Taste[e]
0.	15.0	0.71	5.17	7.20	—	Golden yellow	Normal	Normal	Normal
12.5	15.0	0.70	5.31	7.55	103.4	,,	,,	,,	,,
20.0	14.5	0.69	5.30	7.70	105.2	,,	,,	,,	,,
25.0	14.5	0.67	5.30	7.90	108.2	,,	Slightly better	Slightly gritty	Slightly inferior
33.0	14.0	0.63	5.35	8.50	116.5	Slightly whitish brown	,,	Gritty	,,
50.0	13.5	0.58	5.40	9.30	127.4	,,	,,	Very gritty	,,

a—75% extraction flour passing through 10XX
b—From Australian soft white wheat
c—Thickness of unbaked biscuits–0.25 cm.
d—Diameter of unbaked biscuits–5.25 cm.
e—As compared to control based only on maida.

satisfactory. No difference was observed in bread making quality of the blends containing milo flours processed either in Kamas hammer mill or *chakki*. It may thus be inferred that milo flour milled by simple technique of polishing and grinding could be used for blending with *maida* without sacrificing its bread making quality.

Utilisation of milo in biscuit preparation: Results presented on quality evaluation of biscuits (Table 10) made from different blends of *maida* and milo flour indicate clearly that milo flour could be used upto 20 per cent without affecting the quality of biscuits. It was observed that use of milo flour beyond 20 per cent level made the product excessively brittle and somewhat gritty to taste. As expected, the raise of the biscuits decreased as the level of milo flour increased. This was also reflected in the increase in the spread factor from 100.0 to 127.4. This increase was minimum upto 20 per cent level of incorporation of milo flour.

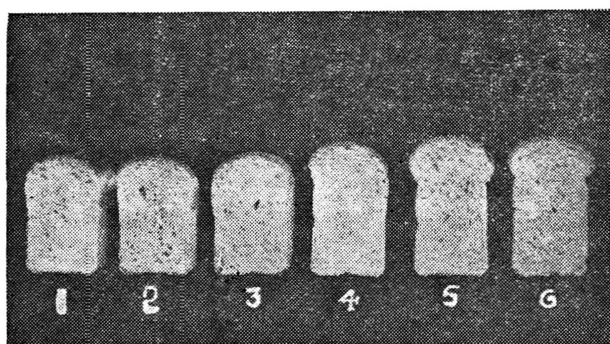

Fig 2. Effect of different levels of sodium stearoyl lactylate (SSL) on the quality of bread. 1, Control-maida; 2, 3, 4, 5, 6, 3:1 blend of *maida*-milo flours containing 0, 0.3, 0.4, 0.5 and 0.6% SSL respectively.

Conclusion: The present investigations are of considerable importance when viewed in the light of increasing stress on the use of composite flours in regions where significant quantities of cereals other than wheat are produced or imported to meet food shortages. Using indigenously available machinery a simple polishing and grinding technique has been worked out in the laboratory for obtaining acceptable quality milo flour. Possibilities have been shown on the utilisation of 10-25 per cent processed milo flour in bakery products which will be helpful in extending the wheat supplies, especially during food scarcity situations.

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Comparison of Lactose Synthetase Activity of α-lactalbumin and Galactosyltransferase from Cow, Buffalo and Goat Milk

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The A and B proteins of lactose synthetase enzyme (UDP-D-galactose: D-glucose. 1-galactosyltransferase E.C. 2.4.1.22) from cow, buffalo and goat milk have been isolated. The lactose synthetase activity of alpha-lactalbumin (B protein) and galactosyltransferase (A protein) from the three species of milk have been compared. Crude samples of α-lactalbumin also showed enzymic activity in presence of A protein. The partially purified A protein contains hexose, hexosamine and sialic acid. Thus it was identified as a glycoprotein enzyme.

Lactose synthetase (E.C.2.4.1.22) catalyzes the formation of lactose and this reaction requires two proteins namely α-lactalbumin (B protein) and galactosyltransferase (A protein). The enzyme lactose synthetase has been shown to exist as a microsomal enzyme in mammary glands of lactating cow, guinea pigs[1] as well as in soluble form in bovine milk[2]. It has been shown that individually A and B proteins of lactose synthetase system do not show enzymic activity, however, recombination of A and B protein shows the enzymic activity[3]. Comparisons have been made of the lactose synthetase activity from milk of different species. Schmidt and Ebner[4] compared the lactose synthetase activity of α-lactalbumins isolated from pig, goat, sheep and human milk in presence of bovine A protein. Ley and Jennes[5] demonstrated that α-lactalbumin prepared from cow, goat, deer, pig, rat and human milk exhibited lactose synthetase activity in presence of bovine A protein. Tanahashi *et al.*[6] have also compared the lactose synthetase activity of A and B proteins from milks of bovine, sheep, goat and human.

In light of these observations, a study was undertaken with the objective of isolating α-lactalbumin and galactosyl transferase from the milk of cow, buffalo and goat and comparing their lactose synthetase activity.

Materials and Methods

Samples of milk were collected from cows, buffaloes and goats maintained at National Dairy Research Institute, Karnal. Standard preparation of α-lactalbumin was from Nutritional Biochemicals Corp., Cleaveland, Ohio, USA. β-Nicotinamide adenine dinucleotide reduced form, phosphoenolpyruvate, uridine 5-diphosphogalactose and pyruvate kinase were obtained from Sigma, USA. All other chemicals were of analytical reagent grade.

Preparation of α-lactalbumin: Crude samples of α-lactalbumin (B protein) were isolated from milk according to Armstrong *et al*[7]. Acid whey from cow, buffalo and goat milk were dialysed separately against several changes of distilled water, concentrated and freeze-dried. The dried samples of whey (0.8-1.0 g) were then chromatographed on Sephadex G-75 (2.6 cm $\times$ 84 cm column) as suggested by Hopper and Mckenzie[8] using imidazole-HCl buffer pH 6.3 at room temperature. Fractions rich in α-lactalbumin were then chromatographed on DEAE-Sephadex A 50 (1.5 cm $\times$ 20 cm column) and eluted with a linear gradient of NaCl (0 to 0.12M) in 0.05M imidazole—0.043M HCl buffer (pH 6.3). Protein was determined by ultraviolet absorption at 280 mμ. Fractions rich in protein were dialysed against distilled water and freeze dried.

Carbohydrate determination of A protein: Hexose was determined as a mixture of mannose and galactose[9], hexosamine as glucosamine hydrochlordie[10] and sialic acid as N-acetyl neuraminic acid[11].

Preparation of galactosyltransferase: Samples of crude galactosyltransferase (A protein) from milks of cow, buffalo and goat were prepared using the method of Brodbeck and Ebner[3]. Subsequent partial purification of three samples were done by DEAE-cellulose and Sephadex G-150 (3.0 cm $\times$ 125 cm column[12]) chromatography. The samples were eluted with 20 mM tris-HCl-5mM $MgCl_2$ of pH 7.4. The individual fractions of two protein peak fractions thus obtained were mixed, dialysed against distilled water and then freeze-dried.

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Polyacrylamide gel plate electrophoresis: Freeze-dried samples of ∝-lactalbumin and galactosyltransferase were examined for their homogeneity and electrophoretic behaviour by a modified discontinuous polyacrylamide gel plate electrophoresis, as described by Mathur and Srinivasan[13] and by vertical polyacrylamide gel electrophoresis.

Measurement of lactose synthetase activity: Lactose synthetase activity of ∝-lactalbumin and galactosyltransferase samples were determined spectrophotometrically as described by Brodbeck and Ebner[3] and Nagasawa *et al*[14]. The activity was measured by coupling the UDP formed to the oxidation of NADH by way of the reactions of nucleoside diphosphate kinase, pyruvate kinase and lactic dehydrogenase.

The standard assay mixture contianed 3 μ mole of phosphoenol pyruvate; 0.45 μ mol of NADH; 0.3 μ mole of ATP; 150 μ mole of tris-HCl; pH 7.4; 5 μ mole of $MnCl_2$; 0.252 μ mole of UDP-D-galactose; 60 μ mole of D-glucose; 1.08 mg of pyruvate kinase; 500 μg of B protein (∝-lactalbumin) or 1250 μg of A protein (galactosyltransferase); and water in a final volume of 3 ml. UDP-D-galactose was omitted from the blank. The mixture was incubated at 25°C for 30 min and heated in boiling water bath for 1 min and filtered. Absorbance of these filtered solutions were then read at 340 mμ in spectrophotometer. The enzymic activity was expressed as nano mole of lactose synthesised per minute.

Results and Discussion

Lactose synthetase activity of ∝-lactalbumin preparation: Lactose synthetase activity of these isolated samples of ∝-lactalbumin was determined using galactosyltransferase (A protein) from cow milk and the results are shown in Table 1. The results indicate that even on an average crude ∝-lactalbumin preparations from cow, buffalo and goat milk showed lactose synthetase activity in presence of A protein of cow milk. ∝-lactalbumin (B protein) of cow milk showed higher activity than buffalo milk ∝-lactalbumin (B protein). The ∝-lactalbumin prepared from goat milk behaves similarly as that of buffalo milk. However, individual variations of lactose synthetase activity in different samples of the three species of ∝-lactalbumin were observed (Table 1).

When freeze-dried whey samples were chromatographed on Sephadex G-75 column two protein peaks (designated for convenience as peak A and peak B) both identical to ∝-lactalbumin were obtained. Further resolution of peak A and peak B on DEAE-A-50 has been shown in Fig 1. The protein fractions of different subpeaks were pooled separately and freeze dried. The lactose synthetase activity of these samples (B proteins) were then determined using three different A proteins and is shown in Table 2. As the amount of protein in the case of A peak of goat ∝-lactalbumin was insufficient, it was not freeze-dried and hence was not chromatographed on DEAE-A-50.

Tanahashi *et al.*[6] observed that lactose synthetase activity was unaffected by the reaction of bovine milk

TABLE 1. LACTOSE SYNTHETASE ACTIVITY OF DIFFERENT CRUDE PREPARATION OF ∝-LACTALBUMIN (B PROTEIN)

Samples*	LSA $\times 10^{-6}$**
C_1	2.5
C_2	2.5
B_1	2.4
B_2	2.7
B_3	1.9
B_4	2.2
B_5	2.1
G_1	2.0
G_2	3.5
G_3	2.7
G_4	2.1

*C, Cow milk; B, Buffalo milk; G, Goat milk

**LSA, Lactose synthetase activity using cow A protein; expressed as n moles of lactose formed per minute.

TABLE 2. LACTOSE SYNTHETASE ACTIVITY OF ∝-LACTABULMIN PREPARATIONS (B PROTEIN)

Samples	Lactose synthetase activity* $\times 10^{-6}$		
	Cow A protein	Buffalo A protein	Goat A protein
Cow ∝-lactalbumin peak A, Sub-peak No. 1	4.5	4.2	3.0
Cow ∝-lactalbumin peak A, Sub-peak No. 2	4.5	4.2	2.1
Cow ∝-lactalbumin peak B, Sub-peak No. 1	3.5	2.1	1.6
Buffalo ∝-lactalbumin peak A, Sub-peak No. 1	2.0	4.2	4.3
Buffalo ∝-lactalbumin peak A, Sub-peak No. 2	2.0	4.2	4.2
Buffalo ∝-lactalbumin peak A, Sub-peak No. 3	2.0	4.2	4.2
Buffalo ∝-lactalbumin peak B, Sub-peak No. 1	1.6	4.2	2.5
Buffalo ∝-lactalbumin peak B, Sub-peak No. 2	3.5	4.2	4.2
Goat ∝-lactalbumin peak B, Sub-peak No. 1	2.8	4.2	4.2

*Expressed as n moles of lactose formed per minute.

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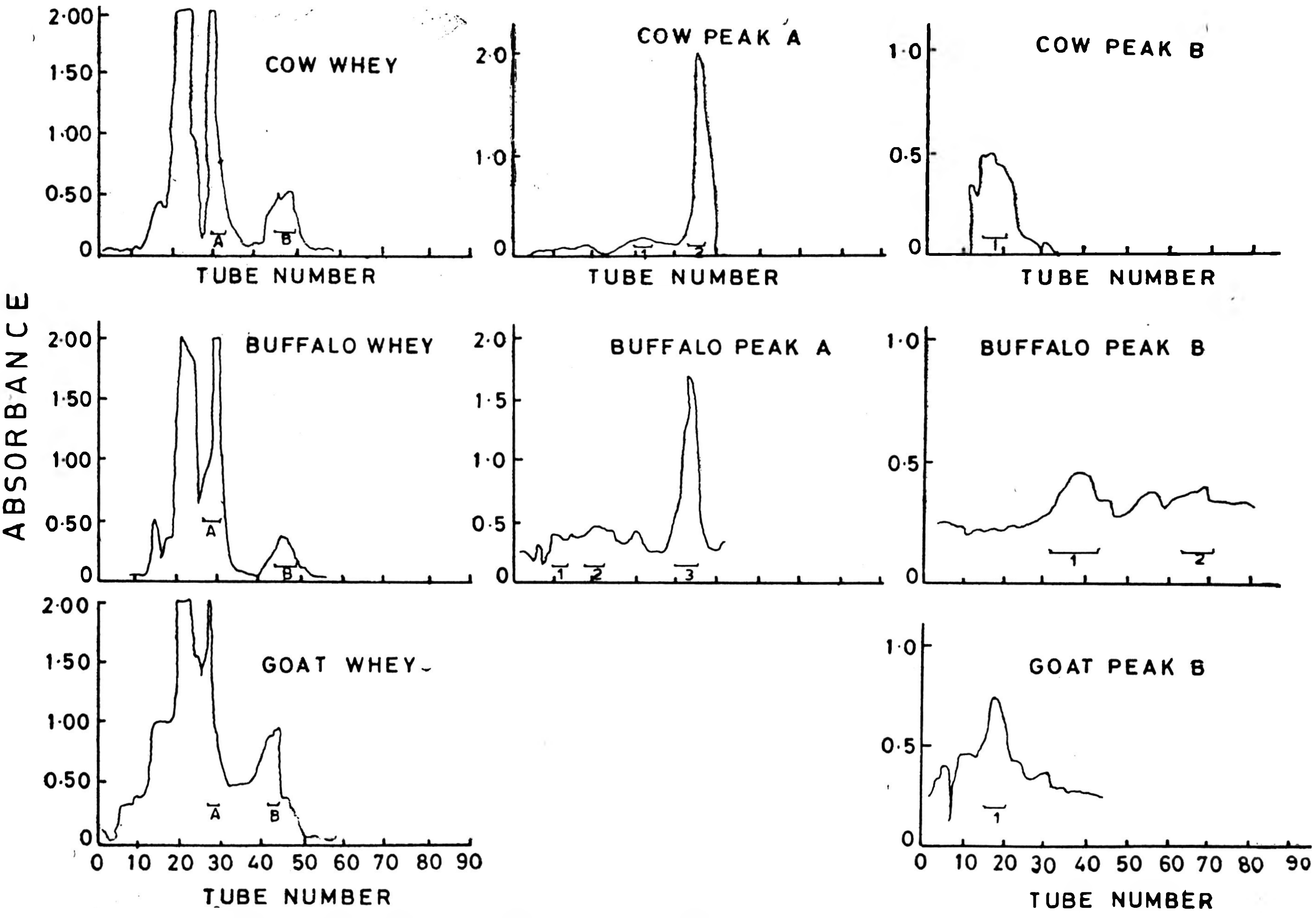

Fig 1. Chromatography of whey proteins of cow, buffalo and goat on Sephadex G-75 and of ∝-lactalbumin therefrom on DEAE-Sephadex A-50

A protein with various purified B proteins from ruminants and non-ruminants. However, the present data (Table 2) suggest that the lactose synthetase activity is diminished to a great extent when buffalo milk B protein is added to cow A protein and to a lesser extent when goat B protein is added to cow A protein. The lactose synthetase activity remains almost unaffected by the reaction of buffalo A protein with various purified B proteins from cow, buffalo and goat milk. When goat A protein is mixed with cow, buffalo and goat B protein, the lactose synthetase activity is lower for cow and almost similar for buffalo and goat B protein. However, Ley and Jennes[5] compared the affinity of the B protein of six species (cow, goat, deer, pig, rat and human) for bovine A protein and observed different ranges of affinities among the species.

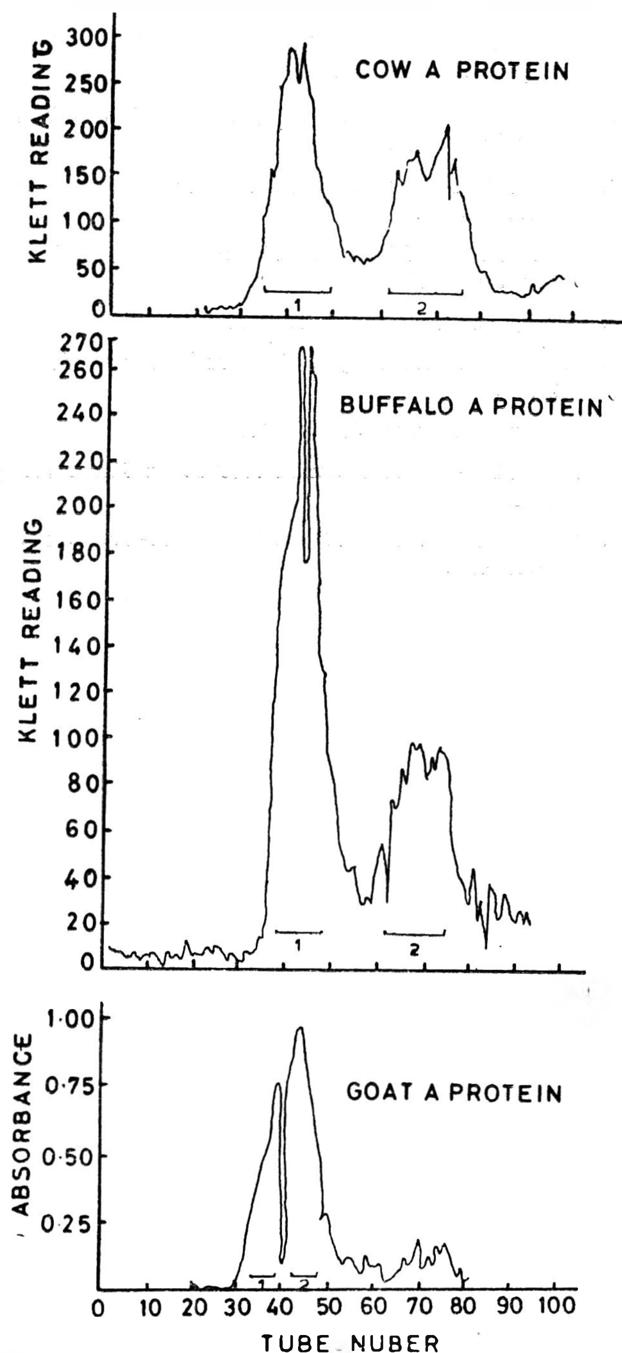

Fig 2. Chromatography of A protein from cow, buffalo and goat milk on Sephadex G-150.

Lactose synthetase activity of galactosyltransferase: The resolution of the three A proteins on Sephadex G-150 is shown in Fig. 2. It was observed that two major protein peaks were obtained in each case. These protein rich peaks were collected separately and freeze-dried. The lactose synthetase activity of these samples then determined using standard bovine ∝-lactalbumin (B protein). The activities of different fractions are recorded in Table 3.

Carbohydrates of A protein: Table 4 shows carbohydrate content of A protein preparations. Results of carbohydrate analysis revealed that A protein contains hexosamine, hexose and sialic acid, thus it can be called a glycoprotein enzyme. Nagasawa *et al.*,[14] showed that human A protein contains sialic acid 1.1; galactosamine 5.03; glucosamine, 2.47; galactose 2.36; mannose 0.87; and fucose 0.87 g per 100 g dried A protein. However, values for hexosamine, hexose and sialic acid in bovine A proteins as shown in Table 4 are

TABLE 3. LACTOSE SYNTHETASE ACTIVITY OF GALACTOSYL TRANSFERASE (A PROTEINS)

Samples	LSA$\times 10^{-6}$
Cow A protein, Peak I	2.1
Cow A protein, Peak II	2.2
Buffalo A protein, Peak I	1.5
Buffalo A protein, Peak II	2.0
Goat A protein, Peak I	2.0
Goat A protein, Peak II	2.1

Lactose synthetase activity using standard bovine ∝-lactalbumin LSA, Lactose synthetase activity expressed as n moles of lactose formed per minute.

TABLE 4. CARBOHYDRATE CONTENT OF THE A PROTEIN*

Samples	Hexosamine	Hexose	Sialic acid
Cow A protein, Peak I	0.73	0.42	1.05
Cow A protein, Peak II	0.75	0.65	0.98
Buffalo A protein, peak I	0.28	3.55	0.94
Buffalo A protein, Peak II	0.11	1.06	0.83
Goat A protein, Peak I	0.85	0.79	1.85
Goat A protein, Peak II	1.17	1.85	2.59

*Expressed in g/100g of dry A protein

much lower. Such lower values may be attributed to breed differences. Differences within the three species studied were also significant.

Polyacrylamide gel electrophoresis of ∝-lactalbumin galactosyltransferase: The homogeneity of purified ∝-lactalbumin preparations were examined by horizontal polyacrylamide gel electrophoresis using tris-HCl buffer (pH 8.9) using sample containing 25-200 μg of protein. It was observed that majority of the samples showed presence of one band indicating homogeneity of the samples.

The A protein preparations were examined by vertical polyacrylamide gel electrophoresis using veronal buffer pH 8.6. It was repeatedly observed that the A protein did not migrate in the gel at pH 8.6. This might be due to higher quantity of carbohydrates in the A protein. On paper electrophoresis at pH 8.6 the A protein preparation showed one zone which indicated homogeneity of the preparations. However, in a few cases some trailings were also observed which indicated need for further purification of the enzyme.

Acknowledgement

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Use of Milk Clotting Enzyme of *Absidia ramosa* in Cheddar Cheese Preparation

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Cheddar cheese prepared using a fungal milk clotting enzyme from *Absidia ramosa* was compared with cheeses prepared with Hansen's animal and Meito rennets. During the preparation there was greater fat loss with *Absidia* and Meito rennets than animal rennet. The two fungal enzymes gave lower yields of cheese than animal rennet. All the three types of cheeses showed a gradual decrease in moisture content during the ripening period, Meito rennet cheese showing the slowest rate of decrease. The trend in the change of acidity and pH in *Absidia* and animal rennet cheeses was similar. Although the initial soluble nitrogen content and maturity index of *Absidia* rennet cheese were higher, the rate of ripening was slower than animal and Meito rennet cheeses. The 2.5% TCA soluble tyrosine content of *Absidia* and Meito rennet cheeses were higher than animal rennet cheese. At six months 86% of 2.5% TCA soluble tyrosine was in 12% TCA soluble form in *Ab.idia* rennet cheese and 94% in Meito rennet cheese while it was only 60% in animal rennet cheese. Organoleptically *Absidia* rennet cheese was comparable to animal and Meito rennet cheeses.

Shortage of animal rennet for commercial cheese preparation has laid great emphasis on the need for obtaining a suitable substitute. Several proteolytic enzymes of animal, plant and microbial origin have been tested for this purpose[1-3]. Some microbial enzymes are available commercially and each of them have their own characteristic differences and limitations in their use as rennet substitutes[3,4]. A milk clotting enzyme from *Absidia ramosa* has been used in the present study to test its suitability as a rennet substitute in cheddar cheese preparation. Its performance in cheese manufacture has been compared with animal and Meito rennet.

Materials and Methods

Organism: *Absidia ramosa* isolated from wheat bran and maintained on the slants of Czepek Dox Agar medium supplemented with 0.5 per cent malt extract (Oxoid) by biweekly transfer (in the Bacteriology Division of the Institute,) was used.

Absidia ramosa *enzyme:* The enzyme was obtained by growing the mold on moist wheat bran medium (50%) at 22°C for 96 hr and then extracting the growth with distilled water. This extract was filtered through Hyflosupercel to get a clear filtrate. It was then precipitated with 30 to 60 per cent (w/v ammonium sulphate). The resulting precipitate was dried in a vacuum desiccator and used for cheese making. The enzyme preparation contained amylase, cellulase and lipase in addition to milk clotting enzyme.

Meita rennet (Mucor pussillus *rennet*): This was obtained from Meito Sangyo, K. K., Japan.

Animal rennet: Hansen's rennet powder was used.

Preparation of cheddar cheese: Pasteurized cow's milk adjusted to a fat: casein ratio of 1.0:0.7 with skim milk was taken in a cheese vat and then tempered to 30°C for addition of starter. One per cent of 16 hr old starter culture (LF-40) (Commercial starter received from M/s Hansen's Laboratory, Denmark consisting of an unknown combination *Streptococci*) was added to this milk and further incubated at 31°C until an increase of 0.02 per cent of lactic acid was noted. Rennet solution was then added and mixed. After the coagulation attained desired firmness, it was cut into small cubes and processed further according to the method outlined by Kosikowski[5]. All the three enzymes were found to form a firm clot of milk even without the addition of calcium chloride. Hence calcium chloride was not added to milk in any of the trials.

The curing of cheese was done in cold room maintained at 13±2°C with 80 per cent humidity.

Moisture content: The moisture content in cheese samples was determined by Mojoiner procedure[6].

Total nitrogen: Cheese sample (100 mg) was used for the determination of total nitrogen by Microkjeldahl method.

Sodium citrate: Method of Vakalaris and Price[7] was used.

Tyrosine content: This was determined by taking 2g of cheese sample and making a fine slurry in 10ml of distilled water which was then mixed with 10 ml of 5 or 24 per cent trichloro acetic acid (TCA) and then filtered through Whatman No. 1 filter paper. The tyrosine contents of the filtrates were determined by the method of Lowry *et al*[8].

Fat: Milk fat was determined as per ISI method[9]. For cheese, 1g sample was taken and the fat was determined according to modified Rose Gotlibs method[10].

Acidity: For testing milk acidity 10 ml of milk sample was taken and titrated against 0.1N NaOH with phenolphthalein as indicator. For the determination of cheese acidity 2 g of cheese sample was finely dispersed in 20 ml of distilled water using a mortar and pestle and the mixture was titrated against 0.1N NaOH using phenolphthalein as indicator. The results are expressed in terms of per cent lactic acid.

pH: 10 g of cheese sample was mixed with 10 ml of distilled water and a fine paste was made. pH reading was taken using a Beckman pH meter.

Maturity index: It was expressed by using the values of water soluble nitrogen and the total nitrogen as per the following formula:

$$\text{Maturity index} = \frac{\text{Sodium citrate—HCl soluble nitrogen} \times 100}{\text{Total nitrogen}}$$

Sensory evaluation: The organoleptic evaluation of experimental cheeses was conducted by a panel consisting of eight judges. The cheese samples were tested for body, texture, taste, smell and acceptability.

Results and Discussion

Changes during manufacture: Results presented in Table 1 give a comparative picture of performance of the three enzymes during the manufacture of cheddar cheese. Eight grams of *Absidia* rennet was required to bring about clotting of 100 kg of milk in 30-45 min. Lesser quantities were required in the case of animal and Meito rennets. The quantity of enzymes required for clotting of milk varies considerably with different rennets[11a,11b,12] and the increased quantity required in the case of *Absidia* rennet was due to the use of crude enzyme preparation which contained in addition to milk clotting enzyme other constituents like amylase, lipase and cellulase.

The initial acidity of whey immediately after cutting the curd was 0.12 per cent in all the three types of cheeses. There were some variations in the acidity values during cooking and cheddaring and the final acidities at the time of milling were 0.38 per cent in the case of animal rennet cheese, 0.39 to 0.44 per cent in the case of *Absidia* rennet cheese and 0.35 to 0.40 per cent in Meito rennet cheeses. The similarities observed in acid development showed that the three enzymes were neither stimulatory nor inhibitory to starter cultures.

The fat loss in whey was 0.4 to 0.5 per cent with *Absidia* enzyme, 0.5 to 0.7 per cent with Meito rennet and 0.1 per cent with animal rennet. The probable causes for higher fat loss in whey in the case of microbial rennet cheese are (*i*) the lowering of the surface tension of the fat globules by lipases present in these enzymes; and (*ii*) the effect on the protein lipid interactions caused by these enzymes resulting in reduced retention of fat in the curd. Higher fat loss in whey has been recorded with *M. pusillus* enzyme by Kikuchi *et al.*[11b] and Antila and Apola[13a,13b].

The yield of green cheese was 10.4—11.9 kg with *Absidia* rennet, 12.5 kg with animal rennet and 10.5—10.8 kg with Meito rennet. The lower yields of cheese in the case of microbial enzymes are obviously due to higher lipolytic activity as well as higher proteolytic activity resulting in greater loss of proteins including those in whey than in animal rennet. The lower moisture content of the *Absidia* rennet cheese (Table 2) may also be another reason for lower yield. Higher protein loss in whey has been reported by Kikuchi *et al.*,[11a,11b] in the case of *M. pussillus* rennet. Tsugo *et al.*,[14] however, obtained similar yields of cheese with Meito rennet despite its higher proteolytic nature than in animal rennet. Lower yields of cheese have also been reported with *Endothia* rennet, *Mucor miehei* enzyme and *M. pussillus* rennet[15-18].

Changes during ripening: The moisture content decreased from 38.72 to 35.67 per cent in the case of *Absidia* rennet cheese; from 41.9 to 39 per cent in the case of animal rennet cheese and 39.91 to 38.75 per cent in Meito rennet cheese during the six-month period of ripening (Table 2). Due to the loss of moisture the fat content recorded a rise and the final values were 29.27, 28.50 and 25.25 per cent for *Absidia*, animal and Meito rennet cheeses respectively. In these respects the changes brought about by *Absidia* rennet in cheese were closely similar to those brought about by animal rennet, Meito rennet[11a,11b] Endothia rennet[19] Suparen and Rennilase[19-21].

The acidity of all the three types of cheeses initially recorded an increase upto 30 days of ripening indicating the accumulation of primary breakdown products such as lactic acid and other lower volatile fatty acids. Subsequently there was a fall in acidity and a rise in pH value from 5.20 to 5.57 in the case of *Absidia* rennet cheese, 5.23 to 5.60 in animal rennet cheese and 5.13 to 5.75 in Meito rennet cheese during the six months period of ripening (Table 3). This rise in pH again indicated that the desired changes were taking place in the cheeses due to the action of the rennets on the primary decomposition products causing accumulation of compounds like neutral carbinols, soluble peptides and basic amino acids which contribute to the texture. flavour and taste of the cheese. In this respect also *Absidia* rennet was similar to animal and other microbial rennets[14,11a,11b,22].

The 5-day sodium citrate-HCl soluble nitrogen (SN)

TABLE 1. COMPARATIVE CHEESE MANUFACTURERS DATA OF MILK CLOTTING ENZYMES

Manufacturing stages	Hansen's rennet	Absidia rennet			Meito rennet	
	I	I	II	III	I	II
Milk acidity (% lactic acid)	0.17	0.17	0.16	0.16	0.16	0.16
Rennet						
(g/100 lit)	3.0	8.0	8.0	8.0	3.0	2.0
Clotting time (min)	45	30	45	35	20	25
Cutting						
Time after renneting (min)	55	40	55	45	30	35
Whey acidity (% lactic acid)	0.12	0.12	0.12	0.12	0.12	0.12
Cooking						
Period (min)	40	40	40	40	40	40
Fat loss in whey (%)	0.1	0.4	0.5	0.4	0.7	0.5
Cheddaring						
Initial acidity (% lactic acid)	0.14	0.14	0.14	0.14	0.13	0.13
Final acidity ,,	0.36	0.37	0.29	0.32	0.20	0.32
Period (hr-min)	3.15	3.00	3.30	3.15	5.00	4.00
Milling						
Time after renneting (hr-min)	5.20	4.50	5.35	5.10	6.40	5.45
Milling acidity (% lactic acid)	0.38	0.42	0.39	0.44	0.35	0.40
Salt (g/100 kg milk)	300	300	300	300	300	300
Cheese yield* (kg)	12.5	11.9	10.4	10.7	10.5	10.8

100 kg pasteurized cow milk adjusted to a fat: casein ratio of 1.0:0.7 tempered to 30°C and inoculated with 1% of a starter culture LF-40 with an acidity of 1%. Development of acid was allowed for 30 min after which rennet was added.

*After pressing at 1.5 kg/sq.ft. for 24 hr. Milk was pasteurized by HTST method.

TABLE 2. CHANGES IN MOISTURE AND FAT CONTENTS DURING RIPENING OF CHEESE

Rennet used	Ripening period (days) 5		30		75		180	
	Moisture (%)	Fat (%)	Moisture (%)	Fat (%)	Moisture (%)	Fat (%)	Moisture (%)	Fat (%)
Animal rennet[1]	41.90	20.00	39.77	23.40	39.60	28.90	39.00	28.50
Absidia rennet[2]	38.72	20.60	36.85	28.03	36.34	29.33	35.67	29.27
Meito rennet[3]	39.91	21.30	39.75	25.60	39.25	25.25	38.75	25.25

1. Values of a typical trial 2. Mean of 3 trials 3. Mean of 2 trials.

TABLE 3. CHANGES IN pH AND TITRATABLE ACIDITY DURING RIPENING OF CHEESE

Rennet used	Period of ripening (days) 5		30		75		180	
	pH	Acidity*	pH	Acidity*	pH	Acidity*	pH	Acidity*
Animal rennet[1]	5.15	0.625	5.20	0.75	5.40	0.60	5.60	0.55
Absidia rennet[2]	5.20	0.73	5.28	1.00	5.30	1.07	5.57	0.82
Meito rennet[3]	5.23	0.78	5.38	0.88	5.45	0.75	5.75	0.63

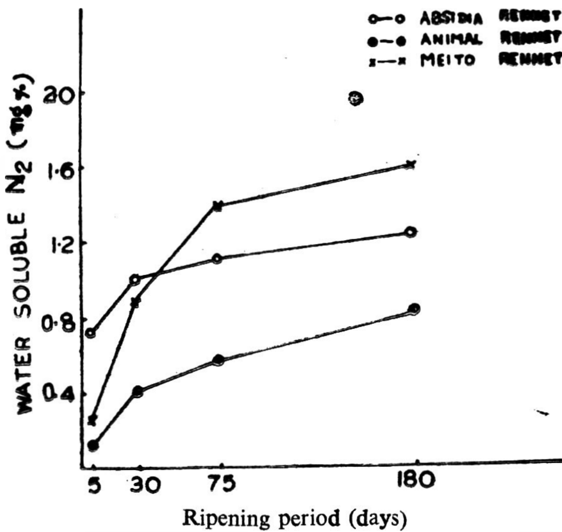
*Per cent lactic acid.

1. Values of a typical trial. 2. Mean of 3 trials. 3. Mean of 2 trials.

TABLE 4. SENSORY EVALUATION OF CHEDDAR CHEESE AFTER EIGHT MONTHS OF RIPENING

Characteristics	Max points	Scores obtained with different types of rennets							
		Animal rennet	Absidia rennet				Meito rennet		
		I	I	II	III	Mean	I	II	Mean
Body and Texture	40	24.5	30.0	23.5	25.5	26.3	26.5	18.5	22.5
Taste	40	18.5	20.5	19.0	19.5	19.7	20.0	13.0	16.5
Smell	40	16.5	21.0	21.0	19.0	20.3	20.0	12.0	16.0
Total score	120	59.5	71.5	63.5	64.0	66.3	66.5	43.5	55.0
	A	7	7	8	7		6	6	
Acceptability	NA	1	1	0	1		2	2	

A = Acceptable NA = Not acceptable

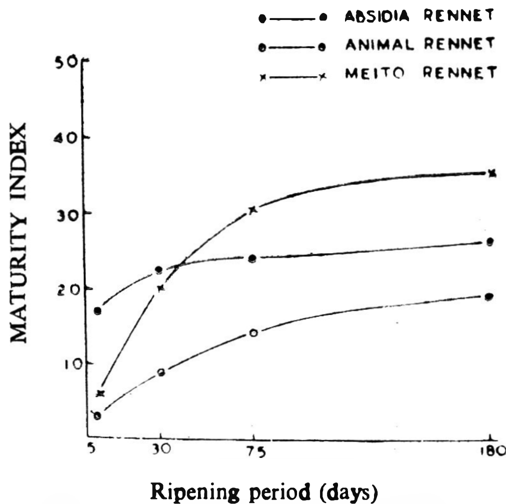


Ripening period (days)

Fig 1. Changes in soluble nitrogen during ripening of cheddar cheese

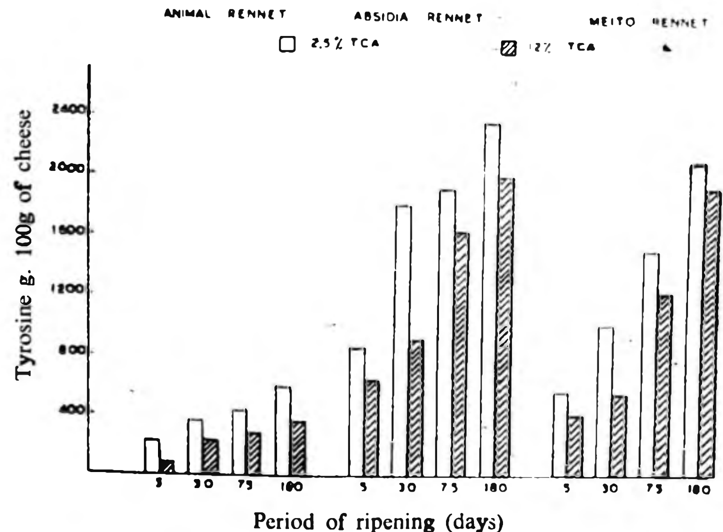
content was relatively high (0.73 per cent) in *Absidia* rennet cheese as compared to Meito (0.22 per cent) and animal (0.11 per cent) rennet cheeses (Fig. 1). During subsequent ripening the rate of increase in SN was highest in Meito rennet cheese. *Absidia* and animal rennet cheeses gave almost parallel increase in SN although higher in the former. This indicates higher protein degradation intially with *Absidia* as compared to animal and Meito rennets. But tertiary proteolysis was higher with Meito rennet than animal or *Absidia* rennets. Similar observations have been made by Kikuchi *et al.*,[11a11b] with Meito and Hansen's rennets.

Maturity index as measured by the ratio between soluble nitrogen and total nitrogen was also higher initially in case of *Absidia* rennet cheese than with Meito or animal rennet cheeses. The rate of increase was maximum with Meito cheese. *Absidia* and animal rennet cheeses again showed somewhat parallel rate of increase from 75 to 180 days. The former cheese however



Ripening period (days)

Fig 2. Variation of maturity index during ripening of cheddar cheese



Period of ripening (days)

Fig 3. Changes in TCA soluble tyrosine during ripening of cheddar cheese

showed higher values of maturity index (Fig. 2). Higher rates of increase in maturity index as compared to animal rennet have been reported in the case of Meito and Endothia rennets.[14,15,22] *Absidia* rennet was more close to animal or *Mucor miehei* rennet in its tertiary proteolysis than Endothia or Meito rennets. *Mucor miehei* rennet was reported to show slowest increase in maturity index[13] as compared to Meito and animal rennets.

The initial 2.5 per cent TCA soluble tyrosine content was higher in the case of *Absidia* rennet cheese (870 mg/100g) than animal (220 mg/100g) and Meito (570mg/100g) rennet cheeses. After six months ripening also 2.5 per cent TCA soluble tyrosine was highest in *Absidia* cheese (2350mg/100g) followed by Meito (2100mg/100g) and animal rennet (600mg/100g). Nearly 86 per cent of 2.5 per cent TCA soluble tyrosine was in 12 per cent TCA soluble tyrosine form at six months in the case of *Absidia* rennet cheese and 94 per cent in Meito rennet cheese while only 60 per cent in animal rennet cheese (Fig. 3). The breakdown ratio between 2.5 per cent TCA soluble tyrosine and 12.0 per cent TCA soluble tyrosine was initially 74 per cent in *Absidia* rennet cheese, 72 per cent in Meito rennet cheese and 37 per cent in animal rennet cheese. These results also confirm that tertiary proteolysis was slower in the case of *Absidia* rennet than in other two enzymes.

Absidia rennet cheese was comparable in respect of body, texture, flavour and acceptability to that of animal and Meito rennet cheeses. (Table 4)

By purification of the enzymes and suitable modification in the manufacturing techniques of cheese it would be possible to minimise initial proteolysis and get normal yield and also prevent the development of bitterness.

Acknowledgement

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Studies on the Preparation of Wort from Barley Malt and Degermed Maize Using Microbial Enzymes

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Barley malt, an expensive commodity compared to barley grain, is invariably used with or without adjuncts in brewing. In order to reduce the cost of inputs, an attempt is made to replace malt partially by degermed maize and prepare wort using microbial enzymes during mashing. The quality of wort is compared with that of all-malt wort. Wort of suitable specific gravity with desirable characteristics such as colour, and carbohydrates, can be prepared using a combination of malt (30%) and degermed maize (70%) with microbial enzyme. The minimum alpha amino nitrogen centent of wort in the malt maize combination is inadequate for the growth of yeast and needs modification to maintain the level.

Barley malt with or without adjuncts is the chief raw material used in conventional brewing[1]. Malt produced from barley grains involves operations like steeping, germination and kilning. Kilning operation during malting is highly energy consuming. As a result, the cost of malt practically becomes double that of barley. The increased cost of inputs results in the increased cost of the finished product, namely, beer. This has necessitated technologists all over the world to think of alternative methods of producing wort, partially substituting malt by barley grain and using microbial enzymes to act upon the protein and starch of the grain[2-10]. Wort so produced is expected to possess all the qualities noticed in all-malt wort and on fermentation is expected to yield beer comparable to that produced in the conventional way. Laboratory studies were, therefore, initiated partially to replace malt by degermed maize. The qualities of wort prepared are reported in the paper.

Materials and Methods

Raw materials: Degermed maize and commercially available malt were used.

Enzymes: Commercially available (Indian proprietory brands) fungal protease and bacterial alpha amylase were used. They were found quite active when tested by conventional methods[11].

Bacterial amylase was used in the liquefaction of the maize starch; protease was added during mashing of the maize malt mixture.

Degermed maize was analysed for moisture, total nitrogen and starch by the methods described in AOAC[12]. Total carbohydrates were determined according to the method of Yadav *et al*[13]. Alpha amino nitrogen was estimated by the EBC ninhydrin method of Lie[14]. Specific gravity was determined using Pycnometer at 20°C. Infusion method was adopted for mashing.

Mashing procedure: Barley malt was replaced by degermed maize at four levels, viz., 95, 90, 85 and 70 per cent and mashed as follows:

Degermed maize flour (30 mesh) was added to water at 65°C and was allowed to rest for 30 min. Alpha-amylase (0.25 g per cent) was added and mixed. The temperature was gradually raised to 82°C at the rate of 1°C per min and maintained at that temperature for 15 min and boiled for 5 min and cooled to 60°C.

Malt flour (30 mesh) was added to water at 42°C and was allowed to rest for 15 min and the temperature was increased at the rate of 1°C per min upto 50°C and allowed to rest for 15 min. The temperature was raised at the rate of 1°C per min to 60°C. Gelatinised maize starch was added to the malt mash and the mixture was maintained at 60°C for 60 min. The temperature was raised to 70°C at the rate of 1°C per min and held at that temperature for 10 min. In the same way the temperature was raised to 80°C and after holding the mash at that temperature for 10 min, the extract was tested for the absence of starch by iodine reaction. A quick boiling was given, cooled and the weight was adjusted to the original weight and filtered through a Buchner funnel. The residue was sparged with water at 80°C and the filtrate collected. Sparging was continued till the washings had no soluble solids. The volume was measured and the extract was sterilised at 1.1 kg/cm^2. The clear extract was analysed.

In order to study the effect of pH on mashing of maize flour, the pH of the mash was adjusted appropriately prior to the addition to barley malt mash. Where the effect of protease was studied, the enzyme was added to the maize-malt mixed mash.

TABLE 1. THE QUALITY OF EXTRACT FROM DIFFERENT COMBINATIONS OF BARLEY MALT AND DEGERMED MAIZE

Physical and chemical characteristics	Barley malt (5%) + Degermed maize (95%)	Barley malt (10%) + Degermed maize (90%)	Barley malt (15%) + Degermed maize (85%)	Barley malt (30%) + Degermed maize (70%)
Sp. gr.	1.05	1.05	1.047	1.047
pH	6.5	6.5	6.5	6.5
Volume (ml)	500	510	540	620
Colour	Pale yellow	Pale yellow	Yellowish brown	Brownish yellow
Total carbohydrates (g %)	10	10	10	10
α-amino N (mg/lit.)	12	16	20	40

Results and Discussion

Degermed maize on analysis was found to contain 10.23 per cent moisture, 7.70 per cent (DWB) protein and 82.07 per cent (DWB) starch.

The quality of wort (extract) derived by replacing malt at four different levels is shown in Table 1.

The worts of the first two combinations had raw and unpleasant flavour while that of 15 per cent malt had a mild malt flavour. Wort got by 30 per cent malt had better pleasant flavour. The first two combinations were discarded and further experiments were carried out using 15 and 30 per cent malt and 85 and 70 per cent maize respectively.

Alpha amylase degrades the starch and thus reduces the viscosity of maize mash. It was of interest to study the viscosity changes of the mash at different concentrations of the enzyme. Alpha-amylase at concentrations of 150, 200 and 250 mg per cent was added to 15 and 30 per cent malt and the viscosity was measured (Table 2).

Increased concentration of α-amylase at two levels of malt affects the viscosity of the mash. A considerable reduction is observed when malt at 30 per cent level is used for mashing. The viscosity values at 200 and 250 mg per cent level are practically same indicating that even 200 mg per cent alpha-amylase would be adequate during mashing. No change in volume, specific gravity, carbohydrate and alpha amino nitrogen contents was observed in worts derived from combinations of 30 per cent malt and 70 per cent maize when mashed with 200 or 250 mg per cent alpha amylase.

The effect of pH on the quality of worts was studied in the two combinations at 15 and 30 per cent levels of malt and the results are indicated in Table 3.

It can be seen from Table 3 that both the contents of alpha amino nitrogen and carbohydrates of wort in the 30 per cent malt combination are more than the other one. pH 6.0 seems to have a better effect on the conversion of starch and protein. The flavour of the wort in the combination of 30 per cent malt and 70 per cent maize was much better than in the other.

TABLE 2. EFFECT OF ENZYME CONCENTRATION (ALPHA-AMYLASE) ON THE VISCOSITY OF THE MASH

Physical characteristics	Alpha-amylase concn (mg %)					
	Barley malt (15%) + Maize (85%)			Barley malt (30%) + Maize (70%)		
	150	200	250	150	200	250
Viscosity (CP)	152.5	102.5	100.0	100.0	72.5	70.0
pH	6.5	6.5	6.5	6.5	6.5	6.5

TABLE 3. EFFECT OF PH ON THE QUALITY OF WORT

Physical and chemical characteristics	Barley malt (15%) + Maize (85%)			Barley malt (30%) + Maize (70%)		
	pH 6.0	pH 6.5	pH 7.0	pH 6.0	pH 6.5	pH 7.0
Sp. gr.	1.049	1.047	1.050	1.052	1.048	1.046
Volume (ml)	660	575	500	—	550	600
Colour	YB	YB	YB	BY	BY	BY
Carbohydrates (g, %)	10.2	10.5	11.1	13.77	11.11	11.00
α-amino N (mg/lit.)	30	30	30	65	60	55

YB: Yellowish brown; BY: Brownish yellow

TABLE 4. EFFECT OF PROTEASE ON THE QUALITY OF WORT

Physical and chemical characteristics	Barley malt (15%)+Maize (85%)	Barley malt (30%)+Maize (70%)	All-malt wort
Sp. gr.	1.047	1.048	1.047
pH	6.3	6.3	5.7
Volume (ml)	575	625	—
Colour	YB	BY	BY
Carbohydrates (g %)	12.2	12.2	9.2
α-amino N (mg/lit)	50	80	151.2

YB: Yellowish brown; BY: Brownish yellow.

Soluble nitrogenous compounds of wort are important for the yeast during fermentation. The level of alpha amino nitrogen in all-malt wort for the growth of yeast is expected[15] to be 140 mg/lit. The laboratory worts never showed a value more than 60 mg/lit. It was felt that the proteolytic enzymes in barley malt used in the experiments were either not potent enough or quantitatively insufficient to act upon proteins of barley malt and maize. Therefore, a protease enzyme at a level at 50 mg per cent was incorporated during mashing and the resulting wort was analysed chiefly for alpha amino nitrogen content. The added enzyme had a wide range of activity between pH 3.0 and 10.0. The results are shown in Table 4.

An increase in the alpha amino nitrogen and carbohydrate contents in the worts indicates the usefulness of the addition of external source of protease enzyme. The increased level in the carbohydrate content in the two cases may be attributed to the amylase and cellulase contaminating enzymes present in the added protease enzyme.

The quality of wort derived from malt-maize combination compared with all-malt wort indicates that the alpha-amino nitrogen level in all-malt wort is more than in the worts of malt-maize combination.

Conclusion: Wort of suitable specific gravity with desirable characters such as colour and carbohydrates can be prepared using a combination of malt (30 per cent) and degermed maize (70 per cent) with microbial enzymes.

The minimum alpha amino nitrogen content of wort in the malt-maize combination is inadequate for the growth of yeast and needs modification to maintain the level.

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Bread and Chapati Making Quality of Indian Durum Wheats

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Twenty-eight varieties of Indian durum wheats and their blends with a soft aestivum wheat- ***Chhoti lerma,*** **have been studied for bread making and chapati making quality. Varieties** ***Local red, Amrit, A*****-206,** ***A*****-1-1-2-3,** ***HD*****-4519 and** ***HD*****-4530 as such had excellent bread making quality and may be categorised as bread type durums. Ten varieties—*****Local red, Amrit, NI*****-5759,** ***A*****-1-8-1,** ***HI*****-7597,** ***WL*****-1002,** ***HD*****-4519,** ***HD*****-4530,** ***HD*****-4502 and** ***A*****-9-30-1, when blended with** ***Chhoti lerma*** **even at 15% level, gave excellent bread.**

Chapatis made from 93-95% extraction flour (***atta*****) from 14 durum varieties as such, were of excellent quality; while most of the** ***Chhoti lerma*** **blends with different durums at 15-25% level gave excellent chapatis. Unlike for bread wheats, no correlation was observed between the Farinograph water absorption of the flour and the chapati making quality. Only** ***HD*****-4530 and** ***HD*****-4519, when used as such or in blend with** ***Chhoti lerma*****, were unique in that they gave excellent bread as well as chapatis.**

Durums in India are cultivated on large scale in central and peninsular zones, to the extent of 2-3 million tons. They are mainly converted into semolina, which is used in various sweet and savoury dishes served as breakfast or snack items.

On the basis of Farinograph data, it has been observed earlier that proteins of some of the durums are similar to those of aestivum wheats which are considered suitable for bread making.[1,2] Commercially grown Indian aestivum wheats are medium-hard and are not well suited for bread making. The poor bread making quality of many Indian aestivum wheats has been attributed to their low protein content of poor quality and low diastatic activity[3]. Earlier studies[1] have indicated that Indian durums have high values for protein content (10.5-15.7 per cent), diastatic activity (302-443 mg maltose/10 g flour) and water absorption (62.0-75.0 per cent). It was therefore, considered worthwhile to explore the possibility of improving the bread making quality of aestivum wheats by blending them with durums for correcting the deficiencies in the flour quality.

Indian wheats are generally suitable for chapati making. Traditionally, in some regions of India, aestivum wheats are blended with small quantities of durums for improving their chapati making quality. No information is available on the bread or chapati making quality of durum wheats as well as durum-aestivum blends. The work carried out on the bread and chapati making quality of 28 varieties of Indian durums and their blends with aestivum wheat as compared to Canadian durums is presented in this paper.

Materials and Methods

Raw materials: Twenty-eight Indian and 3 Canadian durum varieties were conditioned overnight to 16.5 per cent moisture level and milled in a laboratory mill (Model MLU-202) to obtain white flour (*maida*). *Maida* sample, milled from two aestivum spring wheats—*Sharbati sonora* and *Chhoti lerma*—conditioned to 15 per cent moisture level were used as controls. The former variety, a commercially grown medium hard wheat, containing 12.2 per cent protein was used as positive control, as it is generally considered suitable for bread making. The latter variety containing 9.4 per cent protein and known for its poor bread making quality, was used as negative control as well as for blending with durums.

Wheat flour of 93-95 per cent extraction (*atta*) was used for studying the chapati making quality of durums. Different varieties were ground in a Kamas hammer mill (Swedish make, type SLAGY-200A) using a sieve with apertures of 0.8 mm diameter and was passed through 40 mesh Buhler sieve (aperture width-0.42 mm) to remove the coarse fraction mainly consisting of outer bran layers. Good quality commercial Punjab wheat containing 11.9 per cent protein and *Chhoti lerma* were used as positive and negative controls respectively.

Bread making quality: For evaluating bread making quality of durums, Remix formula (based on 100 g *maida*) and procedure of Irvine & McMullan[4] were followed. The dimensions of the standard baking pans used were: 9.5×5.5 cm at bottom and 11.0×6.5 cm at the top and an edge to edge height of 7 cm. For each variety, breads were made in duplicate. Loaf volume of bread was determined by rape-seed displacement method using a loaf-volume meter. In bread evaluation, criteria followed by panel of 6 judges were general appearance, loaf volume, crust and crumb colour, crumb softness, fineness and uniformity of crumb grain, and flavour and eating quality.

Several bread preparation trials using various levels (10, 15, 25, 33 and 50 per cent) of 5 durum varieties were carried out to arrive at their optimum blending capacity with aestivum wheat. Accordingly, 15 per cent of durum was used for blending in case of the remaining varieties in further trials.

Chapati making quality: Different *atta* samples of Indian durums were evaluated for their chapati making quality according to procedures described by Shurpalekar and Prabhavati[5]. Water absorption of the *atta* samples was determined using a 50 g mixing bowl in a Brabender Farinograph set in lever position 1:3. Using the water absorptions arrived at for different varieties, the chapati doughs having an optimum consistency of 500±20 BU were prepared in the Farinograph mixer. Chapatis were rolled from these doughs on an aluminium plat-form using wooden rolling pin and baked for 3-4 min at 162.5°C on a thermostatically controlled hot plate. Chapati making quality of blends of durum and *Chhoti lerma* was also studied. For arriving at the optimum level for blending durum *atta* samples were used at 15, 20, 25, 30 and 35 per cent levels. Chapatis were then assessed according to Shurpalekar and Prabhavati[5] by a panel of six judges. The main criteria considered for evaluation were appearance, texture, number of discrete layers, chewing quality and taste.

Results and Discussion

Bread making quality of durums: The data presented in Table 1 indicate that varieties *Local red*, *Amrit*, *A*-206, *A*-1-1-2-3, *HD*-4519 and *HD*-4530 had excellent bread making quality with a loaf volume of 600 ml or more (Fig. 1). Varieties *NP*-404, *A*-11 and *HI*-7720 were as good as positive control with a loaf volume of 550 ml or more. The above mentioned varieties can, therefore, be categorised as bread type durums, with variety *A*-206 excelling the rest. All the 3 Canadian durums were inferior to the positive control *Sharbati sonora*. Eight of the varieties with a loaf volume of 450 ml or less were adjudged as unsuitable for bread making.

Bread making quality of durum-aestivum blends: The results of preliminary trials to arrive at optimum blending level of durum required to bring about maximum improvement in the bread making quality of aestivum flours are presented in Table 2. Even when durum flour was used at a low level of 15 per cent, the water absorption of the blend showed considerable increase as compared to 52 per cent for *Chhoti lerma*. This increase was dependent on the variety as well as the level of durums used for blending. As durums cost somewhat higher than aestivum wheat, it was considered desirable to use them at minimum possible level. Blending with durum at different levels brought about increase in the loaf volume of *Chhoti lerma*. The increase in the loaf volume was more significant in the case of non-bread type durums such as *Bijaga red*, *Bansi*, *Bijaga yellow*, whereas in bread type durums like *Local red* and *Amrit* the loaf volume of breads based on the blends were somewhat similar to those based only on durums. It is also interesting to note that maximum beneficial effect of blending on the loaf volume was observed at a low level of 15

TABLE 1. EVALUATION OF BREAD FROM DURUM WHEATS AND THEIR BLENDS WITH SOFT AESTIVUM WHEAT

Variety	Durums		Durum-*Chhoti lerma* (15:85) blend	
	Loaf volume[1] (ml)	Overall quality	Loaf volume (ml)	Overall quality[2]
Local red	600	E	600	E
Bijaga yellow	475	F	565	G
Amrit	625	E	600	E
Bijaga red	400	P	540	S
N-59	505	S	510	S
NI-5759	460	F	585	E
A-206	665	E	525	S
A-624	430	P	490	S
A-1-1-2-3	600	E	505	S
A-11	565	G	555	G
A 1-8-1	495	S	625	E
A-9-30-1	475	F	590	E
MPO-157	440	P	460	F
MPO-142	490	S	475	F
MPO-159	520	S	500	S
MPO-161	420	P	475	F
Raj-911	430	P	400	P
Raj-912	475	S	455	F
NP-404	560	G	455	F
HI-7483	510	S	455	F
HI-7747	450	F	505	S
HI-7720	550	G	520	S
HI-7597	425	P	600	E
WL-1002	475	S	600	E
HD-4519	600	E	605	E
HD-4530	600	E	595	E
HD-4502	360	P	605	E
Bansi (commercial)	435	P	550	G
Stewart-63	530	S	555	G
Wakooma	540	S	555	G
Hercules-72	540	S	530	S
Chhoti lerma[3]	450	P	—	—
Sharbati sonora[4]	570	G	—	—

[1]Based on 100 g flour
[2]E-Excellent; G-Good; S-Satisfactory; F-Fair
[3]Aestivum soft wheat used as negative control
[4]Aestivum medium hard wheat used as positive control

TABLE 2. EFFECT OF BLENDING AESTIVUM WHEAT WITH DURUMS ON ITS BREAD MAKING QUALITY

Variety	Blending level of durum																				
	0			10%			15%			25%			33%			50%			100%		
	FWA %	LV ml	OQ	FWA %	LV ml	OQ	FWA %	LV ml	OQ	FWA %	LV ml	OQ	FWA %	LV ml	OQ	FWA %	LV ml	OQ	FWA %	LV ml	OQ
Local red	—	—	—	53.0	530	S	53.8	590	E	54.5	545	G	56.1	540	S	56.6	460	F	62.0	600	E
Bijaga yellow	—	—	—	53.4	500	S	54.1	565	G	56.0	515	S	57.8	505	S	59.0	430	P	68.4	475	F
Amrit	—	—	—	53.9	535	S	56.4	600	E	57.2	570	G	60.0	560	G	61.6	525	S	70.0	625	E
Bijaga red	—	—	—	54.0	505	S	54.8	540	G	56.0	505	S	60.2	480	F	61.4	425	P	70.0	400	P
Bansi (Commercial)	—	—	—	54.0	510	S	55.6	550	G	56.6	475	F	60.4	465	F	61.4	445	P	70.0	435	P
Chhoti lerma[1]	52.0	450	P		—			—			—			—			—			—	
Sharbati sonora[2]	60.0	570	G		—			—			—			—			—			—	

FWA —Farinograph water absorption on 14% moisture basis.
LV —Loaf volume based on 100 g flour.
OQ —Overall quality.
[1] —Aestivum soft wheat used as negative control.
[2] —Aestivum medium hard wheat used as positive control.

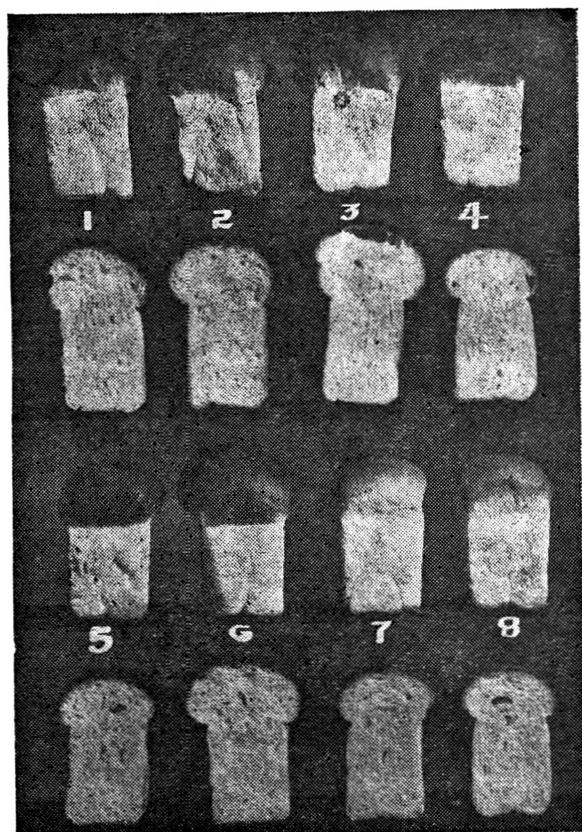

Fig 1. Durum bread of excellent quality

1–*Local red*, 2–*Amrit*, 3–*A*-206 4–*A*-1-1-2-3, 5–*HD* 4519, 6–*HD*-4530, 7–*Sharabati sonora*, 8–*Chhoti lerma*.

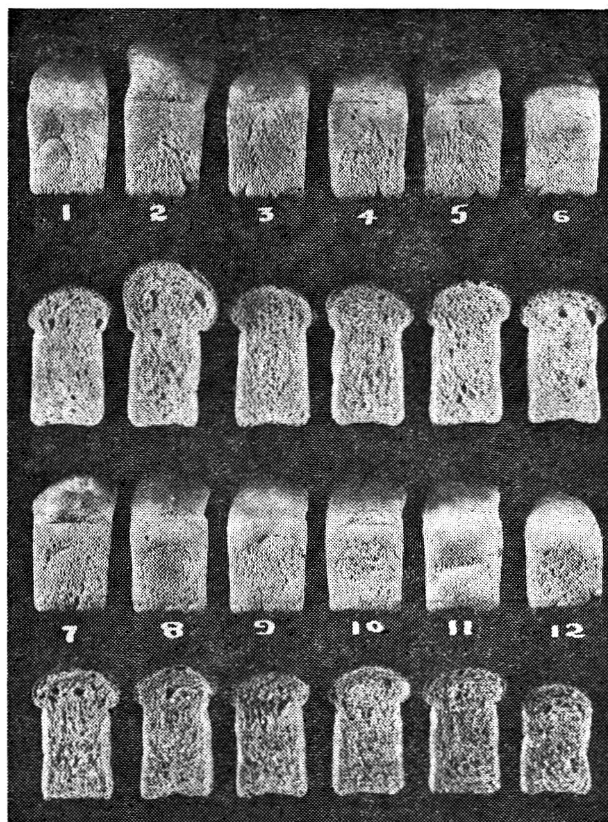

Fig 2. Durum aestivum (15:85) blends with excellent bread making quality

1–*Local red*, 2–*Amrit*, 3–*NI*-5759, 4–*A*-1-1-1, 5–*HI*-7597, 6–*WL*-1002, 7–*HD*-4519, 8–*HD*-4530, 9–*HD*-4502, 10–*A*-9-30-1, 11–*Sharabati sonora*, 12–*Chhoti lerma*.

TABLE 3. CHAPATI MAKING QUALITY OF DURUM WHEATS AND THEIR BLENDS WITH SOFT AESTIVUM WHEAT

Variety	Durums		Durum-*Chhoti lerma* blend	
	F.W.A. ***%	Overall quality	Blending level %	F.W.A. %
Local red	61.0	F	25	58.0
Bijaga yellow	61.8	G	25	58.0
Amrit	60.3	F	25	57.0
Bijaga red	60.9	P	25	58.0
N-59	62.4	S	20	60.0
NI-5759	56.1	E	15	54.0
A-206	64.6	F	20	61.0
A-624	62.2	P	35	60.0
A-1-1-2-3	64.4	E	20	61.2
A-11	62.0	E	20	61.2
A-1-8-1	54.0	F	20	52.0
A-9-30-1	63.0	E	15	51.0
MPO-157	63.0	E	20	60.0
MPO-142	62.0	E	20	60.0
MPO-159	55.0	F	20	53.0
MPO-161	62.0	E	20	60.0
Raj-911	64.4	E	35	60.0
Raj-912	65.0	E	15	59.4
NP-404	63.0	E	15	60.6
HI-7483	62.0	G	15	59.6
HI-7747	64.4	P	20	61.6
HI-7720	63.0	P	30	61.0
HI-7597	55.0	E	15	53.0
WL-1002	56.4	F	20	54.0
HD-4530	57.0	E	15	55.0
HD-4519	59.6	E	15	56.0
HD-4502	59.7	P	25	57.6
Bansi	61.0	E	25	58.0
*Chhoti lerma**	52.0	P	—	—
Punjab (commercial)	**58.0	E	—	—

F.W.A.—Farinograph water absorption on 14% moisture basis.
*Aestivum soft wheat used as negative control
**Aestivum medium hard wheat used as positive control.
*** for getting excellent chapaties

per cent, while both at 10 as well as 25 per cent or higher levels, the loaf volumes were considerably lower. As both bread type and non-bread type durums were included in the preliminary trials, it was inferred that the optimum level of durum blending was 15 per cent.

The data presented in Table 1 also cover the remaining trials for evaluation of bread making quality of other durums when blended with *Chhoti lerma* at 15 per cent level. Majority of the durum varieties used for blending brought about significant improvement in the loaf volume as well as quality of bread, based only on *Chhoti lerma*. Ten of the varieties used for blending gave excellent quality bread (Fig. 2). Varieties *Local red*, *Amrit*, *HD*-4530 and *HD*-4519 besides having excellent bread making quality, also gave excellent bread when blended at 15 per cent level. Even though bread based only on durums—*NI*-5759, *HD*-4502,*A*-9-30-1 nad *HI*-7597 were poor in quality—it is interesting to note that their blends with *Chhoti lerma* gave excellent bread. In contrast, varieties *MPO*-159, *NP*-404 *HI*-7483 and *Raj*-911, on blending, gave inferior quality bread as compared to those based on durums alone. Canadian durums, at 15 per cent blending level gave bread of satisfactory quality with loaf volumes ranging from 530 to 555 ml. This compared favourably with loaf volumes of breads from durums alone.

It may be inferred from these experiments that many durum varieties can be used with advantage for improving the bread making quality of even poor quality Indian aestivum wheats by a simple method of blending with durums at even a low level of 15 per cent. This observation is of considerable importance to the organised bread production industry in India which produces more than a million loaves per annum.

Chapati making quality of durum: The data regarding chapati making quality of durum wheats, their blends with *Chhoti lerma* and water absorption of different *atta* blends to give chapati doughs of desired consistency of 500±20 BU are given in Table 3. The water absorption of blends for obtaining chapati doughs ranged between 53.0 and 61.6 per cent. As against this, the water absorption for chapati doughs based on durums only was 0.8-5.6 per cent more in majority of the varieties tried. Fourteen of the durum varieties had excellent chapati making quality, while 11 varieties gave just acceptable or poor grade chapaties.

As regards suitability of durum-*Chhoti lerma* blends for chapati making, the minimum level of durum required in the blend to give excellent chapaties in most cases ranged between 15 and 35 per cent. As generally observed in bread wheats, no correlation between Farinograph water absorption of *atta* and chapati making quality was observed in the case of Indian durum wheats.

The present studies have high-lighted the fact that apart from conventional suitability for production of paste goods, many Indian durum varieties can be used for improving the bread and chapati making quality of even poor quality aestivum wheats. Use of only 15 and 15-25 per cent durum for blending with such wheats brings about highly significant improvements in the quality of bread and chapaties respectively.

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Physico-chemical, Rheological and Milling Characteristics of Indian Durum Wheats

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Twenty-four Indian and three Canadian durum wheat varieties have been evaluated for physico-chemical characteristics, rheological properties using farinograph and extensograph and the flour milling quality. Of the quality characteristics evaluated, some of the Indian varieties were comparable to or even better than the Canadian durums. The data for varieties *NI*-5759, *A*-11, *HI*-7720, *HD*-4519, *HD*-4530, *A*-9-30-1 were indicative of their potential for the preparation of paste goods; while quality characteristics of many other varieties showed their potential for bread making.

The range and average (in parentheses) values for different quality characteristics were—1000 kernel weight, 34.6-53.1 (43.8) g; pearling index, 13.2-20.5 (16.1); crude protein, 10.6-16.8 (14.6%); wet gluten, 28.2-52.6 (40.7%); pigments, 2.72-6.47 (4.6 ppm); diastatic activity, 302-443 (369) units; damaged starch, 9.3-15.5 (12.7)%; water absorption, 62.0-75.0 (67-9)%; dough stability, 2.0-10.0 (6.1) min.; extensograph area, 10-140 (79) sq.cm. and flour yield, 58.3-75.5 (65.8)%.

Though the production of highly priced durum wheats accounts for about 10 per cent of the total wheat production in India, most of the published literature pertains only to studies on aestivum wheats. Traditionally, semolina milled from durums is used in the preparation of sweet and savoury dishes and vermicelli. However, very little information is available on the quality of Indian durums with special reference to their utilisation in bakery products and the paste goods like macaroni, spaghetti, noodles, etc. Such information is of great importance as durums of certain quality attributes have export potential at a premium price to countries, where paste goods are manufactured and consumed on large scale.

Quality characteristics of only four durum varieties developed in Karnataka State have been reported. However, several varieties of durums developed in the peninsular and central zones under All India Coordinated Wheat Research Programme of Indian Council of Agricultural Research have not been evaluated systematically for their quality and utilisation avenues. As Canadian durums are exported extensively to several European countries, physico-chemical and rheological characteristics as well as milling quality of several varieties of Indian durums as compared to Canadian ones are reported in this paper.

Materials and Methods

Test materials: Twentry-four varieties of Indian durums were procured from different Research Stations of Indian Council of Agricultural Research located in States of Karnataka, Maharashtra, Gujarat, Madhya Pradesh, Rajasthan, Punjab and also from Indian Agricultural Research Institute, New Delhi. *Bansi* wheat—a commercial variety from the local market was also included in these studies. Three Canadian wheats namely *Stewart*-63, *Wakooma* and *Hercules*-72 procured from Plant Science Department of Manitoba University, Canada were used as control.

Physical characteristics: Hectolitre weight were determined by AACC methods[1]. Vitreousness of the wheat kernels was assessed by visual examination. Pearling index was determined according to McCluggage[2] using Corcoran Barley Pearler (London).

TABLE 1. PHYSICAL CHARACTERISTICS AND MILLING QUALITY OF INDIAN DURUM WHEATS

Variety	Hectolitre wt (kg.)	1000 kernel wt (g.)	Vitreous kernels (%)	Pearling index	Total flour yield[1] (*maida*) (%)	Flour recovered[2] (%)	Fine flour fraction[3] (%)
Indian durums							
N-59	82.5	53.1	74	15.7	63.0	12.0	4.5
NI-5759	80.0	47.6	100	16.7	60.2	18.3	6.0
A-206	78.3	41.6	100	18.6	66.4	13.8	4.9
A-624	81.8	45.8	80	15.4	69.3	7.5	9.5
A-1-1-2-3	79.3	42.5	100	14.5	63.9	11.8	6.2
A-11	82.0	43.9	99	16.3	65.8	17.0	6.0
A-1-8-1	73.9	42.4	100	18.8	69.6	10.5	6.2
A-9-30-1	77.9	46.6	100	20.5	61.6	14.3	7.7
MPO-157	77.3	45.5	95	16.6	67.0	8.5	8.0
MPO-142	80.0	35.3	88	14.3	67.0	7.9	15.0
MPO-161	78.3	40.9	85	14.6	63.1	15.5	5.5
MPO-159	76.0	38.0	98	17.6	71.1	15.0	5.7
Raj-911	81.0	51.4	100	15.3	61.1	13.5	6.0
Raj-912	82.3	44.7	99	16.8	60.9	16.5	7.5
NP-404	81.3	50.0	100	16.3	64.4	18.0	2.0
HI-7483	82.3	52.5	98	16.1	65.5	10.0	11.5
HI-7747	81.3	42.3	99	17.2	58.3	16.0	5.0
HI-7720	76.0	34.6	99	16.6	64.6	16.0	7.0
HI-7597	77.3	41.1	99	18.0	75.5	12.3	5.5
WL-1002	74.0	42.3	95	15.2	61.8	10.5	7.9
HD-4519	84.0	43.2	99	13.2	73.0	9.9	7.9
HD-4530	80.3	40.0	100	14.1	74.3	10.5	5.6
HD-4502	77.0	43.5	96	13.7	70.6	13.5	6.6
Bansi	77.0	41.5	100	15.0	62.2	9.0	11.0
Range	73.9–84.0	34.6–53.1	74–100	13.2–20.5	58.3–75.5	7.5–18.3	2.0–15.0
Average	79.4	43.8	96	16.1	65.8	12.8	7.0
Canadian durums							
Stewart-63	82.0	48.0	100	15.6	72.8	10.5	7.5
Wakooma	82.8	45.9	100	13.4	62.8	14.0	6.8
Hercules-72	83.5	48.4	100	13.4	63.7	15.0	6.7
Average	82.8	47.4	100	14.1	66.4	13.2	7.0

[1]Includes flour recovered from shorts

[2]From shorts by passing through 6XX sieve

[3]Passing through 240T sieve.

Milling quality: Flour milling quality was studied by processing 2–kg samples, conditioned overnight to 16.5 per cent moisture, in a Buhler laboratory mill (Model MLU-202). The straight run flour was subjected to sieve analysis on a Buhler plansifter to determine the finest fraction passing through 240 T sieve (aperture width, 45 μ).

As considerable quantity of flour appeared in the shorts fraction for durum wheats, the same was sieved on 6XX sieve (aperture width, 0.2 mm) and the throughs were reground in Kamas hammer mill (Type, Slaggy 200-A) to pass through 10XX sieve (aperture width, 0.13 mm). The *maida* referred to in these studies consists of straight run flour mixed with flour recovered from shorts.

Chemical characteristics: Moisture, total ash, wet gluten, pigments, diastatic activity and damaged starch were determined by AACC methods[1]. The falling number, an index of alpha amylase activity, was deter-

TABLE 2. CHEMICAL CHARACTERISTICS OF INDIAN DURUM WHEATS

Variety	Moisture %	Total ash %		Crude protein %		Wet gluten	Lipoxidase activity[1]	Pigments as β-carotene	Falling number	Diastatic activity[2]	Damaged starch %
	W.W.F.*	W.W.F.	*Maida*	W.W.F.	*Maida*	*Maida*	W.W.F.	ppm	W.W.F.	*Maida*	*Maida*
Indian durums											
N-59	7.3	1.52	0.53	13.0	12.4	40.5	22.1	4.67	589	333	10.0
NI-5759	7.5	1.45	0.68	16.3	14.1	39.9	10.7	5.12	681	425	13.9
A-206	8.5	1.64	0.61	16.6	15.5	49.6	11.3	3.61	748	337	9.3
A-624	7.5	1.64	0.68	11.8	10.5	35.9	4.9	3.61	547	412	15.0
A-1-1-2-3	7.2	1.51	0.82	14.3	12.8	36.1	43.5	3.31	728	373	11.6
A-11	7.4	1.60	0.66	15.7	13.4	44.7	4.6	4.67	669	400	13.5
A-1-8-1	7.5	1.57	0.64	16.1	13.4	52.6	57.1	4.06	823	328	11.5
A-9-30-1	6.8	1.56	0.62	16.1	14.4	49.3	1.8	4.06	823	328	11.5
MPO-142	7.4	1.66	0.74	10.6	9.2	28.8	1.4	6.32	790	341	11.1
MPO-157	7.9	1.56	0.75	15.0	13.2	41.3	26.1	6.17	649	344	10.7
MPO-161	8.4	1.40	0.54	13.3	11.4	32.2	2.0	5.87	668	363	12.0
MPO-159	7.8	1.84	0.72	16.6	14.7	44.4	3.2	6.47	852	352	10.7
Raj-911	8.5	1.92	0.67	14.8	13.3	45.3	1.0	5.87	755	318	13.5
Raj-912	8.9	1.86	0.77	14.9	13.6	38.8	12.8	5.57	822	352	10.7
NP-404	7.0	1.38	0.68	14.4	13.2	40.7	4.0	4.12	560	397	11.6
NI-7483	6.6	1.53	0.75	11.4	10.7	36.2	6.1	3.61	661	375	13.7
HI-7747	6.5	1.62	0.56	16.8	15.7	40.0	1.1	4.21	645	385	12.9
HI-7720	8.1	1.66	0.62	15.3	14.3	42.2	2.2	4.52	603	353	13.0
HI-7597	7.2	1.56	0.70	15.4	14.6	39.8	6.2	4.21	790	385	13.7
WL-1002	8.6	1.83	0.60	13.5	12.0	33.0	9.8	4.21	1000	344	13.3
HD-4519	11.0	1.70	0.60	14.6	13.8	41.7	10.7	3.33	801	438	15.5
HD-4530	10.5	1.63	0.60	14.4	13.7	45.3	4.6	4.85	772	353	13.3
HD-4502	11.9	1.79	0.73	12.9	13.9	34.4	1.2	5.14	585	398	15.5
Bansi	10.0	1.27	0.67	15.9	13.3	46.0	—	2.72	434	356	13.1
Range	6.5–11.9	1.27–1.92	0.53–0.82	10.6–16.8	9.2–15.7	28.2–52.6	1.0–57.1	2.72–6.47	439–1000	302–443	9.3–15.5
Average	8.2	1.60	0.66	14.6	13.2	40.7	10.9	4.57	690	369	12.7
Canadian durums											
Stewart-63	10.8	1.32	0.60	14.6	12.9	45.3	5.9	6.04	437	360	12.2
Wakooma	10.8	1.75	0.68	13.9	13.1	45.1	20.0	5.63	453	435	13.6
Hercules-72	11.1	1.28	0.52	14.4	12.8	48.5	11.2	5.04	541	385	13.5
Average	10.9	1.45	0.60	14.3	12.9	44.6	12.36	5.57	477	393	13.1

*Whole wheat flour

[1]Defined as the activity which in the presence of 5 mg of linoleic acid catalyses the reaction of 1 μg of oxygen in 1 min at 25°C at pH 7.0

[2]Maltose (mg) produced by 10 g flour for 1 hr at 30°C.

TABLE 3. RHEOLOGICAL CHARACTERISTICS OF INDIAN DURUM WHEATS

Variety	Farinograph characteristics				Extensograph characteristics		
	Water absorption (%)	Dough development time (min)	Dough stability (min)	Mixing tolerance index B.U.	Extensibility (mm.)	Resistance to extension B.U.	Area (cm^2)
Indian durums							
N-59	62.0	3.0	4.5	70	100	455	73
NI-5759	69.4	3.5	7.0	50	120	675	100
A-206	62.0	5.0	6.0	10	205	530	128
A-624	69.0	1.5	3.5	113	64	445	64
A-1-1-2-3	68.0	4.0	8.0	50	119	570	97
A-11	68.8	2.5	8.5	50	128	718	130
A-1-8-1	67.2	6.0	6.0	90	118	955	140
A-9-30-1	68.4	6.0	10.0	20	123	1020	149
MPO-157	65.8	2.5	5.0	80	125	290	52
MPO-142	62.8	1.2	2.0	80	94	625	85
MPO-159	70.6	2.5	2.5	90	115	375	53
MPO-161	66.0	1.7	3.5	90	73	390	42
Raj-911	76.0	1.7	3.5	130	88	250	31
Raj-912	66.4	2.5	10.0	30	87	535	64
NP-404	67.0	3.0	7.0	50	114	135	67
HI-7483	63.4	2.0	4.5	70	120	260	63
HI-7747	71.0	1.5	1.5	140	175	240	34
HI-7720	66.2	4.5	15.0	25	103	973	110
HI-7597	68.8	2.0	2.0	80	115	350	47
WL-1002	67.0	2.0	10.0	30	86	760	76
HD-4519	75.0	5.0	10.0	20	86	795	90
HD-4530	68.0	5.0	10.0	30	90	960	88
HD-4502	66.8	1.5	2.0	110	35	200	10
Bansi (Commercial)	74.0	7.5	4.5	70	143	520	110
Range	62.0–75.0	1.5–7.5	2.0–10	10–130	64–205	200–1000	10–140
Average	67.9	3.3	6.1	66	105	501	79
Canadian durums							
Stewart-63	62.0	4.0	6.5	60	—	—	—
Wakooma	68.4	3.5	6.0	40	—	—	—
Hercules–72	64.0	4.5	10.0	25	—	—	—
Average	64.8	4.0	7.5	42	—	—	—

mined using Hagberg's apparatus[1]. Crude protein (N×5.7) was estimated by micro-kjeldahl method. For estimation of lipoxidase activity, Sumner's method[3] was used with the following modifications: 10 g of whole wheat flour were macerated in 20 ml of water using a pestle and mortar. An aliquot of 1-2 ml was taken for the estimation of the enzyme activity, so as to obtain an optical density within the desirable range of 0.2 to 0.4. The reaction time was 5 min.

Rheological properties: Rheological characteristics of *maida* from different varieties were determined using Brabender Farinograph and Extensograph according to AACC procedures[1]. Different dough characteristics like water absorption, dough development time, dough stability, mixing tolerance index, extensibility, resistance and area under the curve were determined from the respective curves.

Results and Discussion

Physical characteristics: It is evident from Table 1 that the average hectolitre weight as well as 1000 kernel weight of Indian durums were slightly lesser than those

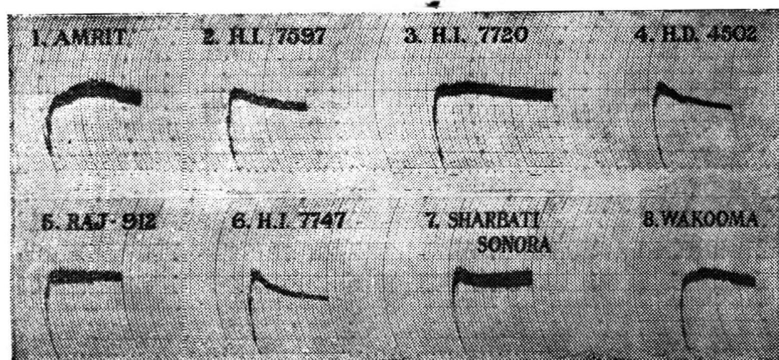

Fig 1. Farinograph curves of durum flours indicating typical characteristics relating to dough development time, dough stability and mixing tolerance index.

obtained for Indian aestivum wheats[5]. This may be attributed to the extra hard nature of the durum as compared to the aestivum wheats.

Chemical characteristics: The data presented in Table 2 show that Indian varieties in general had slightly higher ash content than the Canadian varieties. The average protein as well as wet gluten values for both Indian and Canadian durums were comparable (14.6 & 14.3 and 40.7 and 44.6 per cent respectively).

Interestingly enough, lipoxidase activity displayed very wide variation (1.1-57.1 units). Only 7 Indian varieties had relatively higher content of pigments than Canadian durums—a desirable attribute for use in paste goods. All but one of the Indian varieties had excessively high falling number as compared to Canadian durums. This indicated low alpha-amylase activity of Indian durums.

Indian durums had significantly higher diastatic activity of 369 units (range 203-443) as compared to low value of 210 units (range 133-351) for majority of Indian aestivum wheats[5]. The desirable range of diastatic activity is 225-350 units for bread wheats[6]. As such, durum wheats can be used for blending with aestivum wheats for improving their low diastatic activity. The diastatic activity as well as the damaged starch of Indian durums were comparable with those of Canadian durums.

Rheological characteristics: Some of the Indian durums were less hard as indicated by pearling index. It was, therefore, considered worthwhile to explore the possibilities of using durum flours for bread-making. As such, detailed data have been collected on dough rheology using farinograph and extensograph (Table 3).

Some of the typical farinograph curves of Indian durums indicating extreme characteristics regarding dough development time, dough stability, mixing tolerance index as compared to Canadian durum and an Indian aestivum bread wheat—*Sharbati sonora* are given in Fig. 1. Most of the durums had exceptionally high water absorption of more than 65 per cent which could be attributed to relatively higher protein content as well as higher amounts of damaged starch. Such characteristics coupled with relatively higher diastatic activity of durums are indicative of scope for their blending with soft wheats for obtaining desirable dough characteristics for bread-making.

The typical extensograph curves depicting highly sticky dough of poor strength, a dough of minimum strength, a dough of high resistance and extensibility are given in Fig 2. The different extensograph curves obtained showed that for most of the varieties, extensi-

of Canadian durums. But corresponding values for 6 and 4 varieties of Indian durums respectively were comparable to those of Canadian durums. All but three Indian varieties had some what higher pearling index than those of Canadian durums indicating thereby that in general Canadian durums were relatively harder han Indian durums. All the varieties had amber colour of varying shades. With the exclusion of varieties *N*-59, *MPO*-142, *A*-624 and *MPO*-161, the remaining varieties had 95-100 per cent of vitreous kernels.

Milling quality: Half of the durum varieties tested had good flour yields of 65 per cent or more (Table 1), which compare favourably with those of Indian bread wheats. Only Canadian *Stewart*-63 had a comparable yield. In view of extra hard nature of durums, as high as 7.5-18.3 per cent of the flour has been recovered from shorts, by passing it through 6XX sieve. Similar observations have been made by Finney *et al*[4] in case of Indian aestivum wheats. This observation is further corroborated by considerably lower yields (7 per cent) of flour fraction passing through finest sieve (240T) as compared to 10.3 per cent for aestivum wheats[5]. The average value of the damaged starch for different durum wheats was 12.7 per cent (range: 9.3-15.5 per cent) which was considerably higher than 9.2 per cent (range: 6.2-12.1 per cent)

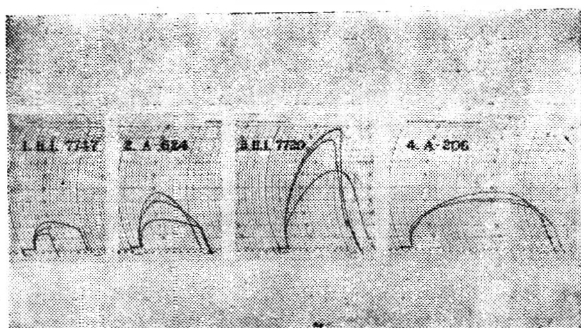

Fig 2. Extensograph curves of durum flours indicating typical characteristics like extensibility (E), Resistance (R), Ratio figure (R/E) and energy.

bility as well as resistance to extension were comparable to those of bread type aestivum wheats. Eleven varieties of durums had areas covering a range of 80 to 150 cm^2 which showed adequacy of flour strength for bread-making.

It may be concluded from the results obtained that many of the Indian durums are comparable to Canadian durums in respect of physico-chemical and rheological characteristics.

Acknowledgement

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Chemical Composition of Sunflower Seed

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The sunflower seeds of the two varieties namely *Sunrise Selection* and *EC* 69874 were analysed for proximate principles, polar and non polar lipids, fatty acids and amino acids. In all ten polar lipids viz. lysolecithin, lecithin, lysocephalin, cephalin, phosphatidyl serine, phosphatidyl inositol, sterol, sterol glycoside, phoshatidic acid and a glycolipid were identified to be present in oils of the two varieties. There was considerable variation in amounts of oleic (37.43 and 47.17%) and linoleic acid (52.18 and 44.17%) in the oil of *Suprise Selection* and *EC* 9874 respectively. The amounts of lysine (limiting amino acid in sunflower seed) in *Sunrise Selection* and *EC* 69874 were 3.563 and 3.286% respectively.

Sunflower is a major oilseed crop of several countries all over the world, particularly of the East European countries. A major improvement in sunflower has been brought about through research in U.S.S.R. and its oil content has been increased from 33 to about 50 per cent in the present varieties mainly by reducing its hull content. Moreover, sunflower oil has been described as a superior quality edible oil by several workers[1-3]. In view of its high oil content and to meet the shortage of vegetable oils, some varieties were introduced in India from U.S.S.R., recently. Since no detailed report on sunflower composition is available, two varieties of sunflower were taken up for detailed study of chemical composition and the data are reported in this paper.

Materials and Methods

Representative samples of two varieties, *Sunrise Selection* and *EC* 69874 were collected from the crop sown in 1973 at Punjab Agricultural University, Ludhiana. The oil content, protein, reducing and non-reducing sugars, crude fiber and ash were estimated by AOAC[4] methods. The oil was fractioned into polar and non-polar lipids and each fraction was then resolved by thin layer chromatography[5]. The developing systems for non-polar lipids and polar lipids were petroleum ether: ether (90:10) and chloroform: methanol: acetic acid: water (65:25: 10:4 v/v) respectively. The different spots were identified by comparing their Rf values with those reported in the literature and with the specific colour reactions of different components with various spray reagents[6,7]. The fatty acid composition was determined by gas liquid chromatography by suing AIMIL Mark II gas chromatograph. Two meter column of copper containing 10 per cent DEGS on celite was used as a stationary phase and the carrier gas was nitrgoen. The temperature of the oven was kept at 190$\pm$1°C and FID, and injector temperatures were maintained at 250°C. Different fatty acids were identified from their retention times and their respective peak areas were directly converted to relative percentages.

The quantitative analysis of amino acids was carried out on amino acid analyser (Beckman-Model 120 C).

Hundred milligrams of the seed was taken in a glass tube, 5 ml of 6N HCl was added to it, sealed and kept at 110°C for 24 hr. The volume of hydrolysate was made to 50 ml and a suitable aliquot (2ml) was dried *in vacuo* and redissolved in deionised water and this procedure of drying[1] *in vacuo* was repeated twice. The residue was then dissolved in 2 ml sodium citrate buffer of pH 2.2, 0.2 ml of this solution was applied on each of the long and short columns of amino acid analyser. Three sodium citrate buffers (pH 3.25, 4.25 and 5.25) were used for the complete amino acid analysis. The buffer flow rate was maintained at 70 ml/hr and ninhydrin flow rate at 35 ml/hour. The column temperature was maintained at 55°C. The Beckman spherical resins PA-35 (short column) and AA 15 (long column) were used. The recovery of different amino acids was found to be±3 per cent and each analysis was carried out in duplicate.

Results and Discussion

The proximate composition of sunflower seed is shown in Table 1. The data reveal that the constituents analysed do not vary much in both varieties. The mean values for dry matter, oil, protein, crude fiber, reducing sugars, non-reducing sugars and ash were 94.22, 39.67, 25.30, 9.80, 0.56, 1.14 and 3.37 per cent respectively. The values are in close agreement with those reported in the literature[8,10]. The oil obtained from the two varieties *Sunrise Selection* and *EC* 69874 was resolved by thin layer chromatography to identify the different components present. The non polar fraction gave 7 spots when the plate was sprayed with 50 per cent sulphuric acid. The Rf values calculated were compared with those reported in literature and were tentatively identified as unresolved mixture, partial glycerides, sterols, free fatty acids, triglycerides, sterol esters and some hydrocarbons. Their identity was further confirmed by co-chromatography with pure standards namely tripalmitin, stigmasterol and its ester, palmitic acid and liquid paraffin. Spot No. 3 and 6 gave violet colouration on spraying with 50 per cent sulphuric acid and heating, thereby confirming that these spots were due to presence of sterols.

The polar fraction of the oil was resolved by thin layer chromatography using chloroform: methanol: acetic acid: water (65: 25: 10: 4). The spraying of the plate with 50 per cent sulphuric acid and heating gave

TABLE 1. PROXIMATE COMPOSITION OF SUNFLOWER SEED

Variety	Dry matter %	Fat %	Protein %	Crude fiber %	Ash %	Reducing sugars %	Non-reducing sugars %
EC 69874	94.14	39.75	24.50	9.76	3.74	0.50	1.25
Sunrise Selection	94.30	39.60	26.10	9.84	3.00	0.62	1.02
Mean	94.22	39.67	25.30	9.80	3.37	0.56	1.14

TABLE 2. IDENTIFICATION OF DIFFERENT POLAR LIPIDS PRESENT IN SUNFLOWER OIL BY REACTION WITH DIFFERENT REAGENTS

Spot No.	Rf value*	50% H_2SO_4	Phospho-molybdate reagent	Ninhydrin	Diphenyl amine	Schiff's reagent	Dragon-dorff's reagent	Identification
1	0.00	+	—	—	—	—	—	Unresolved mixture
2	0.06	+ (pink)	—	—	—	—	—	Sterol
3	0.08	+	+	—	—	—	+	Lysolecithin
4	0.20	+	+	+	—	—	—	Phosphatidylserine.
5	0.26	+	+	—	—	+	—	Phosphatidyl inositol
6	0.32	+	—	+	—	—	—	Lysocephalin
7	0.38	+	+	—	—	—	+	Lecithin
8	0.54	+	+	+	—	—	—	Cephalin
9	0.66	+	+	—	—	—	—	Phosphatidic acid
10	0.70	+ (pink)	—	—	+	—	—	Sterol glycoside
11	0.96	+	—	—	+	—	—	Glycolipid
12	0.99	+	—	—	—	—	—	Pigments, neutral

*Solvent system:—Chloroform: methanol: acetic acid: water (65:25:10:4)

TABLE 3. FATTY ACID COMPOSITION OF SUNFLOWER OIL

Variety	Oil %	Palmitic acid (%)	Palmitoleic acid	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Arachidic acid
EC 69874	39.75	5.61	Traces	4.78	37.43	51.18	Traces
Sunrise Selection	39.60	6.50	Traces	2.16	47.17	44.17	Traces

TABLE 4. AMINO ACID COMPOSITION OF SUNFLOWER SEED

Amino acid	*Sunrise Selection*		*EC* 69874	
	% seed	% protein	% seed	% protein
Lysine	0.930	3.563	0.805	3.286
Histidine	0.632	2.423	0.572	2.336
Ammonia	0.555	2.126	0.537	2.194
Arginine	2.180	8.352	2.050	8.367
Aspartic acid	2.507	9.607	2.080	8.479
Threonine	0.870	3.333	0.790	3.224
Serine	1.540	5.900	0.778	3.173
Glutamic acid	4.447	17.040	4.577	19.194
Proline	0.972	3.752	1.120	4.571
Glycine	1.575	6.064	1.210	4.939
Alanine	1.192	4.569	1.010	4.122
Half cystine	0.120	0.459	—	—
Valine	1.380	5.299	1.320	5.377
Methionine	0.122	0.469	0.190	0.775
Isoleucine	1.077	4.128	1.090	4.449
Leucine	1.590	6.092	1.527	6.234
Tyrosine	0.435	1.666	0.340	1.338
Phenylalanine	1.015	3.889	1.160	4.759

12 spots, the Rf values of which are given in Table 2. For the identification of these spots different spray reagents[6,7,11] were used. These were further confirmed by co-chromatography with standards as unresolved mixture, sterol, lysolecithin, phosphatidylserine, phosphatidyl inositol, lysocephalin, lecithin, phosphatidic acid, sterol glycoside, glycolipid and neutral lipids.

The fatty acid composition of sunflower oil (Table 3) from the two varieties was comparable to that reported in the literature[1,9,13]. While there was not much difference in the amounts of saturated fatty acids (palmitic acid and stearic acid) there was appreciable variation in the amounts of unsaturated fatty acids (oleic, 37.43 to 47.17 per cent and linoleic acid, 44.17 to 52.28 per cent) in the two varieties. A considerable variation in oleic and linoleic acids have been reported[9,13-15] which has been attributed to both climatic and varietal factors[8,13]. Since both the varieties were grown under similar climatic conditions, the variation reported here could be due to the varietal difference.

The amino acid composition of the two varieties is given in Table 4. The mean amino acid composition is quite comparable to that reported by some other, workers[8,16,17]. Lysine is reported to be the limiting amino acid in sunflower. *Sunrise Selection* variety has a little higher lysine (3.563 per cent) as compared to *EC* 69874 (3.286 per cent). Bandemer and Evans[17] and Van Etten *et al*[16] have found the lysine contents in sunflower to be 3.6 and 3.2. per cent respectively.

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Development of Dehydrated Omelette Mix Based on Spray, Foam-mat and Accelerated Freeze Dried Egg Powder

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Dehydrated convenience mixes for omelette were developed based on foam-mat/spray/accelerated freeze dried egg powder and containing dehydrated onion and green chillies, salt, pepper and oil hydrogenated. Studies were made on the storage behaviour of the mixes packed in cans under air as well as nitrogen and in flexible laminated pouches as compressed bars.

Egg powder from acid stabilised melange was found superior to the one made without acid stabilisation. Similarly the mix based on AFD egg powder was found superior to that based on spray or foam-mat dried powder both in organoleptic quality and shelf life. The mixes had a minimum shelf life of about 12 months at room temperature and 9 months at 37°C.

Egg is an important item of the daily menu of the Service personnel. Considerable loss due to breakage occurs during transportation of fresh eggs to forward areas. Further, fresh eggs cannot be supplied to inaccessible regions during times of combat or operations behind enemy lines and as such a dehydrated product would meet the logistic requirements. Although spray dried egg powder is now commercially produced and marketed in this country, it was felt desirable to develop a ready mix for omelette containing all other ingredients like onion, green chillies, fat, salt and spices as well so that minimum time is needed by the soldier or sailor on the preparation of the omelette.

Results of experiments on the development of dehydrated omelette mixes using egg powder made by spray, foam-mat and accelerated freeze drying techniques after different treatments and evaluation of their shelf life are reported in this paper.

Materials and Methods

Processing of eggs: Chicken eggs procured from the local market were washed, broken, churned and filtered as per method described by Iyengar *et al*[1].

The egg melange was desugared using glucose oxidase (crude, BDH make, fungal origin, 1300-1500 units/g) as per the method described by Leo Kline[2], pasteurised at 63°C for 3 min and chilled immediately to 8°C. One batch was dried as such while another was acid stabilised as per the method described by Iyengar *et al.*[1] and then dried. Both the types of powders were used to make the omelette mix for comparative assessment of their acceptability during storage.

Foam-mat drying: The melange was whipped for 5 min using a laboratory stirrer with a whipping attachment, the foam was spread on perforated aluminium trays, dried in a cabinet drier at 60-70°C for 30 min and then finish dried to below 3 per cent moisture using a desiccant.

Spray drying: The chilled melange was dried in a spray drier (portable universal laboratory model—Zahn-Ravo-Rapid, W. Germany, with an evaporation capacity of 1-3 lit/hr of water) with an inlet air temperature of 140-150°C and product temperature of 70-80°C, using a nozzle spray and compressed air at a pressure of 1-2 kg/cm^2. The material was fed at a rate of 2.5 to 3.0 kg/hr.

Accelerated freeze drying(AFD): Accelerated freeze drying was carried out using a SOCALTRA laboratory model radiant type freeze dryer by the method standardised earlier in this laboratory. The melange was frozen in trays, sliced into pieces of uniform size (approx. 2.5×2.5×1.25 cm), deep frozen to –20°C and then freeze dried keeping the surface temperature at 55°C.

Processing of other ingredients: Onion was dehydrated as rings by the method described by Gururaja Rao *et al.*[3] and green chillies as bits as per method described by Jayaraman *et al.*[4] and were used as such.

Formulation of omelette mix: Omelette mix comprising egg powder, 74; oil hydro, 5; dehydrated onion rings, 12; dehydrated green chillies, 2; salt powder; 5; and white pepper powder, 2 per cent was made using the various types of egg powders. Sodium bicarbonate was added at a level of 1.5 per cent in case of acid stabilised egg powders to maintain a pH of 8-8.5 in the reconstituted egg as suggested by Iyengar *et al*[1]. The formulation was thoroughly mixed in a dry mixer. Scale of production used in the study was approximately 4-5 kg of mix per batch.

Packaging and storage evaluation: Samples of the mix were packed in 301×206 (8 oz) cans as such and under

nitrogen (35 g per can). They were also compressed into single serve bars of 35 g each using a laboratory model 12 ton Carver hydraulic press. A pressure of 1000 psig with a dwell period of 5 sec was found optimum. Immediately on removal from the mould, the bars were heat sealed in an inner wrap of MST cellophane (300 gauge) followed by an outer wrap of kraft paper (60 BC)—aluminium foil (0.02 mm)—polythene (150 G) laminate (PFL).

Analysis and storage studies: The proximate composition of the omelette mix was determined by the AOAC methods[5]. Free glucose content of the egg powder was determined by the method of Folin Wu[6].

Samples were stored at 0°C, room temperature (25-30°C) and 37°C and examined periodically by a taste panel for colour, flavour and reconstitution characteristics. Scoring was done using a 9-point hedonic scale with freshly made omelette graded as 9. Reconstitution of the mix into omelette was done by mixing it with 2-2½ times its weight of tap water, keeping aside for 5 min, pouring the batter over a greased pan and shallow frying as usual.

Non-enzymatic browning was measured with a Lovibond Tintometer and the colour expressed in yellow and red units. Peroxide value (PV) and free fatty acid (FFA) content of the fat were estimated by standard AOCS methods[7].

Change in the solubility index (SI) of the egg powder in omelette mix was measured by the Haenni's method[8] to get a comparative picture of the deterioration occurring during storage. The mix was sieved through 30 mesh to remove dried green chillies and onions and the solubility done on the remaining powder. Sufficient quantity of the mix containing 1 g of egg powder was taken and due allowance was made for the salt present in it.

Results and Discussion

The omelette mix had a typical proximate composition (per cent) of moisture, 1.9; protein ($N \times 6.7$), 36.0; fat, 38.7; ash, 8.2; crude fibre, 1.5; and carbohydrate (by difference) 13.7. The egg powder had a residual glucose content of 0.16 per cent.

Among the egg powders made by the three techniques, AFD powder was rated best in appearance and texture of omelette followed by spray dried and foam-mat dried powders.

Acid stabilisation yielded a powder of more appealing yellow colour compared to the faintly pinkish yellow colour of the nonstabilised powder although the texture and flavour were similar in both initially. Acid stabilisation also gave better texture and colour to the reconstituted product during storage. In view of this only the data on acid stabilised powder are presented below.

Data on the peroxide value and free fatty acid content of fat in the mix prepared by spray drying and freeze drying methods and stored at RT and 37°C are given in Fig 1, 2, 3 and 4. The figures for the mix based on foam-mat dried powder have been omitted for brevity since they were more or less similar to those for the spray dried mix.

The organoleptic scorings of the samples during storage are given in Table 1. Samples kept at 0°C retained their initial organoleptic score throughout the period of study and showed no signs of oxidative rancidity or browning. They served as control for assessment of RT and 37°C samples.

The maximum period of acceptability of the samples kept at RT and 37°C more or less coincided with the period for maximum development of PV. FFA values did not show any influence on the acceptability.

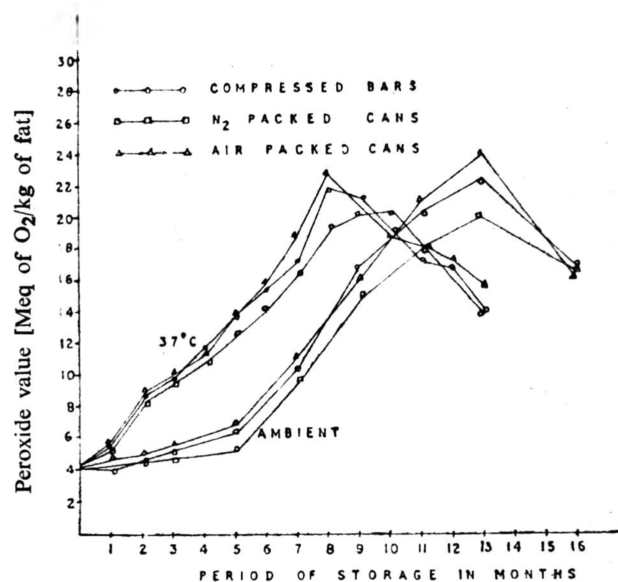

Fig 1. Peroxide value of omelette mix (with spray dried egg powder) stored at ambient temparature and at 37°C

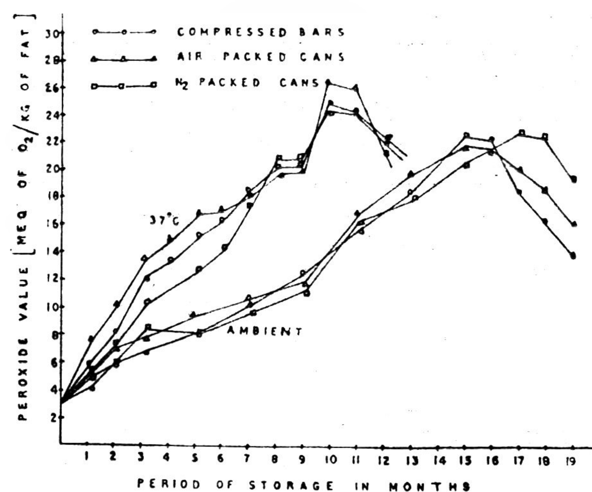

Fig 2. Peroxide value of omelette mix (with AFD egg powder) stored at ambient and at 37°C

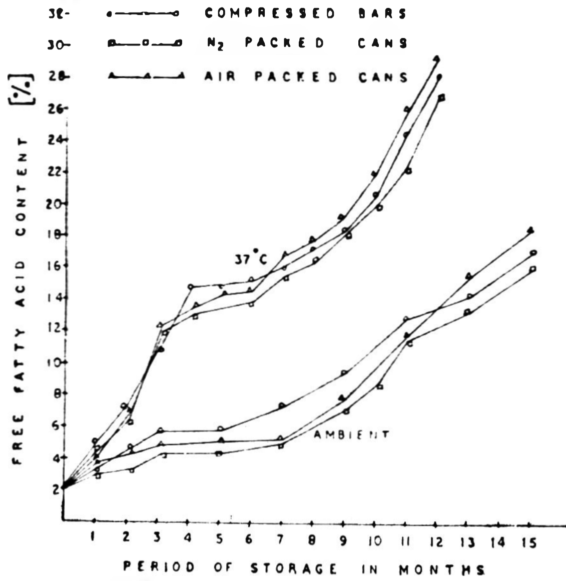

Fig 3. Free fatty acid content of omelette mix (with spray dried egg powder) stored at ambient temparature and at 37°C

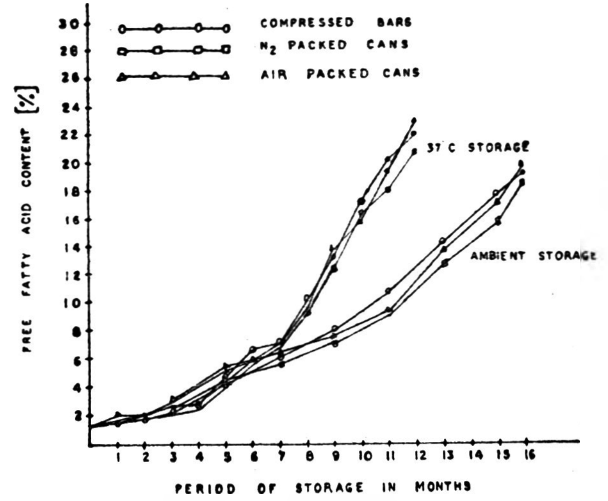

Fig 4. Free fatty acid content of omelette mix (with AFD egg powder) stored at ambient and at 37°C

TABLE 1. OVERALL ACCEPTABILITY OF OMELETTE MIX MADE OF DIFFERENT EGG POWDERS (ACID STABILISED) DURING STORAGE (9-POINT HEDONIC SCALE)

Period of storage (months)	Foam-mat dried egg powder						Spray dried egg powder						AFD egg powder					
	Ambient storage			Storage at 37°C			Ambient storage			Storage at 37°C			Ambient storage			Storage at 37°C		
	Can (air)	Can N_2	Bar	Can (air)	Can N_2	Bar	Can (air)	Can N_2	Bar	Can (air)	Can N_2	Bar	Can (air)	Can N_2	Bar	Can (air)	Can N_2	Bar
0	7	7	7	7	7	7	7	7	7	7	7	7	8	8	8	8	8	8
3	7	7	7	7	7	7	7	7	7	7	7	7	8	8	8	8	8	8
6	7	7	7	6	6	6	7	7	7	6	6	6	8	8	8	7	7	7
9	6	6	6	5	5	4	6	6	6	5	5	4	7	7	7	6	6	5
12	5	6	5	4	4	3	5	5	4	4	4	3	6	6	5	5	5	4
15	4	5	4	—	—	—	4	4	3	—	—	—	4	5	3	—	—	—

TABLE 2. NON-ENZYMATIC BROWNING IN OMELETTE MIXES MEASURED USING LOVIBOND TINTOMETER*

Egg powder used	Period of storage (months)	Room temperature			37°C		
		Can air pack	Can N_2 pack	Compressed bar	Can air pack	Can N_2 pack	Compressed bar
Spray dried	0	0 4, 1 3	0.4, 1.3	0.4, 1.3	0.4, 1.3	0.4, 1.3	0.4, 1.3
	6	0.5, 1.5	0.4, 1.5	0.4, 1.6	0.8, 2.3	0.8, 2.2	1.0, 2.3
	12	0.6, 2.0	0.8, 2.2	0.6, 2.0	0.9, 2.5	0.9, 2.3	1.0, 2.6
Foam-mat dried	0	1.1, 4.2	1.1, 4.2	1.1, 4.2	1.1, 4.2	1.1, 4.2	1.1, 4.2
	6	1.3, 4.3	1.2, 4.3	1.2, 4.3	1.9, 5.2	1.9, 6.0	1.6, 5.2
	12	1.6, 4.6	1.3, 4.5	1.3, 4.5	2.2, 5.5	2.1, 6.1	1.7, 5.6
AFD	0	1.0, 3.4	1.0, 3.4	1.0, 3.4	1.0, 3.4	1.0, 3.4	1.0, 3.4
	6	1.1, 3.2	1.1, 3.3	1.0, 3.6	1.2, 3.3	1.1, 3.4	1.1, 3.4
	12	1.3, 3.8	1.2, 3.8	1.2, 3.8	1.4, 4.3	1.4, 4.2	1.4, 4.6

*First and second figure represent red and yellow units respectively

Omelette mix made using AFD egg powder had the maximum shelf life of 12 months both at RT and at 37°C with or without gas packing compared to 12 and 9 months respectively for mixes using both spray dried and foam-mat dried powders. The AFD mix remained organoleptically superior to the other two during the period of storage.

Compression of the mix into bars did not offer any extra advantage. The bars were organoleptically rated inferior to gas and air packed mixes although the time taken for maximum PV development in bars was more than that for air packed but less than that for gas packed mixes. There was no significant difference between the air packed and gas packed mixes and as such the mix can be packed in paper-foil-polythene laminate pouches.

Table 2 gives the figures for browning measured in terms of Tintometer units in the initial mix and at the end of 6 and 12 months. The data generally were in agreement with the visual observations.

Solubility index values for nitrogen packed omelette mix during storage showed that in general the samples were unacceptable below a Haenni's value of 78.5 which occurred after a period of about 10-12 months at RT and 37°C in case of AFD mix and 12 and 8 months respectively in spray dried and foam-mat dried mixes.

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Effect of Amylase Supplements on the Rheological and Baking Quality of Indian Wheats

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The effects of cereal malt and fungal amylase supplements on the rheological and baking quality of flours of two commercially important Indian wheats, 'WG 357' and 'Kalyan Sona', were compared. The flours contained 9.3 and 9.0% protein, and 0.43 and 0.48% ash, respectively. Compared with the fungal amylase, the cereal malt supplements considerably decreased the peak viscosity of the flours. The degree of softening of dough increased as the level of alpha-amylase was increased to 22.2 SKB/100 g flour. Bread with increased volume, crust colour, medium fine grain, and soft-texture was obtained by adjusting the level of alpha-amylase to 11.1 SKB/100 g flour and without any sugar in the formula.

The baking quality of a number of Indian wheats was recently investigated by Finney *et al*[1]. The importance of damaged starch in relation to the maltose values and water absorption capacities of those flours was emphasised by Tara and Bains[2], and by Tara *et al*[3]. The falling number values of those wheats were reported to be high by Tara and Bains[4] indicating deficiency of alpha-amylase. Flours with such values pose risk of diminished loaf volume and too dry a crumb when used for baking. Since information regarding the effect of amylase supplements on the rheological and baking quality of indigenous wheat is lacking, this investigation was undertaken. It is shown that amylase supplementation considerably improved the bread making potential of the flours of indigenous wheats.

Materials and Methods

The samples of two extensively grown varieties of

wheat, viz., 'WG 357' and' Kalyan Sona' were obtained from the farms of the Punjab Agricultural University, Ludhiana.

Milling: Weighed aliquots (5 kg) of each variety were conditioned at 15.5 per cent moisture content, and kept for 48 hr prior to milling in the Brabender quadrumat laboratory mill to obtain representative flours of the wheats.

(*a*) *Barley malt flour:* The barley malt was coarsely ground and bulk of the husk was removed by sifting and by blowing. During regrinding, the endosperm particles were further reduced and sifted through a 60 mesh sieve to obtain an overall extraction of about 65 per cent of the enzymatically active flour.

(*b*) *Wheat malt flour:* 'WG 357' wheat was steeped at 15°C to a moisture content of 43 per cent and germinated for five days at the same temperature, followed by drying and milling into an enzymatically active flour of 72 per cent extraction.

(*c*) *Fungal amylase* (*Rhozyme* 33): This was a commercial brand of fungal amylase (*Aspergillus oryzae*) obtained through the courtesy of M/s Rohm and Hass Co., Philadelphia, USA.

Yeast: Tower brand compressed bakers yeast (*Saccharomyces cerevisiae*) was obtained fresh through a local bakery for use in the various baking tests.

Shortening: A commercial brand of hydrogenated fat (melting point 3. 7°C) was used in the test baking formula.

Farinograph curves: The constant flour (50 g, 14 per cent moisture) method of the AACC[5] was followed. The farinograph curves of flour without the amylase supplement were centered on the 500 B.U. line. The curves of flours containing 11.1, 22.2 and 44.4 SKB/100 g flour from each supplement were developed by using the farinograph water absorption (FWA) of the control to see if there were changes in dough consistency. The curves were interpreted for dough development time (DDT) stability and degree of softening (BU).

Amylograph curves: The flour-water ratio was adjusted to obtain an amylogram within the 1000 BU scale. A concentration of 57.5 g flour/450 ml water was used in the various tests. The level of alpha-amylase per 100 g flour varied from 0.0 to 11.1 SKB units derived from the cereal malts and 0.0 to 44.4 units in case of Rhozyme 33.

Baking quality: The cereal malt flours were mixed intimately with the flours to provide 11.1, 22.2, 44.4 SKB/100 g flour. In the case of Rhozyme 33, the enzyme was added as aqueous solution at the time of dough mixing.

Baking formula: Straight dough method on the lines of the method used by Finney *et al*[1] optimized with respect to water absorption and mixing time was used with and without the amylase supplements.

Formula ingredients	
Flour (14 per cent moisture) (g)	100
Yeast (compressed) (g)	2.25
Salt (g)	1.5
Shortening (g)	1.0
Potassium bromate (ppm)	10
Amylase supplement (SKB)	0.0-44.4

Fermentation and baking schedule	
Fermentation	2 hr 30 min
Remixing	30 sec
Recovery	25 min
Proofing	55 min at 30°C
Baking	25 min at 232°C

The doughs were optimally mixed and finished at 29-30°C. The test loaves were also prepared with variable sugar levels (0.0-6.0 g/100 g flour) but without using amylase supplements.

Loaf volume: This was measured by the rape seed displacement method of Binnington and Geddes[6] after the loaves were cooled to room temperature. The loaves were also scored for crust colour and crumb characteristics.

Analytical tests: Moisture, ash, protein, gluten, sedimentation value and diastatic activity of the flours were determined according to the AACC methods[5].

Damaged starch: This was determined according to the method of Donelson and Yamazaki[7] as adopted by the AACC[8].

Alpha amylase: This was determined according to the method of the International Association of Cereal Chemistry (ICC) as elaborated by Perten[9].

Results and Discussion

The flour characteristics are given in Table 1. The ash content of 'WG 357' flour was lower than that of 'Kalyan Sona' by 0.05 per cent when milled under similar conditions. The diastatic activity of 'Kalyan Sona' flour was higher than that of 'WG 357', because of the higher damaged starch content, the substrate for the amylase action. The sedimentation values in relation to the protein contents were low. Similar low values for flours of Indian wheats in relation to their protein contents were reported by Bains and Irvine[10] and by Finney *et al*[1]. The flours failed to reveal the presence of alpha-amylase activity as tested by the ICC method[9].

Farinograph curves: Tested at the water absorption of the control, the dough development times of the amylase supplemented flours decreased slightly at the level of 11.1 SKB units. There was a clear indication of the softening of the dough ascribed to the increased amylase supplementation (Table 2). The degree of softening increased considerably as the level of supplementation increased to 22.2 and 44.4 SKB units. The

TABLE 1. THE COMPOSITION OF FLOURS OF WG 357 AND KALYAN SONA VARIETIES OF WHEAT

Variety	Ash %	Protein (N×5.7) %	Wet gluten %	Diastatic activity (mg maltose/10 g)	Damaged starch (%)	Sedimentation value (ml)
WG 357	0.43	9.3	32.9	182	7.3	22.3
Kalyan Sona	0.48	9.0	34.6	218	8.4	22.1

*14% moisture basis

TABLE 2. EFFECT OF AMYLASE SUPPLEMENTS ON THE FARINOGRAPH CURVE CHARACTERISTICS OF FLOURS OF WG 357 AND KALYAN SONA, VARIETIES OF WHEAT

Supplement	WG 357				Kalyan Sona		
	α-amylase (SKB/100 g flour)	DDT (min)	Softening (B.U.)	Stability (min)	DDT (min)	Softening (BU)	Stability (min)
Nil (control)	0.0	5.0	33	8.10	5.5	27	9.8
Barley malt	11.1	5.0	65	3.50	5.5	40	7.5
	22.2	4.5	85	3.75	3.5	80	4.6
	44.4	4.0	85	—	3.5	130	1.6
Wheat malt	11.1	4.5	55	5.20	5.0	40	7.9
	22.2	4.25	100	3.20	5.0	75	5.3
	44.4	4.0	—	—	—	115	1.2
Rhozyme 33 (fungal)	11.1	4.0	75	4.50	5.0	40	4.0
	22.2	4.0	105	2.50	5.0	110	1.2
	44.4	3.0	140	<500 B.U.	4.5	150	<500 B.U.

DDT: Dough development time.

TABLE 3. EFFECT OF AMYLASE SUPPLEMENTS ON THE PEAK PASTE VISCOSITIES OF FLOURS OF WG 357 AND KALYAN SONA VARIETIES OF WHEAT

Supplement	α-amylase (SKB/100 g flour)	Variety WG 357	Peak viscosity BU decrease (%)	Variety Kalyan Sona	Peak viscosity BU decrease %
Nil (control)	0.0	935	—	820	—
Barley malt	5.5	285	69.5	200	75.6
	11.1	200	78.6	120	85.3
Wheat malt	5.5	300	67.9	200	75.6
	11.1	200	78.6	140	82.9
Rhozyme 33 (fungal)	11.1	820	12.2	690	15.8
	22.2	730	21.9	670	18.2
	44.4	650	30.4	570	30.4

softening of the dough reckoned after mixing for 10 min decreased perceptibly with the level of the supplementation. The results indicated the importance of adjusting the water absorption and the alpha-amylase levels for obtaining dough of greater rheological stability.

Amylograph curves: The effect of various amylase supplements on the peak viscosities of flour pastes is shown in Table 3 and the nature of amylograms in Fig 1a, b and c. There was a steep fall in the viscosity when the cereal malt supplements provided even 5.5 SKB units/100 g flour. The peak viscosity of 'WG 357' decreased by 69.5 per cent compared with 79.6 per cent of 'Kalyan Sona'. Doubling the cereal alpha-amylase level to 11.1 SKB units, further reduced the viscosity by another 100 BU in case of 'WG 357' flour compared with 60-80 BU of 'Kalyan Sona'. The effect of Rhozyme 33 on the paste viscosities was not marked even when the concentration was eight times (44.4 SKB) to that of alpha-amylase The peak viscosity decreased from 935 to 650 BU in case of 'WG 357' flour and from 820 to 570 BU in case of 'Kalyan Sona'. These results emphasized the safety factor associated with the use of

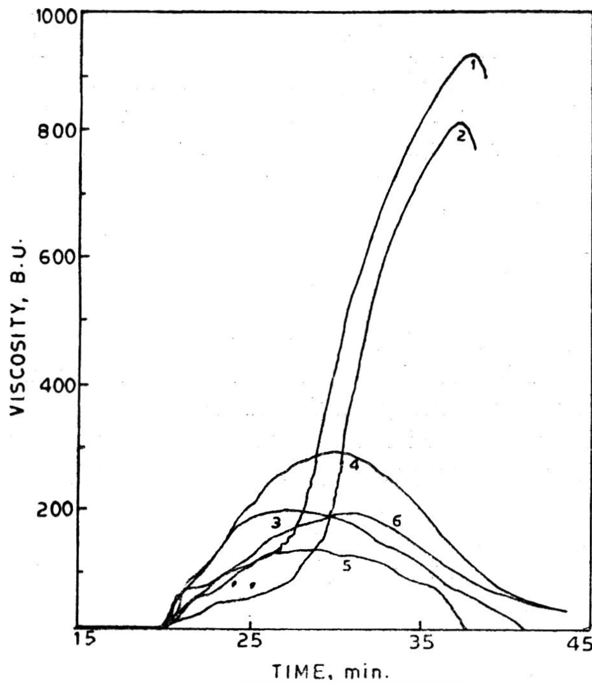

Fig 1 (a) Effect of malted barely supplement on the amylograph curves

SKB 0.0, 5.5, 11.1;
WG 357, 1, 4,3; Kalyan Sona, 2, 6, 5.

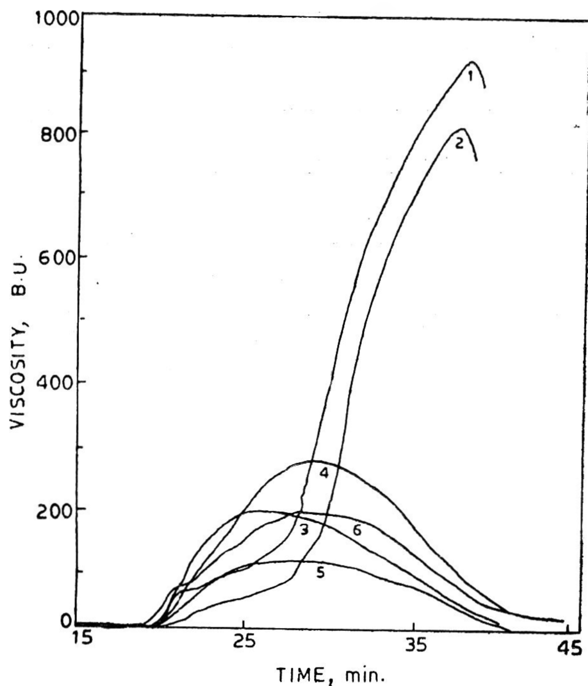

Fig 1 (b) Effect of malted wheat supplement on the amylograph curves

SKB 0.0, 5.5, 11.1,
WG 357, 1, 4, 3; Kalyan Sona, 2, 6, 5.

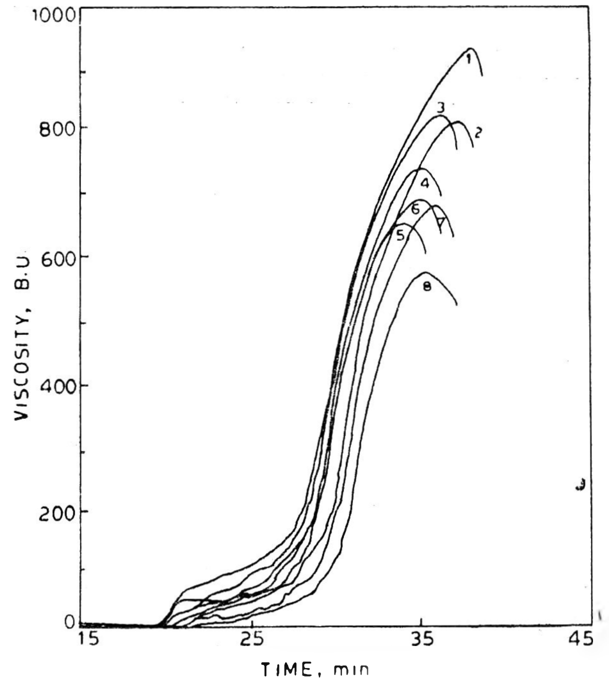

Fig 1 (c) Effect of rhozyme 33 supplement on the amylograph curves

SKB 0.0, 11.1, 22.2, 44.4;
WG 357, 1, 3, 4, 5; Kalyan Sona, 2, 6, 7, 8.

fungal alpha-amylase supplement being relatively more thermolabile than malt amylases for adjusting the paste viscosity for improved baking performance. The flours of some improved Indian wheats were previously shown by Bains and Irvine[10] to produce inordinately high paste viscosities. The alpha-amylase activity of the flours was hardly detectable by the ICC method[9]. Further, the high falling number values of Indian wheats reported by Tara and Bains[4] indicated deficiency of alpha-amylase in the flours. This is a constraint

Table 4. Effect of amylase supplements on the loaf volumes and dough handling properties of flours of WG 357 and Kalyan Sona varieties of wheat

Variety	α-amylase (SKB/100 g flour)	Loaf volume (ml) Barley malt	Wheat malt	Rhozyme 33	Dough
WG 357	0.0	525	525	525	Non-sticky
	11.1	610	610	585	,,
	22.2	610	625	610	Slightly sticky
	44.4	620	635	615	Sticky
Kalyan Sona	0.0	535	535	535	Non-sticky
	11.1	585	585	605	,,
	22.2	585	590	580	Slightly sticky
	44.4	585	585	595	Sticky

TABLE 5. EFFECT OF SUGAR LEVEL ON THE LOAF VOLUME AND DOUGH HANDLING PROPERTIES OF FLOURS OF WG 357 AND KALYAN SONA VARIETIES OF WHEAT

Variety	Sugar (g/100 g flour)	Loaf volume (ml)
WG 357	0.0	525
	3.0	580
	4.5	585
	6.0	610
Kalyan Sona	0.0	535
	3.0	585
	4.5	590
	6.0	585

The dough was non-sticky in all the treatments

affecting the bread-making quality of the flour of indigenous wheats.

Bread quality: Results showing the effect of the amylase supplements and of variable sugar levels in the formula without the supplements on the baking properties of the flours are given in Tables 4 and 5. From the loaf volume data, it is seen that there was a very favourable response of the flours to various supplements even at a level of 11.1 SKB/100 g flour. The volumes remained more or less constant beyond the 11.1 SKB equivalent of the supplements. The doughs containing the higher amounts of the supplements tended to be sticky compared with the control. The crusts of the control loaves were light-brown compared with the brown crust of loaves baked with the amylase supplements or when the formula contained 3.0 to 6.0 per cent sugar. The crumb of 'WG 357' loaves including the control was adjudged softer than that of 'Kalyan Sona'. The loaves exhibited fine to medium fine grain, creamy yellow colour and easy to slice. The doughs containing Rhozyme 33 tended to be less sticky compared with those of cereal malt supplements. The crumb texture was generally hard and tended to be crumbly, the grain medium coarse when sugar was used in the formula. The results indicate the possibility of producing equally satisfactory bread without using sugar (Table 5). This is of economic importance to the baking industry as satisfactory bread can be produced from the flour of indigenous wheats without using sugar. The alpha-amylase acts on the damaged starch substrate causing rheological instability in dough system depending on the extent of dextrinization of the starch. To minimize this, Farrand[11] suggested controlling of the alpha-amylase level in relation to the amount of substrate that is damaged starch content in the flour. Reviewing the amylolytic factors, Maninder and Bains[12] pointed out that the flours of Indian wheats were characterized by a high damage to starch, very low alpha-amylase activity, medium protein content and high water absorption capacity. Improvement of the baking quality of such wheats is possible as seen from the foregoing discussion of the results through a judicious use of the amylase supplement keeping in view the rheological properties of the dough.

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A THURSTONE—MOSTELLER MODEL FOR PALATABILITY OF CHAPATIES OF SOME IMPROVED BREAD WHEATS

A Thurstone—Mosteller model evaluation was done for five improved varieties of wheat for evaluating the palatabilities of chapaties made from these wheats. The varieties did not differ significantly among themselves for palatability.

An earlier study conducted with four improved wheat varieties using a modified Bradley-Terry model for fractional pairs showed that it can be used to discriminate differences in taste of chapaties[1]. In this type of fractional pairs the splitting was not symmetrical and hence the experiment was not balanced in the varieties. Hence a balanced type of fractional pairs has been developed and the same is put to test in this work. In this balanced set each variety is occurring an equal number of times so that precision remains the same for each.

Five improved varieties of wheat viz. *Kalyan sona* (1), *Sonalika* (2), *Choti lerma* (3), *Sharbati sonora* (4) and *N. P.* 718 (5) were tested for palatability by the new method of symmetrical pairs. These pairs were coded as (1,2) (2,3) (3,4) (4,5) and (5,1).

Chapaties made out of two varieties having only marginal taste difference were offered to a group of people. Of these one was a coded variety. When a taster was able to distinguish the coded variety from the other, he was selected. Five such tasters who could discriminate the coded variety were selected for the panel.

Complete set of symmetrical pairs as well as orders of presentation of each pair were randomised using a random number table. Each judge was asked to enter the result in a proforma in the following manner. Suppose A is preferred to B by a judge he must give score '1' to 4 and '0' to B. If both of them are preferred or if he is unable to distinguish their tastes, he must give 0.5 score to each A and B. The judges were instructed not to discuss their reaction among themselves and to start with pair 1 and work towards the last pair. Three replications were made and in each replication a separate randomisation was used.

The method of analysis follows the Thurstone—Mosteller model for symmetrical pairs with ties. The model is developed for estimating the relative strength of treatment stimuli which makes provision for tied observations. It postulates a subjective continuum over which sensations are jointly normally distributed with equal standard deviations and zero correlations between pairs. Mosteller[2] showed that the assumption of zero correlations may be relaxed to an assumption of equal correlations with no change of method. Without further loss of generality, we may let the scale of sensation continuum be so chosen that the difference of any two stimuli responses has unit variance. Let d denote the difference of the true stimulus responses of a pair of treatments. Then under this model the probability distributions of the difference of the two responses is normal with mean d and unit variance. The original Thurstone—Mosteller Model prohibits the declaration of ties. We extended the model with ties to the case of symmetrical pairs and applied the model for analysis of data from the experiment. The theoretical developments of the model are given by Sadasivan and Sundaram[3].

The data for preferences by the judges pooled over replications are given in Table 1.

TABLE 1. DATA FOR PREFERENCES BY THE JUDGES

Pair	$n_{i\cdot ij}$	$n_{o\cdot ij}$	$n_{j\cdot ij}$	Total
(1, 2)	5	3	7	15
(2, 3)	6	4	5	15
(3, 4)	6	3	6	15
(4, 5)	4	3	8	15
(5, 1)	6	2	7	15
Total	27	15	33	75

From the given data we calculate $a_{ij}=(n_{i\cdot ij}+n_{o\cdot ij})/n$ and $a_{ji}=(n_{j\cdot ij}+n_{o\cdot ij})/n$ for each of the pairs. These are presented in the form of a 5×5 matrix.

Let this matrix be denoted by A. Since no treatment compared with itself (a_{ii} is undefined) we give the value zero to all the main diagonal elements. Similarly since a treatment is compared only with two others and not the rest, we give the value zero to the corresponding column or row where its association is not there. Thus we obtain the matrix,

$$A=\begin{vmatrix} 0 & 0.533 & 0 & 0 & 0.600 \\ 0.667 & 0 & 0.667 & 0 & 0 \\ 0 & 0.600 & 0 & 0.600 & 0 \\ 0 & 0 & 0.600 & 0 & 0.467 \\ 0.533 & 0 & 0 & 0.733 & 0 \end{vmatrix}$$

For each of the non-diagonal elements in the matrix A we determine $\sin^{-1}$. $(2\,a_{ij}-1)$ or $\sin^{-1}(2a_{ji}-1)$ as the case may be using table of Rao *et al*[4].

The matrix $(\sin^{-1}(2a_{ij}-1)$ is found to be

$$\begin{vmatrix} 0 & 0.066 & 0 & 0 & 0.201 \\ 0.432 & 0 & 0.432 & 0 & 0 \\ 0 & 0.201 & 0 & 0.201 & 0.0 \\ 0 & 0 & 0.201 & 0 & -0.066 \\ 0.066 & 0 & 0 & 0.496 & 0 \end{vmatrix}$$

From this the G_{ij} values are obtained by the relation $G_{ij}=\frac{1}{2}(\sin^{-1}(2a_{ij}-1)+\sin^{-1}(2a_{ji}-1)$ and is found to be

$$\begin{vmatrix} 0 & 0.249 & 0 & 0 & 0.134 \\ 0.249 & 0 & 0.317 & 0 & 0 \\ 0 & 0.317 & 0 & 0.201 & 0 \\ 0 & 0 & 0.201 & 0 & 0.215 \\ 0.134 & 0 & 0 & 0.215 & 0 \end{vmatrix}$$

The estimate r is the mean of the G_{ij} values and it is obtained as 0.225.

Again H_{ij} values are obtained by the relation $S_i'-S_j' =(\sin^{-1}(2a_{ij}-1)-\sin^{-1}(2a_{ji}-1)/2=H_{ij}$.

The resulting skew symmeric matrix is

$$\begin{bmatrix} 0 & -0.183 & 0 & 0 & 0.068 \\ 0.183 & 0 & 0.116 & 0 & 0 \\ 0 & -0.116 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -0.281 \\ -0.068 & 0 & 0 & 0.281 & 0 \end{bmatrix}$$

The vector $X'Y$ is the vector of row sums in (H_{ij}). The vector $S_i^*(i=2,...5)$ with $S_1^*=0$ is obtained by

$$\begin{bmatrix} S_2^* \\ S_3^* \\ S_4^* \\ S_5^* \end{bmatrix} = \frac{1}{5}\begin{bmatrix} 4 & 3 & 2 & 1 \\ 3 & 6 & 4 & 2 \\ 2 & 4 & 6 & 3 \\ 1 & 2 & 3 & 4 \end{bmatrix}\begin{bmatrix} 0.299 \\ -0.116 \\ -0.281 \\ 0.213 \end{bmatrix} = \begin{bmatrix} 0.100 \\ -0.099 \\ -0.183 \\ 0.015 \end{bmatrix}$$

We find that $S_1^*=0$ $S_2^*=0.100$, $S_3^*=-0.099$, $S_4^*=-0.183$ and $S_5^*=0.015$.

So the ratings are

1. *Sonalika*. 2. *N.P.* 718. 3. *Kalyan sona*. 4. *Choti-lerma* and 5. *Sharbati sonora*.

Using asymptotic theory the standard error for r and S_i's is $\sqrt{1/2n}=\sqrt{1/30}=0.182$, where n is the number of times a pair is repeated.

Using this we find that the varieties are not significantly differing among themselves for palatabiliy. The tie is also not significant. However the trend shown by the varieties is worth noting.

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HEAT INDUCED CHANGES IN BUFFALO MILK SERUM PROTEINS

The effect of various heat treatments on the denaturation and destabilization of buffalo milk serum proteins was studied at heat treatments between 65 and 100°C for a holding time of 10 min. All the heat treatments employed caused both denaturation and destabilization of the serum proteins.

In case of acid whey, buffalo proteins were less denatured and destabilised than the cow serum proteins. Rennet whey proteins from buffalo milk were more denatured and destabilised than the cow proteins. Amongst the ultracentrifugal whey (UCW) obtained at different forces of gravity, denaturation and destabilization were maximum in UCW obtained at the maximum force of gravity. In case of UCW also, the buffalo serum proteins were more vulnerable to heat treatments than the cow UCW proteins.

The heat induced changes on serum proteins are of great importance so far as the processing of milk is concerned. Various investigators[1-4] have either studied such changes in milk itself or in isolated serum proteins. Rowland[1] observed that the coagulation of the serum proteins is pH dependent and that maxiumm coagulation of albumin and globulin occurred at pH 4.7–4.8. Later this observation was confirmed by Freimuth[2]. Kenkare *et al*[3] introduced a procedure to determine the destabilisation of whey proteins by centrifuging the serum at 1000×g for 30 min. On this basis the serum proteins in acid precipitated whey was much less stable to heat treatments than in the ultracentrifugal serum. Guy *et al*[4] observed that the proteins of cottage cheese whey was comparatively labile to heat treatments. The present study was undertaken to investigate the effect of various heat treatments on acid whey, rennet whey and ultracentrifugal whey, obtained from buffalo milk in relation to its destabilization and denaturation. Experiments with cow milk was also run for comparative purposes.

Buffalo milk used was from the Murrah breed and cow milk was from Red Sindhi, Sahiwal and Tharparkar breeds, maintained at the Institute herd. Prior to the preparation of the serum, cream was separated by using a cream separator.

Acid whey was prepared from skim milk by adjusting the pH to 4.6 with 1 N hydrochloric acid at 37°C. The precipitated casein was removed by centrifugation at 1000×g for 30 min and the supernatant passed through cotton wool. The pH of serum was adjusted to 6.8 by 1 N sodium hydroxide.

Rennet whey was prepared from skim milk by treating it with a solution of Hansen's rennet (50 mg per ml), at the rate of 0.1 ml per 10 ml of skim milk. After the curd was set the coagulam was strained and the filtrate cen-

N.D.R.I. Publication No. 76-79.

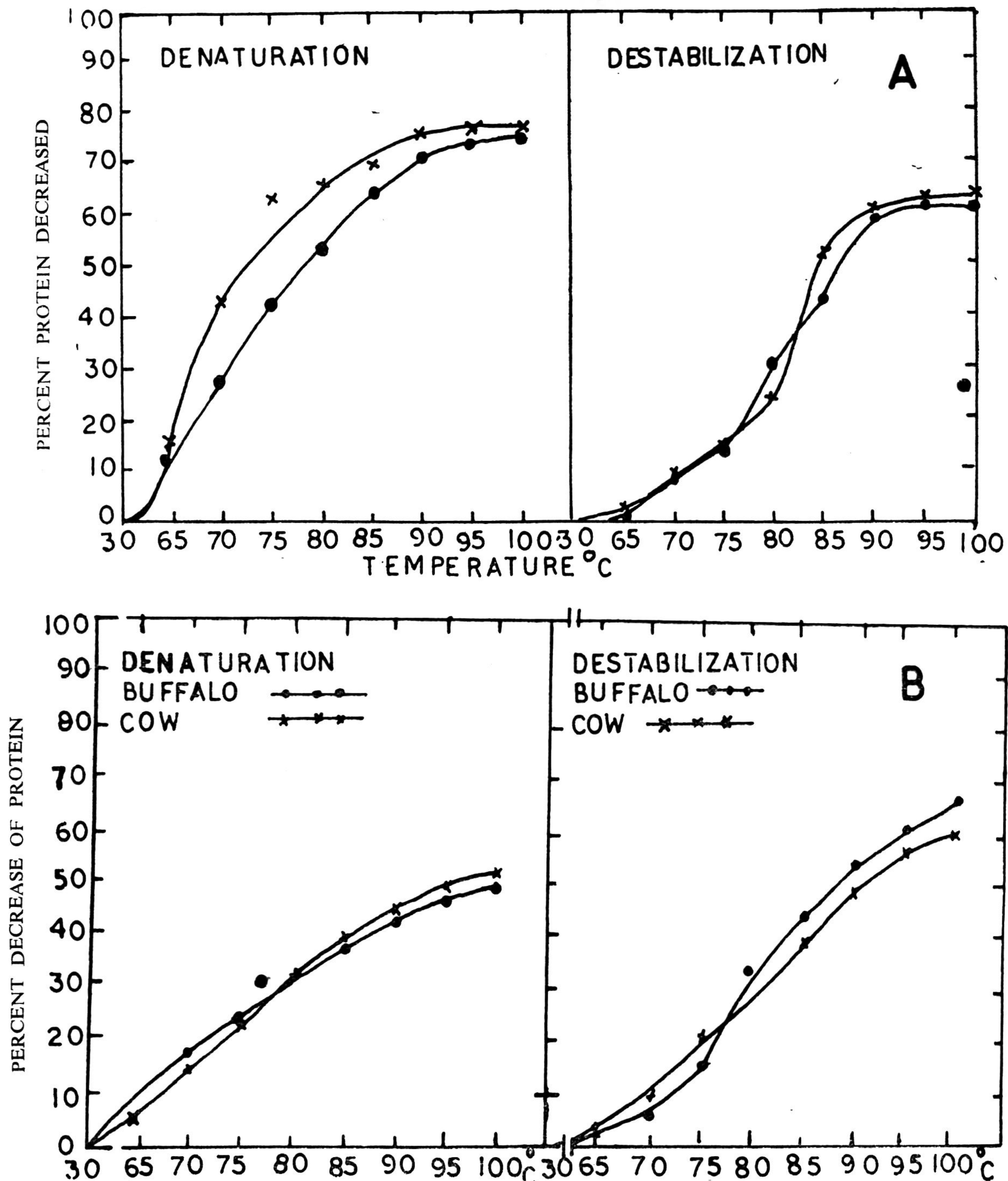

FIG. 1. Heat induced changes in buffalo and cow milk serum proteins.
A, Acid whey; B, Rennet whey.

trifuged at 1000×g for 30 min. The supernatant was used for the experiments.

Ultracentrifugal whey (UCW) was obtained by centrifuging skim milk at 11,739 × g, 46,956 × g and 105,000×g for 30 min at room temperature in a Spinco Beckman, Model L preparative ultracentrifuge. The supernatant was removed using a hypodermic syringe without disturbing the fluffy layer. The extent of heat destabilisation was evaluated by the procedure of Kenkare *et al*[3]. Protein denaturation on heat treatment was assessed by the nitrogen fractionation procedure of Aschaffenburg and Drewry[6]. All whey samples, in duplicate, were heated between 65 to 100°C for a holding time of 10 min and cooled immediately to room temperature in ice-cold water.

Acid whey: Results depicted in Fig. 1 A clearly

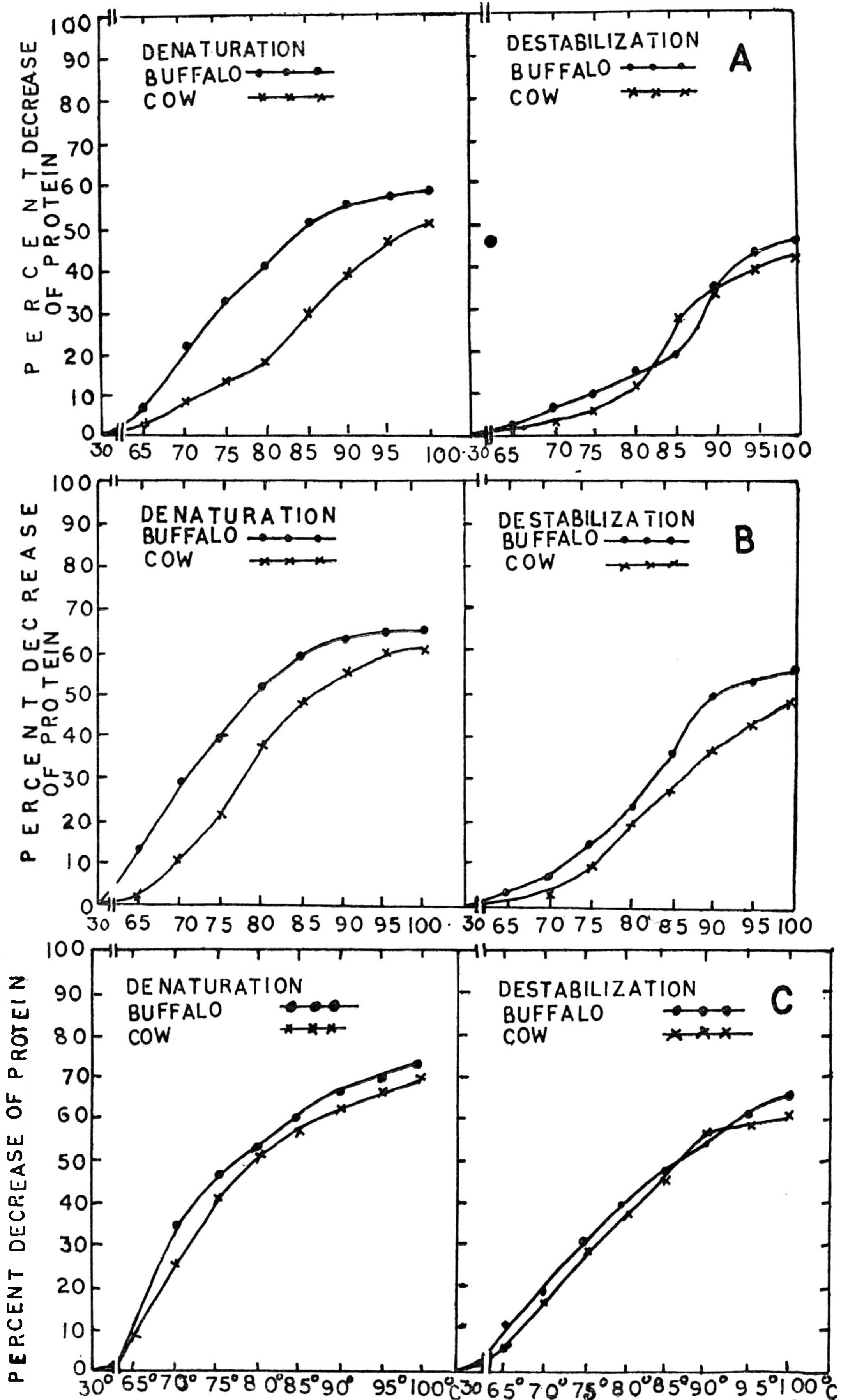

Fig 2. Heat induced changes in ultracentrifugal whey (UCW) in buffalo and cow milk. A, UCW obtained at 11,739×g; B, UCW obtained at 46,956×g; C, UCW obtained at105,000×g.

indicate that serum proteins in acid whey undergo denaturation on heat treatment, the extent of denaturation being proportional to the heating temperature. Between buffalo and cow acid whey, cow whey was denatured to a greater extent. Beyond 90°C, the rate of denaturation was almost constant.

In case of destabilization, the difference between buffalo and cow samples was not very perceptible. However, it may be noted that the extent of destabilization of the whey proteins at a given temperature, was less than that of denaturation upto a heating temperature of 90°C.

Rennet whey: Proteins present in rennet whey undergo both denaturation and destabilization on heat treatment as is evident from the results in Fig. 1 B. At lower temperature of heating, buffalo whey proteins were more denatured whereas destabilization was more at higher temperature compared to cow whey proteins. Above a heating temperature of 80°C, this phenomenon was observed. Unlike acid whey, the extent of destabilization of proteins was more in rennet whey.

Ultracentrifugal whey: Results obtained on heat treatment of proteins in UCW at different centrifugal force are presented in Fig. 2A, B and C. It appears that the proteins of UCW from buffalo milk had undergone more denaturation and destabilization than that of cow milk protein in UCW. Such difference was more significant in the denaturation phenomenon and with UCW obtained at lower ultracentrifugal force. The extent of denaturation and destabilization was maximum in protein of UCW obtained at highest centrifugal force.

Data obtained with rennet whey and UCW clearly reveal that whey proteins as they exist in buffalo milk serum are more vulnerable to heat treatments in terms of both denaturation and destabilization than cow milk serum proteins. These phenomena had earlier been observed with whole milk[7]. The increase in the percent denaturation of UCW proteins with the removal of casein micelles, clearly supports the fact that casein micelles exert protective action to the whey proteins from undergoing denaturation and destabilization[5]. More denaturation of buffalo UCW proteins observed at lower force of gravity, could be explained in terms of the larger amounts of casein micelles sedimenting than from cow milk at that speed[8,9].

The authors are grateful to Dr. D. Sundaresan, Director of the Institute, for his interest in this study.

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24th May 1976

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PROTEOLYSIS OF CASEIN BY RENNET FROM FISTULATED COW, BUFFALO AND GOAT

Tryptic and peptic activities of rennets from fistulated cow, buffalo calves and goat kid were determined and compared with the proteolytic activities associated with Hansen rennet.

Peptic activity of cow and goat rennets were higher than that of buffalo rennet. Hansen rennet generally showed lower peptic activity. Tryptic activity of rennet preparations from fistulated cow, buffalo or goat was higher than that of Hansen rennet.

The presence of rennin alongwith other gastric enzymes has been reported in abomasum of calves[1]. Work done in this Institute[2] on manufacture of rennet from fistulated calves has shown that the abomasal juice possessed higher tryptic activity than peptic. No regular pattern in the activity profiles of pepsin and trypsin with the age of animal was observed[3]. There exists a paucity of information in relation to the proteolytic action of rennets from fistulated animals. Since level of these enzymes is likely to have an impact on the quality of cheese, it was considered worth while to gain some knowledge in this respect. The present communication delineates preliminary data of such an endeavour.

Whole acid caseins from cow, buffalo and goat milks constituted the substrates. The procedure according to Gupta and Ganguli[4] was followed for preparing such caseins. Milk was skimmed and diluted with equal quantity of water. Casein was then precipitated by dropwise addition of 10 per cent acetic acid with constant stirring till the pH reached 4.6 After allowing sometime for settlement of precipitated casein, the whey was removed by filtering through a muslin cloth. The casein was again washed a number of times with distilled water to remove acid and other soluble materials. The wet casein was then blended three times with acetone to remove moisture and traces of fat. Subsequently, such casein was treated with ether. It was then filtered through a Buchner funnel using suction and the fine particles of

TABLE 1. PEPTIC ACTIVITY OF RENNET PREPARATIONS

Type of rennet	mg tyrosine liberated/g casein*		
	Cow casein	Buffalo casein	Goat casein
Cow fistulated	17.21	13.95	11.47
Buffalo fistulated	8.99	9.31	6.2
Goat fistulated	8.06	19.53	12.40
Hansen	9.34	8.68	9.72

*Results are average of four trials

TABLE 2. TRYPTIC ACTIVITY OF RENNET PREPARATIONS

Type of rennet	mg tyrosine liberated/g casein*		
	Cow casein	Buffalo casein	Goat casein
Cow fistulated	8.99	8.99	16.86
Buffalo fistulated	6.20	9.34	15.19
Goat fistulated	8.07	9.92	16.43
Hansen	3.72	6.2	12.5

*Results are average of four trials

casein thus obtained were spread over a filter paper, air dried and powdered into fine grains. Powdered rennets (vacuum dried) from fistulated cow and buffalo calves and goat kid were evaluated for their proteolytic activity and these were compared with that of Hansen rennet.

Tryptic and peptic activities were determined using a 2 per cent casein solution at pH 7.0 and 2.0. Rennet solution (0.5 ml) (3 mg/ml) was added to 2.0 ml of 2 per cent casein solution and the reaction was allowed to continue for 30 min at 30°C. It was then terminated with 5 ml of 5 per cent TCA. Protein was estimated in the filtrate by Lowry's[5] method. Results are expressed as milligram tyrosine released per gram of casein.

Data presented in Table 1 show peptic activities of various fistulated rennet preparations on whole caseins from the milk of cow, buffalo and goat. The release of peptides by cow fistulated rennet with cow casein seems to be highest (17.21) followed by buffalo and goat-caseins (13.95 and 11.47, respectively). Further, a notable preference of goat fistulated rennet for buffalo casein as substrate is evident (19.53) whereas with buffalo fistulated rennet the peptic activity on cow and buffalo casein was to the same extent. However, it can be seen that the peptic activity of cow and goat fistulated rennets was invariably much higher than buffalo rennet. The peptic activity of Hansen rennet with the three types of casein seems to be of almost the same order but generally lower when compared to the fistulated rennet preparations. Berridge[1] was of the view that calf fed on milk secreted only rennin in abomasum and that secretion of pepsin became pronounced with the commencement of solid food ingestion. As against this, Henschel *et al*[6] opined that pepsin was present alongwith rennin in the abomasal juice of fistulated calves. *In vitro* studies carried out by Eweebeck and Jaeger[7] showed that peptic breakdown was rapid and complete in homogenized and boiled milk. While manufacturing cheddar cheese with pepsin and rennet Emmons *et al*[8] observed that the mixture (1:1) of rennet and pepsin produced cheese of high quality, essentially equal to that made with rennet, and that pepsin should not be used alone.

A perusal of the results in Table 2 will show that tryptic action of cow fistulated and goat fistulated rennets on goat casein was distinctly higher (16.86 and 18.43) than that with either cow or buffalo casein. Further, even rennet from fistulated buffalo calves showed preference for goat casein (15.19) followed by buffalo casein (9.34). A similar trend could be observed with Hansen rennet as well (12.5). Ganguli *et al*[9] have observed that hydrolysis of caseins with pepsin, trypsin, papain and pancreatic homogenate was lower in the case of buffalo milk than with cow milk. Haurowitz *et al*[10] compared the *in vitro* digestion of casein and a number of other proteins-(both native and denatured) using trypsin. It was observed that trypsin digested the denatured proteins faster than the proteins in the native state. Stephen[11] while studying proteolysis of milk proteins by trypsin observed that acid casein isolated from heated milk did not behave differently from that of the casein from raw milk in proteolysis and irrespective of the species. Berridge *et al*[12] observed that the cheddar cheese prepared by them using rennet from fistulated cow calf was satisfactory. More recently, Mathur and Bhalerao[13] and Mathur *et al*[14] reported changes in cheddar cheese during ripening of cheese made with fistulated rennet preparations. It was observed that water soluble nitrogen (main index of ripening) showed a constant increase. Whey proteins decreased while proteose peptone and non-protein content showed an increase during ripening.

The author is grateful to Dr. N. C. Ganguli, under whose guidance the work was carried out for the Ph.D. degree. Sincere thanks are also due to Dr. D. Sundaresan Director of the Institute for his interest in the study.

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COLORIMETRIC DETERMINATION OF CHLORAL HYDRATE IN TODDY*

A simple spectrophotometric method for determination of chloral hydrate in toddy has been standardised. Fourteen market samples of toddy contained chloral hydrate ranging from 200 to 480 mg/litre.

Chloral hydrate a non-barbiturate with sedative action is a common adulterant in toddy. Estimation of this compound in drugs[1-3] and alcoholic beverages[4] has been reported. The reagents used in these studies have been quinaldine ethyl iodide, 2-thio-barbituric acid, pyridine (Fujiwara reaction) and the determination involved either distillation or adsorption of chloral hydrate on activated charcoal.

A simple method for the estimation of chloral hydrate has been standardised over tintometry[2] and is now applied for its determination in toddy using a colorimeter. The chromophore formed has an absorption maximum at 535 nm.

Apparatus: Spectronic-20 (Bausch & Lamb) or equivalent.

1. *Chloral hydrate:* A stock solution of this was prepared by dissolving 100 mg of pure chloral hydrate in distilled water and diluting to 500 ml in a volumetric flask. A solution containing 20 μg of chloral hydrate per ml was prepared by suitable dilution with distilled water.

2. *Sodium hydroxide—reagent grade:* 40 per cent W/V aqueous solution.

3. *Pyridine (B.D.H. laboratory reagent):* Freshly distilled over KOH pellets (should not give any colour when heated with alkali).

Chloral hydrate solution in the range of 5 to 40 μg was pipetted into test tubes (16 cm$\times$2 cm) and the volume made upto 2 ml with distilled water. Pyridine (7 ml) and sodium hydroxide solutions (1 ml of 40 per cent) were added to the samples using a Vacupet. The contents were mixed by shaking and the tubes were then placed in a water bath maintained at 80°C for 5 min and later cooled in running water to room temperature. The pink coloured complex was then separated from aqueous layer using a long needled syringe, clarified by filtering through Whatman No. 1 filter paper and the colour was measured in Spectronic 20 at 535 nm within 20 min. The blank contained distilled water instead of chloral hydrate and all other components were also added (Fig. 1).

Several variables such as effect of temperature, time of heating and level of alkali were studied and it was found that the maximum intensity of colour was developed at 80°C in 5 min with 1 ml of 40 per cent sodium hydroxide. The colour was stable upto 20 min and there after it decreased. Malhotra *et al.*[2] described a tintometer method for the estimation of chloral hydrate which was very subjective. Kamat *et al.*[4] reported a sensitive method for estimating chloral hydrate using 2-thiobarbituric acid. But the authors confined themselves for the recovery of this chemical in alcoholic beverages. The time required for colour development was 100 min at 37°C with optimum pH; besides, the method involved either distillation or adsorption on activated charcoal for preparation of the sample.

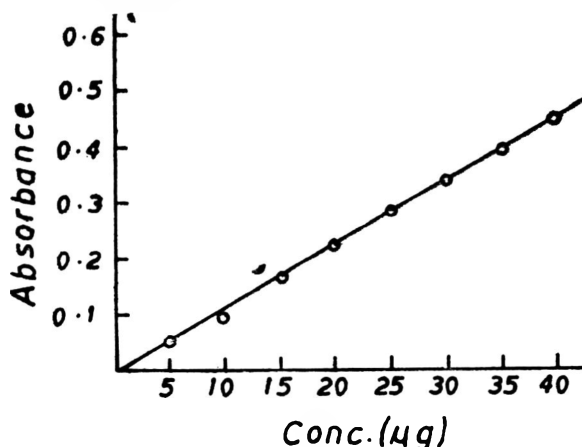

Fig 1. Standard curve of chloral hydrate at 535 nm.

*Toddy is the fermented sap or exudate of plants like wild date, Palmyra, coconut, sago, etc. used as alcoholic beverage.

TABLE 1. RECOVERY OF CHLORAL HYDRATE FROM TODDY SAMPLES

Chloral hydrate		
Added (mg)	Recovered (mg)	% recovery
107.00	105.00	98.13
114.00	112.00	98.24
118.00	115.00	97.88
127.00	120.00	95.08
236.00	231.00	97.89
152.00	152.00	100.00
312.00	304.00	97.59
892.00	882.00	98.64

The method described here is simple, and suitable for the estimation of chloral hydrate in toddy in a short time.

Chloral hydrate at different levels was added to toddy free of chloral hydrate and estimated according to the method developed and the percentage recovery of the same was determined. The results are shown in Table 1.

The per cent recovery is almost quantitative indicating that there are few, interfering substances in toddy which affect colour development. Fourteen market toddy samples collected in the city of Mysore were analysed for their chloral hydrate content. In all the samples, chloral hydrate was present in amounts ranging between 200 and 480 mg per litre.

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Nutrition Technology of Processed Foods: by Nicholas D. Pintauro, Noyes data Corporation, Park Ridge, New Jersey, London, England 1975, pages 332.

This book is based on U.S. Patents in the area since 1961 and deals with the nutrition technology of processed foods. It consists of the following 10 chapters; (1) Cereal Products; (2) Enriched and Dietetic Breads; (3) Rice and Potato Products; (4) Milk and Dairy Products; (5) Nutrition Beverages; (6) Low Cholesterol Egg Products; (7) Salt Substitution; (8) Complete Diets; (9) Special Fortification Processes and (10) Special Processes and Products. In addition it has Company Index, Inventor Index and US Patent Number Index. Each Chapter contains information gleaned out from a number of US Patents and contains sufficient details for any one to plan the experiments.

Nutrition technology is a vague phrase and perhaps there can be more than one definition. For the purpose of the book patent literature, which discusses both the ingredients and technology of food processing specifically designed for their nutritive benefits, has been used. It includes "processes designed to preserve the nutritional quality of foods; formulations, ingredients, supplements or other additives intended to improve the nutritional quality of foods; or where nutritional values were given which might then be used for labelling purposes".

There is a wide coverage of the subject which encompasses animal foods, foods from vegetable sources, protein beverages, inorganic nutritents, etc. Although certain foods such as low calorie foods may be of interest to Western Society only, there are many other foods described which will be of interest in the Indian context also.

Perhaps it can be said with some justification that a published patent conceals more than it discloses. Subject to this limitation one may agree with the publishers that this book describes "the number of technical possibilities available, which may open up profitable areas of research and development". The book will certainly be found useful by Food Scientists and Technologists because information is available in a single compilation on a wide variety of foods.

M. S. Narasinga Rao

Recent Studies on the Thermophysical Properties of Foodstuffs: International Institute of Refrigeration, 177, Boulevard Malesherbes, 75017, Paris, pp. 345.

Knowledge about thermophysical properties of foodstuffs is undoubtedly an essential pre-requisite for work in the field of application of cold to the science of food technology. A large number of workers in different institutions all over the world have been working in collecting basic data on the thermophysical properties of different types of food materials. The International Institute of Refrigeration has brought out several such workers in the field on a common platform at a meeting of their Commissions B1 (Thermodynamics and transport processes); C1 (Freeze-drying-Cryobiology-Medical applications) and C2 (Food Science and Technology) at Bressanone, Italy, in September 1974 and have documented and pulished all the reports and discussions held at these meetings.

The Publication contains 41 reports in all—29 in English and 12 in French. They have been divided into 7 sections. Section 1 deals with Heat transfer during the change of state of foodstuffs. Of the 5 papers in this Section, 2 deal with computer application, one on heat transfer in general, one on freezing times and one on heat transfer coefficients. Section 2 deals with heat transfer problems in cooling and freezing and has 9 papers in all. Section 3 deals with heat transfer during freeze drying of foodstuffs and has 8 papers starting from the theory of sublimation to drying time calculations. Section 4 has 8 papers bearing on thermal properties of foodstuffs particularly thermal conductivities. Section 5 has 9 papers dealing with structure and microstructure of food products. Section 6 has 3 papers dealing with formation and growth of ice crystals. Section 7 gives a gist of the round table discussions that followed the meeting of the Commissions.

There will be wide variations in the thermal properties of different food materials depending on variety, time of harvesting, location, climate, etc., and it is difficult to give accurate data on any one product. But an indication of the probable average values could be arrived at with the help of the available data.

The most interesting part of the report is the round table meeting and the conclusions arrived at. The Panel for the round table consisted of several experts. After discussing the various methods used for collecting the data on thermophysical properties of foodstuffs, the Panel came to the following conclusions.

(1) A very high degree of accuracy does not seem necessary if physical data are used for thermal design.

(2) Availability and accessibility of experimental data are the most important requirements which are to be met through intensive international

cooperation and efficient classification procedures.

(3) Whatever method is developed, if it is to be useful to Engineers, it must be relatively simple and useable under a wide range of conditions.

(4) Since a large number of institutions are working on this problem all over the world, the IIR or the ASHRAE should act as a liason between them by forming a working group, whose task is to furnish contacts in this field to all those who are in need of information on the physical and thermal properties of food and related compounds. Another solution is either publication of a Bibliography or Handbook or organisation of a data bank with easy methods of manual or automatic filing and retrieval of information by a computer. A list of organisations gathering such information is also given at the end.

All the papers are in the nature of research publications and these and the discussions that followed the presentation of the papers will be very useful to researchers in the field of cold and frozen storage of food materials. The data would also be useful to designers of plant and machinery to cold and frozen storage.

S. K. LAKSHMINARAYANA

Food Emulsions–Food Science Series: Vol. 5—Edited by Stig Friberg, Swedish Institute for Surface Chemistry in Stockholm, Sweden, 1976; pp. 488, Price: 48.50 U.S. $.

The present volume consists of eight chapters of which Chapters 1, 2, 3 and 6 deal with basic aspects of emulsion science, and Chapters 4, 5, 7 and 8 deal with applied aspects of the food emulsion. Adequate information regarding the basic aspects relevant to each of these Chapters have been presented before elaborating the applied aspects.

In Chapter 1, Friberg presents the physico-chemical aspects of emulsion stability and theories propounded to explain them are briefly reviewed. This is followed by a list of references for further reading. In the second Chapter K. Karsson describes the recent advances in the crystal and liquid crystal structures of lipids under different experimental conditions by X-ray and phase analysis. This area. I believe, would get increasing attention from physicists and physical chemists because of the uniqueness of lipids in regard to their structures which have not been anticipated in theoretical classification of possible states of order—amorphous state of a perfect crystalline state. N. Krog and J. B. Lauridsne in Chapter 3 have elaborated extensively the manufacture, properties, application of some food emulsifiers, their behaviour in aqueous solution, their role in food emulsions, their physical state and their dependence on the interaction with starch components. In Chapter 6 the physical properties of the lipids and different types of interaction between protein and lipids are discussed by R. P. Rand. The information collected from NMR study in this area is lacking.

In Chapter 4, K. G. Berger deals at length on the applied aspects of ice-cream processing, including discussion on the chemistry of ingredients, structural and textural aspects of ice-cream. These are mostly taken from the results of E. M. study. A detailed analysis has been made of ice-cream structure, its dependence on ingredients and processing. T. S. Shepherd and R. W. Yoell in Chapter 5 present a detailed account of the technology of cake emulsion which I believe will be of interest to both housewives or bakers and to scientists who want to improve the quality of cake. Relevant basic information has also be n presented in this Chapter. E. Grof and H. Bauer in Chapter 7 deals with basic and applied aspects of milk and milk products which include characteristics of the fat globules, stability of the milk emulsion for long period and its physical properties, and mode of homogenisation. The arrangement of sub-heads is very thoughtfully done as in other Chapters. Meat emulsion aspects presented in Chapter 8 by J. Schut provide equally useful information.

In conclusion, I would say that this book is extremely useful for a food research laboratory. Every chapter of the book contains very useful information and the presentation has been very thought provoking. This book probably, for the first time, has been able to bring-out the role of crystals or liquid crystals in aqueous medium from the surfactant molecules which determine emulsion properties of a system. Binding and the printing are attractive. A few printing errors in the book could have been avoided.

P. K. NANDI

New International Dictionary of Refrigeration: Published by the International Institute of Refrigeration, 177 Boulevard Malesheaber, 75017, Paris, France. pp. 600. Price: 300 F.F.

This new International Dictionary is a revised edition of the first one published by the same organisation in 1961. This second edition contains 2400 elements corresponding to 3000 expressions used in the field of Refrigeration, Air-conditioning and allied fields and is

given in seven languages viz. English, French, Russian, German, Spanish, Italian and Norwegian.

Since the publication of the first dictionary, the field of refrigeration has been considerably enlarged during the past 15 years and includes such fields as Cryogenics and Freeze Drying. The Dictionary is in two parts; the first part consists of all concepts and expressions methodically grouped together subjectwise in 15 Sections ranging from basic data to industrial applications and covers all subjects one is likely to encounter in the field of Refrigeration & Air-conditioning. The second part consists of indices in each of the languages giving a reference to the expressions in the first part. It is thus a very useful reference book on the shelf of all scientific workers in the field of Refrigeration, Air-conditioning, Cryogenics, Freeze Drying and other allied fields. A work of this nature conforming to rules of several international organisations involves considerable compilation work with international cooperation and the I.I.R., Paris, is to be commended for bringing out such a useful reference work.

S. K. LAKSHMINARAYANA

Guide to English Language Publications in Food Science and Technology: by S. Green, Food Trade Press Ltd, London, England, 1975.

This fourth revised edition of the book list attempts to provide a comprehensive information on primary, secondary and other serial publications, conference and seminar proceedings, surveys and reviews, directories, handbooks, encyclopedias, dictionaries, patents, standards and food legislation, reference and text books published in the area of food science and technology upto 1974 (?). The method of arrangement of titles is very helpful but in a few situations multiple entries lead to confusion in understanding the exact nature of the publication.

The author has attempted to provide an exhaustive list of source materials under very widely distributed categories in Food Science and Technology, but quite a few of them are left at large. Among these are Packaging Abstracts, Review of Applied Entomology, Food Composition Tables for Use in East Asia, Encyclopedia in Food Technology, English-German Dictionary of Food Science, Dictionary of International Food and Cookery Terms, Proceedings of International Symposium on Proteins (CFTRI), Tropical Products Institute publications—to mention a few.

Although the author has attempted to internationalise the publication, still leaving apart a couple of countries, this proves to be a poor source material for publications in Food Science and Technology for any other country. This is quite possible when we realise the limitations in knowing the publications of other countries and in most cases in compiling such a publication, it is natural to be carried away by the internationally established publishers. It would be more appropriate if each country prepares a similar booklist covering exhaustively of what has been published in their own country in the area of Food Science and Technology which in turn can be used to prepare an international booklist having relevance to each and every country. A word about food standards specifications and food legislation. As these are very essential in international trade and are innumerable in number it would have been more helpful if addresses of organisations and bodies dealing with these in different countries had been furnished so that they could be procured from the concerned countries.

However, the publication under review will be of immense use to those who are in the initial stages of establishing a library in Food Science and Technology and will serve as a checklist for those who have already established a library in this area.

K. A. RANGANATH

The Mango (Mangifera indica *L.*)*: Harvesting and Subsequent Handling and Processing—An Annotated Bibliography*, by J. C. Caygill, R. D. Cooke, D. J. Moore, S. J. Read and H. C. Passam, Tropical Products Institute, 56/62 Gray's Inn Road, London WC1X 8LU, 1976, pp. 124, Price: £ 2.10.

This exclusive annotated bibliography on mango is based on survey of literature from 1960–74 scanned from various abstracting journals. Pertinent earlier references have also been included.

For facility, the subject matter has been included under ten chapters with a preface at the beginning of each chapter viz., harvesting and distribution, storage and ripening, storage disorders and diseases, use of radiation in fruit storage, technology, composition, flavour, preservation, processing, by-products and marketing. For each reference, authors name in alphabetical order and full title is given followed in most cases by brief annotations.

This bibliography serves as a source book for those connected with mango storage and processing.

S. RANGANNA

Food from Waste: Edited by G. G. Birch, K. J. Parker and J. T. Worgan, Applied Science Publishers Ltd., London, 1976; pp. 301; Price: £ 16.00.

The book represents a compilation of the 20 papers with reports of discussions presented at an Industry—University Cooperation Symposium organised under the auspices of the National College of Food Technology. University of Reading, England. Papers presented and discussions cover 291 pages; leaving the rest for an index. Food From Waste is the main theme of the Symposium. Hence, the first two papers deal in a general way with two aspects of this problem; namely, present world situation and an over view. The area covered relates to the concept of producing food from waste, availability of raw-material with respect to quantity and distribution and indicates how economics is the overriding factor in the decision to implement any process.

The remaining eighteen papers are brought under four sessions with a chairman for each session. Four papers are presented in Session One, dealing largely with the utilization of wastes from crop plants, carbohydrates and molasses either to upgrade these by microbial processing or alternatively for production of yeast and protein. The use of fungi as the up-grading agents offers many advantages; particularly in the context of production of protein from carbohydrate wastes.

In session two we have five papers, dealing with microbial production of oils and fats, algal proteins, food from waste paper and carbohydrates derived from potato wastes. The data provided under microbial production of oils and fats, indicates the feasibility to produce a cheap microbial fat in the present context of world's available fat resources. Four papers are listed in session three and cover the areas of leaf protein, protein from starch mill effluent and meat, poultry and fish processing plants. The author on leaf-protein coucludes "that while the importance of circumventing waste is gaining ground, it is more important to avoid producing waste in the first place". The rest of the papers deal with starch, meat and dairy industry effluents. Even though the authors of these three papers have indicated the possibility of recovering protein from all these effluents yet the economic feasibility for large scale production still remains to be worked out.

The last and fourth session has an assorted assemblage of five papers. The more appropriate papers in this session deal, with, nutritional and toxicological evaluation of novel feed and socio-economic implications of producing food from wastes.

The theme selected for this symposim "Food from Waste" and now presented in the book from is welcome and timely. It would have been more purposeful if the papers had been classified either on the basis of the main component of the substrate or on the nature of the effluent. As matters stand, papers on protein from carbohydrates are to be found in Session 1, 2 and also 3. There are in all four general papers which again are distributed somewhat randomly both at the begining and again in session four. These are matters of minor importance in a book which is otherwise beautifully brought out. The present work brings under one cover, the expertise of the leading workers in the field with the added interest of discussions under each paper: Today all of us are involved in reducing waste and also for re-utilisation of waste for feed or food. The editors deserve our thanks for bringing out such a timely and useful publication.

T. N. RAMACHANDRA RAO

New Editor of Journal of Food Science & Technology (1977–1979)

The Executive Committee of the Association of Food Scientists & Technologists (India), is happy to announce the nomination of Dr. D. Rajagopal Rao as the New Editor of the Journal of Food Science and Technology from 1st January 1977 for a period of 3 years.

Dr. D. Rajagopal Rao had his initial academic training at Andhra University, Waltair, from where he graduated with B.Sc. (Hons) and took his M.Sc., degree in Chemical Technology. After research work in Biochemistry for an Associateship at the Indian Institute of Science at Bangalore, he took Ph.D. in Biochemistry in 1958 at Philadelphia, U.S.A. Later he was a research associate at the University of California Medical Centre, San Francisco; a visiting fellow at the John Curtin School of Medical Research, Australian National University, Canberra and recently as Senior Fellow of the Alexander Von Humboldt Foundation at the University of Colonge, West Germany. He was also associated with Hindustan Lever Ltd., for about a year as a Research Manager, involved in the development of infant foods, dairy products, etc.

Dr. D. Rajagopal Rao has been in the Central Food Technological Research Institute, for more than a decade, and his research interests have been mainly in the area of proteins and amino acids.

Bangalore Chapter

The Bangalore Chapter of the Association organised a Symposium on "Strategies for Storage, Handling and Processing of Food Grains in the Present Day Context", from 3rd to 5th September 1976 at the Conference Hall of the West End Hotel, in Bangalore. The Symposium was inaugurated by the Union Minister for Agriculture, Shri Jagjivan Ram, and was presided over by the Minister for Agriculture in Karnataka State, Shri Chickegowda. The Souvenir for the occasion, was released by Srimathi Eva Vaz, Minister for Food, Karnataka State Government.

The deliberations of the Symposium took place in four technical sessions: (*i*) Storage and Conservation of Food Grains; (*ii*) Transport and Handling of Food Grains; (*iii*) Processing of Food Grains; and (*iv*) Logistics of Build Up and Maintenance of Dynamic Buffer Stocks.

The Symposium was significant in several ways. The theme was of very great importance because of the recent decision of the Union Government to build up buffer stocks, to overcome dependence of the country on imports of food grains, and as a measure for improving the productivity of the Indian agricultural system, ensuring ultimately food security. The problems that emerge from such a national policy had not been discussed in an open forum up till now. The Symposium provided a platform for the much-felt need for such a discussion. The response from several sectors who are concerned in this, programme, was very encouraging. The Food Corporation of India, which is the major executive body in the programme of grain procurement, gave its full cooperation, by participating in the discussions. Several of the senior officers of the Karnataka State Government, presented papers and took part in the discussions. Research Institutes like, the Central Food Technological Research Institute, Mysore, Defence Food Research Laboratory, University of Agricultural Sciences Bangalore, sent delegations to the conference. The Food Department of the Ministry of Agriculture, the Modern Bakeries, the Flour Millers Association and other organizations cooperated and took part in the deliberations. Representatives from the Madras region, Hyderabad and Trivandrum Chapters of the Association attended the Symposium and took part in the discussions.

The Plenary Session at the conclusion discussed the resolutions and drafted the recommendations. Before the conclusion of the Symposium, a Panel discussion on "Rationale and Need for Buffer Stocks", was held. This Session provided for a frank discussion of the ramifications of the philosophy of buffer stocking. The views expressed, highlighted the achievements, but at the same time brought the difficulties encountered in such a massive operation.

Shri Jagjivan Ram, Union Minister for Agriculture inagurating the Symposium on "Strategies for Storage, Handling and Processing of Food Grains in the Present Day Context".

Lt to Rt: M. M. Krishnaiah, Secretary AFST, Dr. V. Subrahmanyan, Project Head, Paddy Processing Research Centre, Tiruvarur, Smt. Eva Vaz, Minister for Food & Civil Supplies, Karnataka, N. Chikkegowda, Minister for Agriculture and Animal Husbandry, Karnataka, M. R. Chandrasekhara, President-AFST, M. K. Panduranga Setty, President, Bangalore Chapter-AFST.

Dr. H. S. R. Desikachar, Project Coordinator, Cereal Processing Technology, CFTRI, Mysore, receiving the Prof. V. Subrahmanyan Industrial Achievement Award for 1975, from Dr. V. Subrahmanyan.

Lt to Rt: M. K. Panduranga Setty, President, Bangalore Chapter, AFST, M. C. Madhura, Councillor, AFST, and M. M. Krishnaiah, Secretary, AFST.

The papers presented were based on expertize and experience. The recommendations evolved from the Symposium, have now been sent to all the important organisations connected with the building up of buffer stocks.

The Association is maintaining a close watch on the follow-up operations and where necessary is willing to provide its cooperation to the Government and other agencies in this national activity.

The Proceedings of the Symposium are now being printed and will be published by the Bangalore Chapter. This will include besides the papers presented at the Symposium, the discussions and the recommendations.

Recommendations drawn at the Symposium:

1. The Symposium emphasised the overall need for accumulation of buffer stocks as an essential element in the policy of food management to tide over lean years, to overcome the need for import of food grains and as an important strategy for overall agricultural development of the country.

2. It was recognised that the basic objective of this strategy was to provide a ready market to the farmer, ensuring him price, support and at the same time sustaining the distribution system, with the objective of improving the productivity of the Indian agricultural system, ensuring ultimately food security.

To achieve this end effectively it was recommended that buffer stock operations should be linked with support prices, which should be announced in advance of the agricultural season and could be manipulated in such a manner as to increase, maintain or decrease production of particular commodities depending on the need of the time.

3. The Symposium recognised that there are many problems in the accumulation of buffer stocks, like the quality of the grains to be stored, handling, storing, transport and disposing of these. These problems have to be dealt with urgently by competent bodies with accumulated experience and by problem-oriented research.

4. It was also recognised that there is an immediate need for building up of professional and technical manpower to deal with food commodities when stocked on this massive scale. In this effort the universities and other organizations should train the personnel at the graduate and technical level in post-harvest technology.

5. Among the recommendations adopted for implementation to ensure the overall efficiency of the working of buffer stocks the following are of immediate interest.

(*a*) Taking note of the fact that buffer stocks have to last for a long period, the quality of the grain stored becomes very important. It is necessary to educate the farmers to supply clean and dry produce at the time of procurement. The Save Grain Campaign, the extension services of the Agricultural Departments and the Agricultural Universities should intensify this educational programme to provide the know-how to the farmers. At the same time it was recommended that in order to ensure the proper quality of the grain procured, adequate facilities for precleaning and drying should be provided both at the farm and bulk storage levels.

(*b*) It is very necessary that grains procured as a measure of price-support policy, specially under adverse weather conditions, should be checked for quality standard and if these cannot be met, the grain should not be stored for long periods.

(*c*) Under conditions of surpluses, it is often difficult to store grains under ideal storage. To guard against sprouting, discolouration and mould growth, it is necessary to evaluate various simple and quick methods of assessment of the quality indices including mycotoxins and standardize them. For this more intensive scientific research in this field has to be undertaken on a cooperative basis between research institutes and organisations handling the grain. Suitable equipment for on-the-spot checking should be manufactured and made available on a large scale to all those concerned with procurement.

(*d*) To encourage the farmer to offer better grains for procurement for buffer stocks, it was recommended that premium price should be offered for quality grain intended for long storage.

6. The Symposium recognised that several types of storage constructions are used and are made of different types of material, both at the rural level and large scale operation. It was recommended that the use of various materials for construction and types of structures be comparatively evaluated in the light of cost benefit analysis. Suitable structures and materials of construction thus evaluated and found satisfactory both at farmer's level and at bulk level should be given wide publicity.

7. The mechanical drying equipment both at farm level and at the level of large scale operations require improvement in design and efficiency. It was recommended that research institutes and agricultural universities should take up research work on fabrication of such driers and incentive should be provided for such research activities.

8. It was recognised that regulated markets are the appropriate places where quality requirements need be emphasised. It was recommended that machinery for cleaning, drying, grading and bulk weighing of food grains be installed in these markets.

9. It was recognised that contamination of foodgrains with harmful dusts and foreign matter took place in transport. To overcome this it was recommended that for transport of foodgrains special vehicles for both rail and road be designed, and these should be used exclusively for this purpose. For intermediate handling bags are needed. Storage bags for foodgrains should be colour-coded to prevent their use for other harmful commodities.

10. It was recommended that apart from procurement of coarse cereals that are produced in the dry farming areas they should also be adequately protected.

The Symposium appreciated the role of Save Grain Campaign which is actively functioning in some parts of the country. At the same time it was recommended that this programme should be intensified in the rural areas and other organisations like the Food Corporation of India, State and Central Warehousing Corporations should be associated with this campaign. It was recommended that more funds should be earmarked for this campaign in order to attain total coverage of rural areas in the next ten years.

11. Based on the information available on the condition of storage of food grains in the rural areas the Symposium felt that it was now necessary to educate the farmers on proper storage methods and impress on them the nutritional losses which occur if the grains are not stored properly. It further recommended that rural level pest control operators should be trained for work in every taluka and they should be provided with adequate supply of fumigants and rodenticides. The extension activity of the Save Grain Campaign should be geared to this activity. In order to ensure proper storage at the farm level it was recommended that development of economical threshing floor and drying facilities for the farmers should be urgently undertaken.

12. The Symposim discussed the methods of processing of food grains with the object of either extending their use or for enhancing their nutritive value. It felt that there is a need for a natural policy for modernisation of rice milling for higher out-turn of rice and production of oil-rich bran. This is urgently needed in the case of conventional units which do the bulk of the rice milling in the country. Methods developed for the elimination of smell and loss of rice during soaking of paddy for production of parboiled rice should be quickly applied. There is need for more extension work with support from both the center and the state.

13. Priority should be given for the development of the edible rice oil industry with stabilisation of the bran by the methods which have already been developed and successfully applied.

14. There should be greater economy in the processing of wheat with due consideration for the quality of the products. For certain categories of products, admixture with flours of other grains and tubers can also be tried. This will help to extend the uses and reduce the costs. Clean wheat flour with adequate and strong gluten content should be made available for producing bakery products specially bread which have become increasingly popular and successful. There should also be intensive research for further advances in bakery technology for better use of low gluten flour. Bulgar wheat which has already proved to be popular should be produced on a large scale with the techniques already developed in India. Its use should be popularised and a market built up for its use. Side by side with the above, there is also a case for developing toasted products based on wheat. These products can find application in a variety of programmes like mid-day meal feeding programme and special nutrition programme which now largely depend on imported gift foods.

15. Special attention should be paid for the processing of millets for the production of quality products with consumer appeal. Application of scientific work in the line, which has already proved to be successful should be extended.

16. It is of paramount importance that large procurement agencies like the F.C.I. and State Civil Supplies Corporations pay greater attention to the supply of quality products meeting the requirement of consumers. Concerted efforts should be made by the procurement agencies and the scientists in solving the practical problems relating to quality improvement, particularly cleaning and drying of grains prior to storage, so that the consumers' requirements are met.

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

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

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

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