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Methods for Detoxification of Aflatoxins in Foods and Feeds - A Critical Appraisal

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Control of aflatoxins is the need of the hour, since their occurrence in foods and feeds is continuously posing threats to both health and economics all over the world. Besides the post-harvest preventive measures, it is imperative that suitable detoxification methods are developed for inactivating or removing aflatoxins from the contaminated commodities, as the toxins are also produced by *Aspergillus flavus* and *A. parasiticus* even during pre-harvest stages of crop production. Several physical and chemical detoxification methods developed so far have been critically discussed in this review for their advantages and limitations based on certain adopted strategies and specific criteria. Detoxification by microbiological means is also reviewed with a view to knowing the status on potential microorganisms and their enzymes that can degrade aflatoxins to less toxic or innocuous end products. Understanding of mechanisms of aflatoxin detoxification by physical, chemical and microbiological methods will enable establishment of combined treatment procedures to effectively decontaminate, contaminated foods and feeds. Such treatment methods are expected to be cost effective and minimally deleterious to food constituents.

Keywords : Aflatoxins, Foods and feeds, Detoxification methods, Mechanism of detoxification, Nutrient losses, Microbiological degradation, Limitations, Advantages.

Aflatoxins are the most potent toxic, mutagenic, teratogenic and carcinogenic metabolites produced by the species of *Aspergillus flavus* and *A. parasiticus* on food and feed materials (Salunkhe et al. 1987). They infect the food crops, especially the cereals and oilseeds both in pre- and post-harvest conditions of food production (Anderson et al. 1975). The occurrence of these toxins in food and feed materials and their consumption has caused not only health hazards in animals and humans, but also resulted in economic losses, especially to the exporting countries (Salunkhe et al. 1987). Human exposure to these toxins can arise from direct consumption of contaminated commodities, such as corn, peanuts, coconuts, and the products of farm animals (egg, milk and meat), previously exposed to aflatoxins in feed (Park and Pohland 1986; Park 1993). The ratios of feed level aflatoxin to edible tissues are very large and at levels of 75, 2200 and 14000 in milk, egg and liver, respectively (Park and Pohland 1986). Chronic exposure to such small levels of aflatoxin is classified as a group 1 carcinogen (Autrup and Autrup 1992) and is not recommended by the Joint FAO/WHO Expert Committee on Food Additives (Park and Liang 1993). However, the US Food and Drug Administration has currently established action levels (maximum) of aflatoxin at 20 ppb for human foods (except milk), 0.5 ppb for milk, 20 ppb for animal feeds, except for 100 to 300 ppb in some cases of feeds meant for maturing and finishing of meat animals (Park 1993). As per regulations available from 53 countries, the aflatoxin limits vary from 0-50 ppb for all foods (Pohland 1993), whereas for India, it is 30 ppb (Salunke et al. 1987).

From the point of view of health and economics, it is imperative that only very low levels of aflatoxin are admitted. However, achieving such low levels is a difficult task, especially in tropical and third world countries, where the conditions are conducive for the mould to infect and elaborate toxin in foodstuffs, even prior to harvest (Anderson et al. 1975). Varieties of crops that can resist mould infection and mycotoxin production are yet to be developed. Although drying of foodstuffs to safe moisture level (<10.0%) can reduce the post-harvest development of aflatoxins (FAO 1977), the practice is beset with certain problems, like inclement weather conditions, small farm holdings and lack of facilities for drying. Prevention of aflatoxin formation has also been attempted by making use of plant extracts and antifungal agents (Rusul and Marth 1988; Ray and Bullerman 1982). But the process is expensive and tedious, rendering it impractical. Hence, approaches involving physical, chemical and biological methods have been made to detoxify aflatoxins in food and feedstuffs in the recent past. A critical review on these methods is presented in this paper.

Strategies and specific criteria for detoxification

Physico-chemical and biochemical characteristics of aflatoxin B_1 molecule reveal two important

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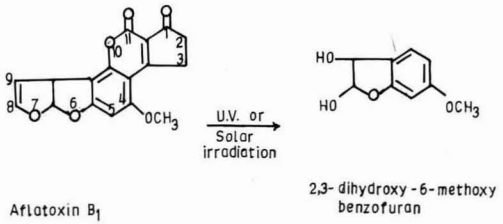
sites for toxicological activity (Heathcote and Hibbert 1978). The first site is the double bond in position C-8.9 of furofuran ring (Fig. 1). The interactions of aflatoxin, DNA and proteins, which occur at this site, alter the normal biochemical functions of these macromolecules, thereby leading to deleterious effects at the cellular level. The second reactive group is the lactone ring in the coumarin moiety. The lactone ring is easily hydrolyzed and is, therefore, a vulnerable site for degradation. Hence, the degradation treatments should be aimed at removing the double bond of the terminal furan ring or in opening the lactone ring. Once the lactone ring is opened, further reactions could occur to alter the binding properties of the terminal furan ring to DNA and proteins.

Among the various aflatoxins that occur in food and feed, aflatoxin B_1 is the most toxic (Eaton and Ramsdell 1992). It has been found that any change in this molecule either by physical, chemical or biological means would reduce the toxicity (Samarajeewa et al. 1990). The relative toxicity and mutagenicity of many of these derivatives of aflatoxin B_1 has been reviewed by Samarajeewa et al (1990). For example, a simple introduction of a hydroxyl group at furofuran ring of aflatoxin B_1 leads to a derivative aflatoxin M_1 *in situ* in cow, sheep and goat before excretion in milk. This toxin is three times less carcinogenic to rainbow trout (Sinnhuber et al. 1970), has the same order of toxicity to ducklings as aflatoxin B_1 (Wogan et al. 1971) and 30 times less mutagenic to *Salmonella typhimurium* (Wong and Hsieh 1976).

Hydration of double bond in furan ring leading to aflatoxin B_{2a} takes place in microsomes of ducklings as well as under acidic conditions (Samarajeewa et al. 1990). This is 200 times less toxic to ducklings (Lillehoj and Ciegler 1969) and 1000 times less mutagenic (Wong and Hsieh 1976). Similarly, physical (irradiation) and other chemical



Aflatoxin B_1

2,3- dihydroxy - 6- methoxy benzofuran

Fig. 1. Degradation of aflatoxin B_1 by UV and solar-radiation (Aibara and Yamagishi 1968)

reactions can lead to different derivatives, which have less toxicity (Samarajeewa et al. 1990).

Consequently, various physical and chemical agents have been used to decontaminate/detoxify aflatoxins from food and feed materials (Detroy et al. 1971; Samarajeewa et al. 1990). Different reaction products are obtained depending upon the treatment process (Samarajeewa et al. 1990). Strategies and specific criteria, established for evaluating the acceptance of a given decontamination procedure (Park 1993), include (a) inactivate, destroy or remove the toxin; (b) not produce or leave toxic residues in food from animals, fed with the decontaminated product; (c) retain the nutritive value and feed acceptability of the product; (d) not alter significantly the properties of product; and (e) if possible, destroy fungal spores.

Physical methods

Physical removal

Physical removal of infected grains (coarse) by hand-picking or electronic colour sorting, density segregation by floatation, removal by milling cereal grains, adsorption-cum-filtration technique for decontamination of groundnut oil, removal by suitable solvents and inactivation by heat and irradiation are some of the methods found to be promising in reduction of aflatoxin levels in contaminated products (Basappa 1983).

An effective method has been reported for reducing aflatoxin in corn (Huff and Hagler 1985) and peanuts (Cole 1989) by floatation and density segregation of toxic kernels (Detroy et al. 1971). A systematic electronic and hand-sorting of contaminated peanuts has been used extensively by the peanut industry in USA to reduce aflatoxin level in peanut products, destined for human consumption. By following a set of strategies like belt separation, shelling, colour and gravity sorting, blanching and colour and re-colour sorting, it was possible to reduce aflatoxin from peanut, containing 217 ppb to a level of 1.6 ppb in kernels amounting to 99.3% removal (Park and Stoloff 1989).

The data presented in Table 1 (Park and Stoloff 1989) are from processing 40 tonnes of contaminated peanuts. It is indicated that a few methods are very encouraging, although the problem of disposing the toxin-rich segregated batch still remains to be solved. However, the method appears to be cost intensive and may not be applicable in under developed countries. In fact, the method of hand-picking of discoloured peanut kernels on a conveyer

TABLE 1. EFFECTIVENESS OF POST-HARVEST AFLATOXIN MANAGEMENT STRATEGIES AT THE PROCESSING LEVEL[a]

Technology	Aflatoxins level, µg/kg	Reduction, %	Cumulative reduction, %
Farmers stock	217	--	--
Belt separator	140	35	35
Shelling plant[b]	100	29	54
Colour sorting	30	70	86
Gravity table[b]	25	16	88
Blanching/Colour sorting	2.2	91	99
Re-colour sorting[b]	1.6	27	99

a Data taken from Park and Stoloff (1989).

b Data based on medium-category peanuts only

belt in some oil mills of South India has reduced aflatoxin levels to a great extent in oil pressed cake. This cake has been used as protein supplement in feeding programmes of school children under CARE.

Various processing procedures, such as the dry and wet milling of corn, can result in lower aflatoxin levels in the final product (Brekke et al. 1975). Germ hulls contained nearly 90% of the toxin. Similarly, when brown rice is milled, about 60-80% of the aflatoxin partitioned with bran and polish and the remaining 20-40% with the rice (Shroeder et al. 1968) (Table 2). This is mainly because aflatoxin occurrence is more in outer layers of the grain than the inner ones. However, the toxin-enriched fractions of bran and germ hulls, which are generally meant for animal feed still pose a threat of health hazard that needs to be tackled.

When peanuts are processed in the expeller mill, it has been found that cake contained about 85-90% of the aflatoxin originally present in kernels and the oil contained only 10-15% (Basappa and Sreenivasa Murthy 1974). On further processing of cake to isolate protein, the protein fraction contained 60-70% of the toxin originally present in cake and rest in the whey and residue (Basappa et al. 1972). These results (Table 2) indicate the possible affinity of aflatoxin to proteins. On studying the state of aflatoxin in raw peanut oil, it was found that 65-70% of aflatoxin went with sediment and 30-35% with the supernatant oil on centrifugation (upto 200,000 g for 20 min), indicating that the toxin is sparingly soluble in oil (Basappa and Sreenivasa Murthy 1977). Mixing 2% Fuller's earth with raw oil and centrifuging resulted in adsorption of toxin to the earth material and the clear oil contained

TABLE 2. DISTRIBUTION OF AFLATOXIN IN VARIOUS FRACTIONS OF CORN, RICE AND GROUNDNUTS. DURING MILLING/PROCESSING

Food material	Processed fractions	Aflatoxin distribution, %	Reference
Corn	**(Dry milling)**		
	Germ hull or Degermer fines	90.0	Brekke et al (1975)
	Grits	10.0	
	(Wet milling)		
	Starch	1.0	Yahl et al (1971)
	Steep liquor	39-43	
	Fibre	35-40	
	Gluten	14-17	
	Germ	6-10	
Rice	**(Brown)**		
	Rice	20-40	Schroeder et al (1968)
	Bran and polish	60-80	
Groundnuts	Cake	85-90	Basappa and Sreenivasa Murthy (1974)
	Oil	10-15	
	Cake (expeller)		
	Protein	60-70	Basappa et al (1972)
	Residual whey	30-40	
	Oil (expeller) (centrifugation 2 lakh g)		
	Sediment	65-70	Basappa and Sreenivasa Murthy (1977)
	Clear oil	30-35	
	Oil (expeller) (filtration-cum-adsorption method)		
	Clear oil	10-15	Basappa and Sreenivasa Murthy (1979)
	Sediment	85-90	

only 10-15% of what was originally present (Basappa and Sreenivasa Murthy 1977). These data are useful in predicting the levels of aflatoxin in various fractions of peanuts in oil industry.

Based on these data, filter pads to suit plate-and-frame filter press in pilot plant/oil mills were prepared and aflatoxin could be removed successfully to the extent of 85% on filtration (Table 3) (Basappa and Sreenivasa Murthy 1979; Basappa 1983). The process know-how has been given to about 16 entrepreneurs in India. A process demonstration in a commercial plant (Maharaja Refineries, Erode, Tamil Nadu) was also made successfully, using only 12 filter pads (2'x2'x1/40') in the plate-and-frame filter press, having 48 pads total capacity.

TABLE 3. COMMERCIAL SCALE DECONTAMINATION OF GROUNDNUT OIL FROM AFLATOXIN USING SPECIAL FILTER PADS

Oil sample	Aflatoxin B_1, ppb	Removal, %
Directly from expeller	130	-
Filtered through cloth (present practice)	130	0.0
Filtered through special filter pads	20	85.0

Quantity of oil handled :2 tonnes per two pads

Source :Basappa (1983)

However, the main disadvantage of the process is that the filtration rate has to be kept low especially in the initial stages for efficient adsorption of the toxin and to avoid probable breakage of pads at high pressure. The pads could be used repeatedly without damaging the integrity of the pads. It was also possible to remove aflatoxin to nearly 100% level by modifying the process, which was developed in a separate project sponsored by M/S Voltas Ltd., Bombay. This has been achieved by pre-treatment of the contaminated oil with Fuller's earth, before filtration through the pads. Shelf-life and organoleptic evaluation trials indicated that the decontaminated oil could be stored upto 4 months with better consumer acceptance than the contaminated oil (Basappa et al. 1985). The economics of the process is very favourable, as it costs about Rs. 0.1/1 of decontaminated oil as against refining process, which costs about Rs. 3.0/1. The refining process also detoxifies aflatoxin, but it is a chemical process and may destroy certain nutrients, unlike the filtration process.

The other chemisorbents such as clay, kaolin, activated carbon and zeolite can bind and remove aflatoxins from liquid media (Park and Liang 1993; Masimango et al. 1979; Miller et al. 1985). Sodium calcium ammonium silicate is also used for adsorbing aflatoxin (Smith et al. 1994). A phyllosilicate clay (Nova Sil), which effectively removed aflatoxin from contaminated peanut oil and prevented its mutagenicity and toxicity *in vitro* has been used in animal feeds to reduce levels of bio-available aflatoxins by selective chemisorption (Park and Liang 1993).

All the above physical removal methods appear to be not only cost effective, but also conform to the specific criteria laid down for decontamination procedures.

Heat inactivation

In general, aflatoxins are resistant to thermal inactivation. Thermal processing of food can only result in moderate reductions in aflatoxin levels. The sensitivity of aflatoxin to heat is governed by environmental conditions. The presence of moisture in foods may enhance degradation of hydrolyzing lactone ring at a critical moisture concentration and temperature. On the other hand, aflatoxins may be protected in foods, in part by their "binding" or association with proteins and other food constituents. The varying degrees of aflatoxin B_1 degradation noted in different foods, subjected to thermal processing demonstrate the role of moisture and other protective factors (Samarajeewa et al. 1990).

Normal food processing and preparation conditions appear to cause partial degradation under laboratory conditions (Table 4). Depending upon the temperature and excess of moisture, the degree of destruction varies from 49 to 82%, as in case of rice cooking (Rehana et al. 1979). In the presence of excess moisture, aflatoxin B_1 is believed to undergo a hydrolytic opening of the lactone ring to form a terminal carboxylic acid, which then undergoes heat driven decarboxylation (Coomes et al. 1966). In case of oil, the destruction of aflatoxin was much less (50%), in spite of heat treatment at higher temperature (Dwarakanath et al. 1969; Peers and Linsell 1975), which indicates that oil contains some protective factors against aflatoxin destruction. Heat stability of aflatoxin and absence of other factors aiding destruction may be the factors responsible for poor destruction in oil. Dry heating like roasting, frying, microwave roasting (household) at higher temperatures and time degraded comparatively less aflatoxin. These processing methods are of practical importance and are relevant in epidemiological surveys in order to assign actual levels of aflatoxin consumption through heat processed foods.

On the other hand, microwave treatment at high energy levels destroyed aflatoxin in peanuts to the extent of 95% (Luter et al. 1982; Staron et al. 1980). Similarly, autoclaving at 121°C for 4 h also destroyed aflatoxin to the extent of 95% in peanut meal (Coomes et al. 1966).

The need to use elevated temperatures and pressures for effective detoxification of contaminated foods hinders the exploration of heat treatment, as a practical means of aflatoxin detoxification. The impairment of nutritional and organoleptic qualities and doubts concerning the generation of toxic pyrolysate at elevated temperatures further

TABLE 4. DEGRADATION OF AFLATOXINS IN FOODS BY HEAT PROCESSING METHODS

Method	Conditions	Food	Degradation, %	Reference
Cooking	Steaming Puffing	Corn	50	Rehana and Basappa (1990)
Normal cooking	Steaming	Rice	49	Rehana et al (1979)
Pressure cooking	121°C, 5 min	Rice	72	Rehana et al (1979)
Autoclaving	121°C, 4h	Peanut meal	95	Coomes et al (1966)
Pressure cooking excess water	121°C, 5 min	Rice	82	-do-
Pasteurization	80°C, 45 sec	Milk 385 ppb AFM_1	64	Purchase et al (1972)
Baking	250°C, 30 min	Wheat bread	50-100	Jemmali and Lafont (1972)
Frying	150°C, 10 min	Peanut	50	Dwarakanath et al (1969)
Roasting	180°C, 30 min	Peanut kernels	80	Lee et al (1968)
Dry roasting	191°C, 15 min	Pecans	60-90	Escher et al (1973)
Microwave roasting	0.7 KW, 8.5 min	Peanuts	48-61	Pluyer et al (1987)
Microwave roasting	1.6 KW, 16 min	Peanuts	95	Luter et al (1982)
Microwave roasting	6.0 KW, 4 min	Peanuts	95	Staron et al (1980)
Spray drying	180°C,inlet 80°C,outlet	Milk 385 ppb AFM_1	87	Purchase et al (1972)
Dry heat	250°C,	Peanut oil	Partial	Peers and Linsell (1975)
Smoking	Smoking to ash	Cigarette tobacco	100	Kaminiski (1970)

AFM_1 = Aflatoxin M_1

discourage conventional heat treatment, as an effective tool for decontamination. However, industrial treatments providing high temperatures and pressures such as steam flaking, extrusion cooking, microwave cooking, popping, and spray-drying have shown promising results (Applebaum et al. 1982 ; Samarajeewa et al. 1990) and suggest redesigning them for effective decontamination of aflatoxin in foods, while achieving other objectives of thermal processing.

Irradiation

The effect of exposure to rediations like, gamma-radiation ultra-violet (UV) and visible light on aflatoxin contaminated foods is represented in

TABLE 5. DESTRUCTION OF AFLATOXIN BY RADIATIONS

Method	Food/ Medium	Destruction, %	Reference
Sunlight	Oil	99	Shantha and Sreenivasa Murthy (1977) Samarajeewa et al (1977)
	Flakes	50	Shantha et al (1986)
	Coconut oil	75	Samarajeewa et al (1977)
	Olive oil	95	Mahjoub and Bullerman (1988)
UV light	Chloroform solution	99	Arthur and Robertson (1970)
	Peanut oil	45	Shantha and Sreenivasa Murthy (1977)
Gamma-ray	Groundnut meal	0	Feuell (1966)
Infrared-light	Groundnut oil	0	Shantha and Sreenivasa Murthy (1977)
Tungston lamp	Groundnut oil	30	Shantha and Sreenivasa Murthy (1977)

Note :Free aflatoxin is more susceptible than the bound form

Table 5. The high energy gamma-radiation, which can penetrate both liquid and solid foods has not been found to be potentially useful in effective degradation of aflatoxin within the permitted dosage of 0.1 Mrad. The overall low degradation noted in foods may probably be a result of limiting moisture concentration of about 15% (Samarajeewa et al. 1990). The production of toxic degradation compounds of AFB_1 poses an additional limitation (Van Dyck et al. 1982). For foods with high moisture (Feuell 1966) treatment with hydrogen peroxide (Patel et al. 1989) combined with gamma-radiation may be a better approach, as the enhanced generation of free radicals may cause efficient reaction with aflatoxin molecules. However, the effects of free radicals on food constituents need to be examined carefully. Although gamma-radiation at 0.3-0.4 Mrad suppressed mould growth and aflatoxin formation (Toofanian and Zare 1987), the present evidences do not indicate the possibility of degrading aflatoxins in foods by it alone.

Aflatoxin B_1 is reported to be highly sensitive to UV radiation at a pH of less than 3 or greater than 10, by affecting the structure of the terminal furan ring and thus eliminating the active binding site (Lillard and Lantin 1970) (Fig. 1). A marked

reduction in contaminated peanut oil was achieved on exposure to long and short wave UV light (Shantha and Sreenivasa Murthy 1977). Aflatoxin M_1 in contaminated milk containing 0.05% peroxides after exposure to UV light at 25°C for 20 min was decreased by 89.1%, compared to a reduction of 60.7% in peroxide-free milk (Yousef and Marth 1987). Oxygen appears to enhance UV mediated free radical degradation of aflatoxin (Benaze and Kiermeier 1972). However, some of the UV degraded compounds of aflatoxin are toxic to the chick embryo (Samarajeewa et al. 1990). This indicates the limited prospects of UV radiation to degrade aflatoxin in foods.

A white light source would better facilitate breakdown of the toxic primary degradation compounds to non-toxic secondary derivatives. Visible light has a wider spectrum of wave lengths. Solar radiation, which possesses energy in the UV and visible spectra has been shown to have a greater efficiency in degrading aflatoxin in foods (Samarajeewa et al. 1990). A 14 hour exposure to sunlight destroyed 77-90% of aflatoxin B_1 added to peanut flakes, whereas only 50% of the toxin was destroyed in naturally contaminated product (Shantha et al. 1986). This is due to unbound and bound forms of aflatoxins, wherein the unbound form is more susceptible to degradation than the bound form. Further, toxins present within the food particles are protected from penetration of solar radiation. Thus, layers of oils of coconut (Samarajeewa et al. 1985), olive (Mahajoub and Bullerman 1988) exposed to sunlight were effectively decontaminated from aflatoxins. Solar-irradiated edible oil was shown to be non-toxic to experimental animals (Samarajeewa et al. 1987; Shantha and Sreenivasa Murthy 1977). However, the exposures to tungsten lamp and infrared light were very ineffective (Shantha and Sreenivasa Murthy 1977).

The above evidence indicates that the double bond in the terminal furan ring of aflatoxin B_1 is the likely site of the photodegradation (Fig. 1). Since this is also the site of aflatoxins binding to nucleic acids and proteins, aflatoxins bound through the C-8, 9 positions are not susceptible to solar degradation.

As solar radiation can penetrate thin layers of liquids like edible oils, it could be efficient means for aflatoxins decontamination. Pilot plant studies on the efficacy of solar degradation of aflatoxin in coconut oil (Samarajeewa et al. 1985) and peanut oil have indicated encouraging results. However, as the thin layers of oil are to be exposed directly to sunlight in decontamination process, commercialisation of the process has limitations in terms of inclement weather conditions.

Removal by solvents

Various solvent systems can be used for the removal of aflatoxins to the extent of 80-99% from different commodities (Table 6) with minimal effects on the quality of material decontaminated. These include 95% ethanol (Rayner et al. 1970) 80% isopropanol (Rayner and Dollear 1968), 96% methoxymethane (Aibara and Yano 1977) and hexane:acetone:water (42.1:56.5:1.4) (Goldblatt 1965), which can act as solvents for the removal. However, these would require elaborate installations with facilities for recovery of solvents and measures to prevent fire accidents. Besides, due to flavour alterations, disposal of toxic extracts and cost factors, these procedures are impractical (Park and Liang 1993). Use of 1% calcium chloride solution (Sreenivasa Murthy et al. 1965), though found to

TABLE 6. REMOVAL OF AFLATOXINS BY SOLVENT EXTRACTION

Solvent system	Conditions	Commodity	Removal, %	Reference
Acetone :Hexane:Water (42.1:56.5:1.4)	Continued column extraction 24 h	Peanut meal	90	Goldblatt (1965)
Aqueous isopropanol (80%)	6 passes of 15 min each at 60°C	Peanut and cotton seed meal	99	Rayner and Dollear (1968)
Aqueous ethanol (90%)	6 passes of 15 min each	-do-	95	Rayner et al (1970;1977)
Methoxy methane (96%)	Stainless steel, pressure proof column at RT under 6 kg/cm²	Peanut powder	99	Aibara and Yano (1977)
Sodium chloride 10% solution	80°C, 30 min	Peanut oil	85	Shantha and Sreenivasa Murthy (1975)
Calcium chloride	pH 6.5 to 7.0	Peanut meal	80	Sreenivasa Murthy et al (1965)

RT = Room Temperature

remove aflatoxins from contaminated peanut meal to the extent of 80%, the extract also removed proteins, thereby limiting its further application. Washing peanut oil with 10% sodium chloride solution removed 85% aflatoxins and found to be simple (Shantha and Sreenivasa Murthy 1975), but an additional step to dehydrate oil is required. It may be mentioned here that treatment of oil with sodium chloride is one of the steps in addition to treatments with alkali, activated charcoal and Fuller's earth, along with steam deodourization and filtration in refining of peanut oil. The refined oil is free from aflatoxins, but the refining process destroys certain vitamins in oil and is cost-effective.

Chemical methods

Structural degradation and inactivation of aflatoxins has been found to be possible by the use of chemicals such as chlorinating agents (sodium hypochlorite, chlorine dioxide and gaseous chlorine), oxidizing agents (H_2O_2, ozone), and hydrolytic agents - acids (organic and inorganic) and alkalis (sodium hydroxide, ammonium hydroxide, potassium hydroxide, etc.) (Table 7). Some of these chemicals are already in use in food industry and are less prone to consumer resistance (Samarajeewa et al. 1990). However, according to Park and Liang (1993), most of these chemicals are impractical and are particularly unsafe, because they form toxic residues or damage nutrient content, flavour, odour, texture or functional properties of the products, although the involved processes can destroy aflatoxin efficiently in the range of 85-100% not only in meals, but also in whole grains.

Ammoniation

Among various chemical processes, ammoniation process has been extensively worked out and largely accepted in spite of certain resulting nutritional losses. Liquor ammonia, gaseous ammonia, *in situ* liberation of ammonia by reaction of urea and urease as well as reacting with monomethylamine and lime have been used (Park and Liang 1993). The noteworthy losses affecting the nutritional quality of ammoniated animal feeds include 10% reduction of protein quality (Thiesen

TABLE 7. CHEMICAL METHODS OF DETOXIFICATION OF AFLATOXINS

Chemical	Conditions	Commodity	Destruction, %	Reference
Hydrogen peroxide	Alkaline 10% slurry 6% H_2O_2, 30 min at 80°C	Ground meal (1200 ppb)	97 (Toxin-free protein isolated)	Sreenivasa Murthy et al (1967)
Ozone	2 h at 100°C	Cottonseed meal (22% moisture)	100	
	1 h at 100°C	Groundnut (30 moisture)	78	Dwarakanath et al (1968)
Ammonia*	40 psig at 100°C 4% NH_4OH, 30 min	Cottonseed meal (400 ppb) (14% moisture)	99	Park et al (1984)
	Ambient 100°C, 1 h NH_4OH	Cottonseed	99	Mashaly et al (1983)
	NH_3 45 psig 93°C,-15 min	Peanuts (10% moisture) (121 ppb)	99	Gardner et al (1971)
	NH_3 20 psig 93-102°C, 1 h	Peanuts meal ((70 ppb)) (10-15% moisture)	97	Masri et al (1969)
Urea + urease	20% urea 2% soybean flour Sunlight 14 h at RT	Peanut flakes 50% moisture (1000 ppb)	85	Shantha et al (1986)
Sodium hypochlorite	0.2 to 5% or 15 mg Cl_2 gas per 100 mg AFB_1 or spiked foods	Spiked foods	100	Sen et al (1988)
		Prepressed peanut meal	50	Mann et al (1970)
Sodium bisulphite	0.4%, 5 min	Milk AFM_1	45	Doyle et al (1982)

* More than 40 research papers published.

RT = Room Temperature, AFM_1 = Aflatoxin M_1; AFB_1 = Aflatoxin B_1

1977), significant drops in lysine and methionine contents (Conkerton et al. 1980; Piva et al.1981) and an irreversible reduction in the degree of unsaturation in lipids (Black et al. 1978). The presence of residual toxicity arising from hydrolyzed products (Schroeder et al. 1985), along with the potential for covalent bonding of aflatoxin B_1 to proteins (Brekke et al. 1979) and the losses in nutritional quality make the ammoniation treatment processes seem less acceptable. However, under defined conditions, the treatment of grains (peanuts, cottonseed, corn) and their meals with ammonia appears to be a commercially viable approach to detoxification of aflatoxins to the extent of 99%, particularly for feed purposes (Park and Liang 1993).

Ammonia degradation proceeds through hydrolysis of lactone ring, and is followed by decarboxylation to produce non-toxic compounds (Fig. 2). Due to severity of aflatoxin contamination in selected agricultural commodities, in various

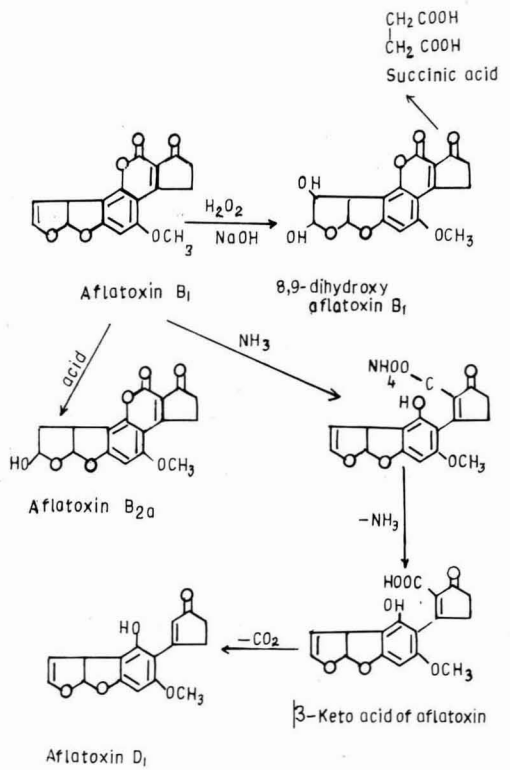


Fig.2. Chemical degradation of aflatoxin B_1 by H_2O_2 (Van Dorp et al. 1963), ammonia (Lee et al. 1974) and acid (Dutton and Heathcote 1968)

TABLE 8. PARAMETERS AND APPLICATIONS OF AFLATOXIN DECONTAMINATION PROCEDURES INVOLVING AMMONIATION[a]

	High pressure high temperature procedure	Ambient pressure atmospheric temperature procedure
Ammonia level	0.2-2.0%	1-5%
Pressure	35-50 psi	Atmospheric
Temperature	80-120°C	Ambient
Duration	20-60 min	14-21 days
Moisture, %	12-16	12-16
Commodities	Whole cottonseed, cottonseed meal, peanut meal, corn	Whole cottonseed, corn
Applications	Feed mill	Farm

a Park et al (1988)

locales, specific decontamination processes have been approved and put into use (Park 1993; Park et al. 1988). Ammoniation of seeds is authorised by Food and Drug Administration of the USA. In USA, the States of Arizona, California and Texas permit the ammoniation of cottonseed products. Mexico and South Africa have approved the procedure for use in corn. Ammonia-treated peanut meal is widely used in animal feeds in Europe and elsewhere. Consequently, the process is routinely used in France, Senegal, Sudan and Brazil. Several member countries of the European Community import ammonia-treated peanut meal on a regular basis.

Ammonia treatment processes for feed mill level and at farm level have been worked out intensively by Park et al (1988). Low ammonia concentration (0.2 to 2.0%) at high pressure (35-50 psi) and high temperature (80-120°C) would require less time (20-60 min), as compared to high ammonia concentration (1-5%) at atmospheric pressure. Ambient conditions needed more time (14-21 days) for treating feed materials, containing 12-16% moisture (Park et al. 1988) at feed mill level and farm level, respectively (Table 8).

Pathological and histopathological examinations conducted on experimental and farm animals, fed on ammoniated meals did not show any signs of aflatoxicosis. Also, there were no differences in egg production and immunological responses in poultry (Samarajeewa et al. 1990).

Treatment with sodium bisulphite

Sodium bisulphite can react with aflatoxins B_1, G_1, M_1 and aflatoxicol to form water soluble products, depending on its concentration, incubation

temperature and time (Hagler et al. 1982). The addition of 0.04 g of potassium bisulphite per 10 ml milk resulted in a 45% reduction in the level of aflatoxin M_1 after 5 min (Doyle et al. 1982). Potassium metabisulphite is a common food preservative and does not appear to pose any consumer resistance problem.

Degradation by acids

Conversion of aflatoxin B_1 to aflatoxin B_{2a} (Fig. 2) was reported in the metabolic system, using rat liver microsomal enzymes (Patterson and Roberts 1970). As the treatment with strong acids may affect food quality, and the resultant aflatoxin B_{2a} still possesses toxicity, the use of strong acids to degrade aflatoxins in foods is discouraged. However, complete degradation of aflatoxins without the production of the toxic derivatives during manufacture of hydrolyzed peanut meals with 3N HCl at elevated temperature and pressure for 12h (Dutton and Williams 1988) and the partial reduction of toxicity noted during fermentation of yoghurt (Megalla and Hafez 1982) and silage (Hafez and Megalla 1982), probably resulting from acidity, encourage further exploration of acid treatment for decontamination of aflatoxins in specific foods.

Degradation by oxidizing agents

Both hydrogen peroxide and ozone are the high potential aflatoxin degrading agents. Different treatments with hydrogen peroxide, which have been reported to almost totally degrade aflatoxin B_1 include 0.5% H_2O_2 at pH 4 in peanut protein isolates (Rhee et al. 1976), 3% in corn (Chakrabarti 1981) and 6% at pH 9.5 for 30 min in peanut meal (Sreenivasa Murthy et al. 1967). These treatments are claimed to cause only mild effects on food quality as supported by the unaltered protein efficiency values. Aflatoxin B_1 is observed to degrade to succinic acid through its dihydroxy derivative, when it is treated with a mixture of hydrogen peroxide and sodium hydroxide (Van Dorp et al. 1963) (Fig. 2). Aflatoxin-free protein isolate was also made and used in miltone preparations at the Central Food Technological Research Institute (CFTRI), Mysore. A world patent on the process of preparation of aflatoxin-free protein was also taken by CFTRI, Mysore.

Ozone effectively degraded aflatoxin B_1 and aflatoxin G_1 in 4% dimethyl sulfoxide at room temperature within a few minutes. The treated products were confirmed to be non-toxic by various methods. It is reported to reduce aflatoxin B_1 levels by 91% in cottonseed meal, containing 22% moisture after treatment at 100°C for 2 h. However, with peanut meal (30% moisture) the reduction was only 78% after exposure to ozone for 1 h (Dollear et al. 1968). Further exploration of this oxidizing agent has not been made.

Microbiological methods

Biologically, aflatoxin B_1 is degraded to several less toxic metabolic derivatives by microbial and animal systems. Various derivatives of aflatoxin B_1 and their relative toxicity and mutagenicity to *Salmonella typhimurium* as compared to aflatoxin B_1 have been reviewed by Samarajeewa et al (1990).

Microbiological inactivation and fermentation have been studied as means of degrading and removing aflatoxins (Mann 1977). *Flavobacterium aurantiacum* (Ciegler et al. 1966; Lillehoj et al. 1971; Hao and Brackett 1988) and selected acid producing moulds have been successfully used to remove aflatoxins from liquid media. Transformation of aflatoxin B_1 to less toxic B_{2a} has been observed in case of fermented foods by *Lactobacillus delbrueckii* (Maing et al. 1973) and yoghurt cultures (Megalla and Hafez 1982; Hafez and Megalla 1982; Rasic et al. 1991) (Fig. 3). It was postulated that the reduction in aflatoxin levels was a result of acid production and subsequent transformation of aflatoxin B_1 to aflatoxin B_{2a}. Transformation of aflatoxin B_1 to aflatoxical (18 times less toxic than aflatoxin B_1) was observed in cases of *Corynebacterium rubrum* (Mann and Rehm 1976) and several species of moulds, belonging to the

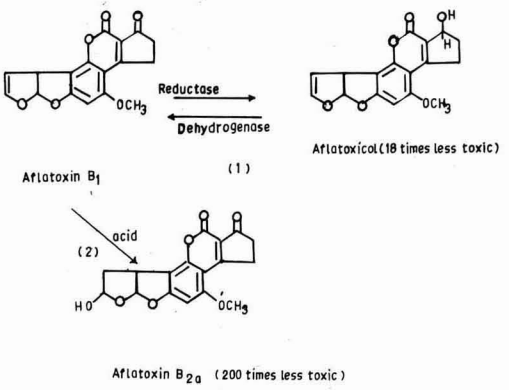


Fig.3. Microbial conversion of aflatoxin B_1 to less toxic aflatoxins (1) *Corynebacterium rubrum* (Mann and Rehm 1976; Hsein et al. 1974; Lee et al. 1981) and *Rhizopus* sp (Mitsuo et al. 1991); (2) *Lactobacillus delbrueckii* (Maing et al. 1973)

TABLE 9. MICROBIOLOGICAL DEGRADATION OF AFLATOXINS

Organism	Medium	Action/ destruction	Reference
Flavobacterium aurantiacum NRRL-B 184	Aqueous	Removal	Ciegler et al (1966)
-do-	Milk, AFM_1 9.9 µg at 30°C, 4 min	Complete removal	Lillehoj et al (1971)
Tetrahymena pyriformis	Liquid	Uptake	Teunisson and Robertson (1967)
Lactobacillus delbrueckii	Soy sauce fermentation	AFB_1 AFB_{2a}	Maing et al (1973)
Yoghurt cultures (Lactobacilli and yeasts)	Milk 1400 ug/kg AFB_1	90% AFB_1 AFB_{2a}	Rasic et al (1991) Megalla and Hafez (1982)
Corynebacterium rubrum	Liquid	AFB_1 Aflatoxicol	Mann and Rehm (1976)
Rhizopus sp.	Liquid	AFB_1 Aflatoxicol	Mitsuo et al (1990;1991)
Rhizopus stolonifer	Liquid	AFB_1 Aflatoxicol	Detroy and Hasseltine (1969)
Helminthosporium sativum			Robertson
Mucor alternans			et al (1970)
Absidia repens			
Doctylum denroides			
Mucor griseo-cyanis			

AFB_1 - Aflatoxin B_1; AFM_1= Aflatoxin M_1; AFB_{2a} = Aflatoxin B_{2a}

genera *Aspergillus, Mucor. Helminthosporium, Rhizopus, Absidia, Trichoderma* and *Doctylum* (Detroy and Hesseltine 1969; Robertson et al. 1970; Mitsuo et al. 1990) (Fig. 3). An enzyme responsible for conversion (reversible) of aflatoxin B_1 to aflatoxicol has been isolated, partially purified and characterised (Mitsuo et al. 1991). Table 9 represents data on the above aspects. However, these microbial transformations have not yet been put into practical applications.

The use of microorganism(s) for degradation of aflatoxins in food or feed may have certain disadvantages, in that the organism would not only utilise the food for its growth, but may also elaborate undesirable compounds. However, the use of selected lactic acid bacteria in food fermentation like yoghurt and fermented milk may be of advantage, In general, such organisms should have GRAS (Generally Regarded As Safe) status. In future, use of isolated aflatoxin-degrading enzymes from such organisms may find application especially in liquid foods.

Conclusions

As per the specific criteria that have been established for evaluating the acceptance of a given detoxification process, it is not possible to fix a single set of physical or chemical treatment conditions for removing/degrading aflatoxins in all types of foods and feeds. The nature of food constituents, environmental conditions such as moisture content, pressure and temperature as well as the processing methods or storage conditions of the foods, all affect the efficiency of aflatoxin removal. The understanding of mechanisms of aflatoxin detoxification by physical and chemical methods will enable establishment of the combined physical, chemical treatment methods to effectively decontaminate aflatoxins in foods. Such treatment methods are expected to be cost effective and minimally deleterious to food constituents.

Some of the physical methods adopted for removal of aflatoxins from peanut and peanut oil appear to be promising on an industrial scale. Ammoniation process for detoxification of aflatoxins in oilseed meals has been widely accepted on a commercial scale, although certain nutrients are destroyed in the process.

Microbial aflatoxin detoxification methods that can transform aflatoxin B_1 to less toxic derivatives are promising and need to be further investigated and incorporated with the above methods.

Besides the above, biotechnological approaches such as replacing aflatoxigenic strains with non-aflatoxigenic ones in the field, incorporation of antifungal genes into crop plants, breeding resistant varieties and prevention of aflatoxin biosynthesis are to be developed and these could perhaps form the long-lasting solutions to the aflatoxin problem.

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Fructose Syrup and Ethanol from Deseeded Carob Pod

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Ripe deseeded carob pod (fruit of *Ceratonia siliqua* L. tree) of 'g-l' cretan carob variety is rich in water-soluble sugars (54.7% on dry weight). The profile of these sugars, in an aqueous carob extract, was sucrose, 77%; fructose, 13.9% and glucose, 9.1% on the basis of the total water-soluble sugars. A liquor containing fructose (10.49% w/v) and ethanol (4.75% v/v) was obtained by selective microbial fermentation of glucose by a glucophile yeast strain of *Saccharomyces cerevisiae* (Sace 1), after 100 h fermentation of aqueous carob extract.

Keywords : Carob beans, Fructose production, Carob sugars, Ethanol, Selective microbial fermentation.

Fructose is the naturally occurring sweetest nutritional sweetener (Zittan 1981). This sugar is 1.5-1.7 times sweeter (Shallenberger 1963), than the common refined sugar (sucrose). Fructose is absorbed from the gastro-intestinal tract more slowly than glucose and hence, reduces the feeling of hunger (Reynolds 1993). It has been employed as an alternative to glucose in parenteral nutrition (Reynolds 1993). Although there is a disagreement with regard to dependance of fructose metabolism on insulin, this sugar might be useful as a sweetener for diabetics (Anon 1980). British Diabetic Association has advised that the daily intake of fructose should be limited to 25 g (Reynolds 1993). So, fructose is used in limited quantities as a source of carbohydrate for diabetic.

Since fructose has become acceptable to dietary health schemes, the requirement for this sugar has increased (Stavropoulos 1985). Therefore, several studies have been carried out to produce fructose at lower cost (Smith 1988; Suntinanalert et al. 1986). Millions of tonnes of high-fructose syrup (approximately fructose:glucose=1:1) are produced annually by partial isomerization of glucose derived from starch, using immobilized glucose isomerase (Smith 1988). From this equilibrium sugar mixture, fructose itself can be produced by a typical method of fructose enrichment, using cation exchange resin to separate fructose from other sugars (Goldstein 1987). Suntinanalert et al (1986) reported that two mutants of *Zymomonas mobilis*, unable to utilize fructose as a sole carbon source, were able to convert sucrose into fructose and glucose, and then ferment only the glucose to ethanol.

The commercial price of fructose is higher than that of sucrose (Statistical Year book of Greece 1988-1993). Fructose extraction from carob bean (fruit of *Ceratonia siliqua* L.) will be of commercial importance in carob producing countries, for example Greece, which imports fructose (Stavropoulos 1985).

The carob tree grows naturally on barren soils, often unproductive for any other type of crop, in most warm regions of the Mediterranean countries, mainly near the coasts (Mitrakos 1968). Carob trees also occur in Rhodesia, parts of the USA, Australia, South America, India, Philippines and other regions, with similar climate to that of Mediterranean countries (Mitrakos 1968; Imrie and Vlitos 1975). The carob bean is a major Greek agricultural product of low commercial price (Kalaitzakis 1979). Greece is the fourth largest carob bean producing country in the world (Marakis 1980).

Carob fruit consists of 63-90% pod and 10-37% seeds, depending on the carob variety (Marakis et al. 1987; 1994). The ripe husk (deseeded carob pod), although rich in water-soluble sugars (upto 60.5%; mainly sucrose, 63-70% on total sugars) (Imrie and Vlitos 1975; Marakis 1992), has a very low crude protein (3-5%) and contains high levels of total tannins (6-27%) (Marakis and Karagouni 1985; Wurch et al. 1984; Wurch 1987; Marakis 1980), mainly the condensed type (Marakis et al. 1993), which minimize the nutritional value of carob pod (Vohra et al. 1966; Tamir and Alumot 1970). Although several studies have been carried out on the improvement of the nutritional value of carob husk (Drouliscos et al. 1976; Marakis 1986; Marakis and Diamantoglou 1990), this agricultural product has not yet been commercially exploited. Therefore, its commercial price still remains quite low.

Deseeded carob pod can be regarded as relatively rich in fructose, considering the fact that

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combined sucrose and free fructose content of the pod is about 28% on carob husk dry weight basis (Marakis and Marakis 1993). Hence, carob husk could be used for fructose production.

This paper describes fructose syrup and ethanol production by selective microbial fermentation of carob glucose by a glucophile strain of *Saccharomyces cerevisiae* (Sace 1), cultured in aqueous carob extract.

Materials and Methods

*Microorganism:*A glucophile strain of *Saccharomyces cerevisiae* (Sace 1), isolated from fermenting must of 'liatiko' variety of the grapes of Crete (Marakis 1986), was used.

Preparation of aqueous carob extract; In this study, the 'g-1' cretan carob variety was chosen from among 15 Greek carob varieties, which have been recognized so far (Marakis et al. 1987, 1994). Two kg of chopped and deseeded carob pod was mixed with 4 l potable tap water and autoclaved for 30 min at 121°C. The slurry was passed through a cheese cloth and the extracted carob pod was resuspended in 2 l water and autoclaved once again. Then, the two extracts were mixed. Carob extract tannins were removed, using the ultra filtration module (Advancierte Purifications and Membran-System Co. GmbH, Julich, Germany). The resulting detannined liquor, containing 20.3% (w/v) total sugars, was used for the fructose and ethanol production. The medium (detannined carob extract), after adjusting its pH to 4.5, was sterilized by autoclaving (15 min, 121°C).

Fermentation procedure; Fermentation was carried out in 5 l Erlenmeyer flasks, containing 4l detannined carob extract, enriched with ammonium sulphate and sodium dihydrogen phosphate to 0.3 and 0.1%, respectively. These flasks, after inoculation with 10^2 yeast cells/ml of medium, were statically incubated at 25°C for 100 h. Each experiment was run in duplicate (5 flasks per run). Samples were taken after 24 h fermentation and sampling was continued until glucose was absent in the culture filtrates. After fermentation, the fermented medium was allowed to settle for 24 h at 10°C. The clear supernatant solution containing fructose and alcohol was decanted, using a plastic tube, into a distillation apparatus for recovery of ethanol.

Analytical methods: Ethanol and total sugars were determined by the methods of Amerine and Ough (1980) and Dubois et al (1956), respectively. Sugar profile determination was done by gas-liquid chromatography procedure, as described elsewhere (Marakis 1992). Sugars were first converted to oximes (STOX oximes-PIERCE) and then silylated to TMS ethers (N-Trimethylsilylimidazole-PIERCE) (Baumgartnet et al. 1986; Pierce 1989). A Perkin-Elmer Sigma 2000 gas-liquid chromatograph, equipped with a flame ionization detector was used. The glass column (2m x 2mm) contained 3% OV-7 + 1.5% OV-22 (Chrompack, the Netherlands) chromosorb WDMCS 80-100 mesh. Chromatographic conditions used include oven temperature 175°C x 0.5 min ▲ 4°C/min ▲ 290°C x 7 min, injector temperature 270°C detector temperature 300°C and N_2 flow rate 20 ml/min.

Results and Discussion

*Water-soluble carob sugars:*A clear decolourised and odourless carob syrup of good quality was obtained after removal of tannins by a microfiltration procedure, which is a low cost intensive procedure, as compared to the high cost chemical processes (Vuataz et al. 1959) or other methods based on expensive ion exchange resins (unpublished data, Greek sugar industry 'ZAAE', private communication).

Deseeded carob pod of 'g-1' cretan carob variety is among the richest Greek carob varieties in terms of total water-soluble sugars (54.7%) and contains three individual sugars : sucrose, 42.1%; fructose, 7.6% and glucose, 5.0% on husk dry weight basis. In the most common cretan carob variety 'g-3', which contains 58.9% total water-soluble sugars, Marakis (1992) found a different sugar profile : sucrose, 37%; fructose, 9.1% glucose, 7.4% maltose, 3.3% and trehalose, 2.1%, on dry weight basis.

The aqueous carob extract of 'g-1' variety used in present studies, contained: sucrose, 77%; fructose, 13.9% and glucose, 9.1% on total water-soluble

TABLE 1. TOTAL AND INDIVIDUAL SUGARS (g/l) AND ALCOHOL CONCENTRATION (%, v/v) MEASURED DURING THE FERMENTATION OF DETANNINED AQUEOUS CAROB EXTRACT.

Fermentation time, h	Sugar concentration, g/l				Alcohol concentration, %, v/v
	Total	Sucrose	Fructose	Glucose	
0	203.0	156.3	28.2	18.5	0.00
24	197.4	148.2	32.1	16.9	0.28
48	190.0	132.9	39.4	17.5	0.61
72	151.4	79.8	65.9	6.0	2.42
90	123.1	32.0	89.4	1.9	3.82
100	105.1	ND*	104.9	ND*	4.75

ND* = Not detected

sugar basis (Table 1). Imrie and Vlitos (1975) detemined seven individual sugars (sucrose, 60%; fructose+ glucose, 30%; xylose + ketose + ceretose + primverose, 10% on total sugars) in aqueous extract from carob pods from Cyprus. Kalaitzakis (1979) determined five sugars: sucrose, glucose, fructose and two non-identified sugars in the husk of a carob variety, which was not specified.

This variation in water-soluble sugars may be due to the different methods of extraction and sugar determination or the use of different carob varieties. According to the data on carob sugar profiles available so far, sucrose is the major sugar constituent (more than 60% on the basis of total sugars).

*Fermentation of detannined aqueous carob extract:*A number of wine yeasts show perferential fermentation of glucose in a mixture of glucose and fructose, e.g., as in grape must (Minarik et al. 1977). The presently used wine yeast strain, *S. Cerevisiae* (Sace 1), belonging to the above category, should be lacking a specific kinase, responsible for the phosphorylation of the fructose.

The presence of glucose in carob extract did not allow the enzyme synthesis or enzymatic activation of the fructose fermentation, as it did in case of other microorganisms, e.g., *Allomyces macrogynus* (Cochrane 1958). S. *cerevisiae* (Sace 1), under static fermentation conditions, hydrolyzes sucrose to glucose and fructose with simultaneous preferential fermentation of glucose to ethanol, thereby leading to fructose accumulation (Table 1). In this way, a liquor containing only fructose (104.9 g/l) and ethanol (4.75% v/v) was obtained after 100 h fermentation. The same yeast strain, in aqueous carob extract of the 'g-3' cretan variety, gave a lightly coloured liquor containing (g/l): fructose, 117.3; glucose, 0.2; maltose, 0.2 and ethanol, 5.8% (v/v) after 120 h fermentation (Marakis and Marakis 1993). Therefore, the carob extract of 'g-1' variety is better than that of 'g-3' variety for fructose syrup production, because i) the fermentation time of 'g-1' carob extract is 20 h shorter than that of the 'g-3' carob extract and ii) the 'g-1' fermented liquor contained only fructose and ethanol.

The fermented liquor of 'g-1' variety, after removal of the ethanol, contained 11.3% (w/v) fructose. Taking into account the fact that fructose is 1.7 times sweeter than sucrose (Shallenberger 1963), the above fructose syrup is equivalent to sucrose syrup, containing 19% (w/v) sucrose. This fructose syrup should find many applications in confectionery, diabetic food and other industries. On the other hand, the fermented product could also be used as a low alcoholic beverage.

Fructose syrup production from carob pods should increase the commercial value of this cheaper agricultural product, thereby resulting in the stimulation of interest of the carob growers for the expansion of carob plantations on barren soils, which areunproductive for any other type of crop. Hence, an upgrading of the environment will take place, because barren, rocky and dry regions, for example in Southern Greece, where the carob tree is naturally distributed, will become green and attractive.

Conclusions

The detannined aqueous non-fermented carob extract, rich in sucrose, can be used, for fructose production. Such a syrup may be suitable for several industrial uses, i.e., sweetener for confectionery products, tin-preserved fruits, pullulan polysaccharide production, sucrose production and other applications (Marakis 1992). An econometric, computeried model need to be constructed on the basis of all the other possible alternatives for the complete utilization of the carob beans, i.e., locust gum (galactomannans) from seeds, SCP production, sucrose crystallization from husk, and other potential utilization avenues, which may show the feasibility of fructose production from carob pods.

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Composition of Some Commercially Available Biscuits

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Twenty five brands of glucose biscuits and seven other varieties of biscuits were analysed for moisture, fat, proteins, total sugars, ash, peroxide value, free fatty acids, sodium, potassium, calcium, iron, manganese, copper and zinc concentrations. Total sugars in glucose biscuits varied from 23.25-31.25% whereas in other varieties, it varied from 17.26-40.42%. Protein content varied between 5.46-8.9%. The concentrations of sodium, potassium, iron and calcium ranged between 800-4950 mg/kg, 450-1720 mg/kg, 38-230 mg/kg and 120-1800 mg/kg, respectively. Differen-ces in the levels of copper, manganese and zinc were not large, ranging between 1-7 mg/kg, 3.5-10.4 mg/kg and 8.2-25.5 mg/kg respectively. Peroxide value and free fatty acids are known to change during storage. The brands having very low fat levels (<5%) invariably had very high peroxide and free fatty acid values. Total fat contents in various biscuits ranged between 1 and 24.6%, while total energy values ranged between 365 and 501 K calories.

Keywords: Glucose and other biscuits, Proximate composition, Mineral composition, Energy value, Peroxide and free fatty acid values.

Biscuits are one of the most popular and widely consumed processed food products in India. Though annual per capita consumption of biscuits is quite low (900 g) as compared to 15-20 kg in developed countries, it has almost doubled from 462 g to 900 g during 1978-92. Total annual production of biscuits both in organised and unorganised sectors is estimated to be around 10 lakh tonnes and annual growth rate is around 7% for general varieties and 20-40% for cream and fancy varieties of biscuits (Agarwal 1994). Though a large number of biscuits varieties varying in shape, size, composition, cost, shelf-life and packing are marketed, it is rather surprising that no data are available on the nutrient composition of various biscuit varieties produced in the country. Therefore, a study on the nutrient composition of some of the popular brands of biscuits available in Mysore market was undertaken, and the results are reported in this paper.

Materials and Methods

Two packets of each of 25 brands of sweet biscuits (glucose type), and seven other varieties were procured from local market within 2 months from the date of manufacture. The contents of the two packets were mixed and powdered in a dry glass mortar and the powdered material was stored in a hermetically sealed paper (42 GSM)-aluminium foil (0.02 mm)-polyethylene (37.5μ) laminate packs at 0°C, till analysed. Moisture, total proteins, petroleum ether extractable fat, total ash, total soluble sugars were analysed by methods reported earlier (Arya and Thakur 1986).

Sodium, potassium, calcium, iron, manganese, zinc and copper were determined by atomic absorption spectrophotometer (Model AA 670 Shimadzu Kyoto, Japan) after wet digestion with sulphuric acid and nitric acid as per Jacob (1958).

Energy values were calculated by using factors of 4, 9 and 4 for total carbohydrates, fat and proteins, respectively.

Results and Discussion

Proximate composition of 25 brands of sweet glucose variety biscuits is given in Table 1. Maximum variation was found in moisture and fat contents, which ranged between 2.47-8.75% and 1.04-14.82%, respectively. Moisture contents in biscuits are mainly governed by the packaging and turnover. The brands which are more popular and therefore have faster turnover invariably, had lower moisture contents. Almost all the brands of glucose biscuits are packed in waxed paper packs and therefore higher moisture in some of the brands may have resulted, due to improper sealing of waxed paper packs. A low moisture content (<4%) is desirable in maintaining crispness in biscuits.

Fat contents in majority of the glucose biscuits ranged between 7-14%, but in a few brands, the values were found to be less than 5% and these brands had energy values less than 380 Kcal/100 g. Fat contents in more popular brands were above 10% and their total energy values ranged between 430-455 Kcal/100 g. Energy values in glucose biscuits ranged between 365-455 Kcal/100 g with a coefficient of variation (C.V.) of 6.5%. Besides influencing energy value, fat contents influence crispness and texture in biscuits. A low fat content is invariably associated with hard texture in sweet glucose biscuits Fat contents in other varieties of biscuits especially cream varieties were much higher (12-24%) and energy values ranged between

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TABLE 1. PROXIMATE COMPOSITION OF GLUCOSE BISCUITS (g/100 g)

,Brand@	Moisture,	Fat,	Proteins,	Total soluble sugars,	Ash,	Energy, Kcal
	g	g	g	g	g	
1	2.47	13.92	7.80	26.79	1.05	455.52
2	3.14	14.05	7.25	25.18	1.10	453.29
3	2.33	11.93	6.32	24.93	0.90	446.73
4	2.81	14.16	7.04	23.25	1.40	453.96
5	3.03	7.87	6.05	28.67	1.14	422.67
6	3.91	8.62	7.81	26.68	1.01	423.42
7	3.80	9.85	7.40	29.25	1.22	429.17
8	4.12	14.82	7.55	27.47	1.20	452.91
9	5.60	12.08	8.11	29.34	1.15	433.40
10	6.21	1.58	7.80	29.01	1.18	378.94
11	3.42	12.10	7.86	29.30	1.20	442.02
12	5.06	11.78	7.13	29.76	1.20	433.86
13	6.46	7.02	7.34	28.67	1.18	404.54
14	6.80	1.61	7.12	27.65	1.05	376.65
15	4.51	9.78	7.02	28.61	0.98	426.94
16	4.98	7.21	6.48	29.12	1.18	411.41
17	5.57	8.42	6.91	28.86	1.09	415.46
18	5.15	12.74	7.90	26.00	0.95	439.30
19	5.00	9.60	7.90	25.40	1.07	423.72
20	4.80	10.50	7.80	26.30	1.05	429.10
21	6.58	12.20	8.90	27.10	1.30	429.48
22	8.75	1.05	7.60	30.00	1.21	365.41
23	7.00	1.04	7.10	31.25	1.20	372.40
24	7.30	5.12	8.20	24.30	1.15	391.80
25	6.36	1.83	7.74	29.56	1.20	378.91
Range	2.47 -8.75	1.04-14.82	6.05-8.90	23.25-31.25	0.90-1.40	365.41-455.52
Mean	5.01	8.84	7.33	27.70	1.13	419.62
Coefficient of variation (%)	32.7	49.8	12.3	7.2	9.6	6.5

@ Brand names withheld.

440-501 Kcal/100 g (Table 2). In biscuit manufacture, vegetable shortening rich in saturated fatty acids is added to improve the storage stability of biscuits by dilution of naturally occurring highly unsaturated wheat flour lipids. Previously also, addition of hydrogenated vegetable oils in

TABLE 2. PROXIMATE COMPOSITION OF OTHER VARIETIES OF BISCUITS (g/100 g)

Varieties@	Moisture,	Fat,	Proteins,	Total soluble sugars,	Ash,	Energy, Kcal
	g	g	g	g	g	
A Chocolate	4.84	23.68	7.42	28.06	1.30	493.84
B Cashew	4.22	24.66	7.41	24.78	1.35	501.02
C Marie	4.08	12.59	7.66	19.96	1.45	440.83
D Pineapple cream	4.20	20.69	5.46	40.42	1.05	482.45
E Orange cream	4.60	20.38	5.66	40.06	1.12	479.02
F Coconut cookies	4.80	19.77	7.44	22.40	1.25	474.65
G Krackjack	3.93	16.69	7.40	17.26	1.70	460.93
Range	3.93-4.84	12.59-24.66	5.46-7.66	17.26-40.42	1.05-1.70	440.83-501.08
Mean	4.38	19.78	6.92	27.56	1.32	476.11
Coefficient of variation (%)	7.7	19.2	12.5	31.3	15.2	3.9

@ Brand name of biscuit withheld.

convenience foods was found to improve their storage stability (Bhatia et al. 1969; Patki and Arya 1994).

Protein contents in sweet glucose biscuits ranged from 6.05-8.9% with a mean of 7.33% except cream filled varieties (D and E) which had protein contents less than 6%.

The total soluble sugars in sweet glucose biscuits ranged from 23.25-31-25% with a mean of 27.70%, whereas in other varieties total soluble sugars ranged between 17.26-40.42%. As expected, cream filled varieties contained higher levels of total soluble sugars (40%), while type 'G' variety contained least total soluble sugar (17.26%). Total ash contents in biscuits reflect the quality of wheat flour and the level of salt used in the biscuit manufacture. In sweet glucose variety, total ash contents varied from 0.90-1.40%, whereas in other varieties, it ranged between 1.05-1.70%. Variations in total ash contents in various brands were not large (C.V., 9.6%).

The concentrations of sodium, potassium, calcium, iron, manganese, copper and zinc in glucose and other biscuits are given in Table 3 and 4, respectively. Despite the fact that sodium chloride is added during biscuit manufacture, the variation in sodium concentrations in glucose biscuits were not large (C.V., 11.9%). Total sodium contents varied between 2100-3580 mg/kg with a mean of 2878 mg/kg. On the other hand, variations were much higher (800-4950 mg/kg) in other varieties of biscuits. Surprisingly, largest variations were found in calcium (120-1800 mg/kg, C.V., 84.6%) and iron (38-230 mg/kg, C.V., 52.6%) contents in glucose biscuits and this may have occurred due to fortification of glucose biscuits with calcium and iron or by the use of milk solids by some of the companies. Calcium levels in Indian biscuits were relatively lower than Australian biscuits (Makinson et al. 1989). Copper contents in Indian biscuits varied between 1.0-7.1 mg/kg with a mean of 3.24 mg/kg and varied considerably (C.V., 41.6%). The

TABLE 3. MINERAL COMPOSITION OF GLUCOSE BISCUITS (mg/kg)

Brand@	Sodium	Potassium	Calcuium	Iron	Manganese	Copper	Zinc
1	2700	1400	310	100	10.4	4.2	18.5
2	2360	1340	320	100	9.4	4.2	18.2
3	2900	1350	530	130	8.6	2.8	25.5
4	2100	450	1800	230	3.5	1.0	8.2
5	2800	1000	380	43	5.5	2.1	18.5
6	3000	1500	360	100	10.0	3.3	16.3
7	3200	1370	310	72	8.2	3.1	16.6
8	3500	1200	390	72	6.6	2.6	12.4
9	2510	1180	380	78	7.1	3.1	12.6
10	2700	1320	310	52	8.8	2.4	16.8
11	3100	1400	480	69	7.1	2.5	13.7
12	2475	1220	410	80	6.2	2.1	12.8
13	2700	1420	370	45	4.8	1.8	14.2
14	3000	1325	320	38	7.2	2.4	16.8
15	2680	1275	330	85	5.8	2.1	14.6
16	3200	1340	300	62	8.7	3.3	12.9
17	2640	1270	300	75	6.6	2.8	14.2
18	3250	1120	120	62	5.2	6.0	12.5
19	2725	1570	1120	70	6.8	4.2	14.2
20	3000	1500	129	65	5.8	5.1	13.3
21	3580	1470	1400	40	7.2	7.1	19.5
22	3100	1230	230	55	6.2	4.0	16.5
23	3200	1275	210	40	5.8	3.4	14.8
24	2950	1350	280	40	75.	3.6	13.2
25	2600	1375	320	51	5.8	1.8	15.6
Range	2100-3580	450-1570	120-1800	38-230	3.5-10.4	1.0-7.1	8.1-25.5
Mean	2878.8	1290	456.3	74.1	7.0	3.2	15.2
Coefficient of variation (%)	11.9	16.4	84.6	52.6	23.4	41.6	21.1

@ Brand name of the biscuit withheld

TABLE 4. MINERAL COMPOSITION OF OTHER VARIETIES OF BISCUITS (mg/kg)

Varieties	Sodium	Potassium	Calcium	Iron	Manganese	Copper	Zinc
A	3100	1620	460	105	6.5	4.8	22.1
B	3000	1720	560	128	9.8	6.0	25.5
C	3100	1650	330	95	9.7	4.6	19.3
D	800	1160	300	116	10.5	4.8	21.0
E	1600	1230	700	110	8.1	4.1	21.0
F	2500	1548	360	120	9.6	5.6	16.5
G	4950	1340	650	110	9.5	4.0	19.5
Range	800-4950	1160-1720	300-700	95-128	6.5-10.5	4.0-6.0	16.5-25.5
Mean	2721.4	1466.8	480.0	112.0	9.1	4.8	20.7
Coefficient of variation (%)	44.6	14.0	30.8	8.8	13.8	14.0	12.4

@ Brand name of the biscuit withheld

concentrations of potassium, manganese and zinc varied to a lesser extent, the concentration ranges being 450-1570 mg/kg, 3.5-10.4 mg/kg and 8.2-25.5 mg/kg, respectively with C.V. ranging from 16.4-23.4%.

From the foregoing discussion, it is evident that various brands of glucose biscuits and other varieties vary considerably in proximate and mineral composition. Largest variations occur in fat (1.04-24.66%), moisture (2.47-8.75%), calcium (120-1800 mg/kg), iron (38-230 mg/kg) and sodium (800-4950 mg/kg).

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Physico-chemical and Functional Properties of Starches Separated from *Bombay halwa*, a Traditional Indian Confection

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Traditionally, *Bombay halwa* is prepared with fermented wheat or refined wheat flour after removal of gluten. To simplify the process and to find out the type of starch suitable for its preparation, *Bombay halwa* was prepared using maize, wheat, pregelatinised maize and waxy maize starches in presence of acid. The changes in the physico-chemical properties of starch occurred during the preparation of *Bombay halwa* have been studied. The starches were separated from the products and the properties were compared with those of the corresponding native starches. There was a decrease in viscosity and degree of polymerization (DP), an increase in solubility and swelling power, indicating the degradation of the starch molecule during the preparation of *Bombay halwa*. Gel permeation chromatographic studies showed a decrease in the relative concentration of amylopectin. There were no additional peaks corresponding to dextrins. But, the increase in concentration of amylose fraction indicated that the degraded fraction of amylopectin eluted along with amylose fraction. However, decrease in the amylose content, blue value and λmax of the iodine complexes showed that the linear molecules were also degraded during the preparation of the product. The extent of degradation was more pronounced, when the concentration of the acid was increased.

Keywords : *Bombay halwa*, Indian traditional sweet, Starch, Acid modified starch, Tartaric acid, Physico-chemical properties.

Traditional sweetmeats occupy an important place in social customs and a sizable amount of fat, milk and farinaceous materials go into their production. One of the traditional cereal-based sweetmeats is *Bombay halwa*. It resembles confectionery jelly in texture except that it contains about 10% fat. It is relished for its cohesive and elastic texture, which is a desirable characteristic. Traditionally, it is prepared from soaked and ground wheat extract or from refined wheat flour *(maida)* after removing gluten, and overnight fermentation by a tedious and time consuming method. Like any other traditional sweetmeats, the texture of the product varies considerably from one manufacturer to another. Information with respect to role or functional properties of the ingredients and process parameters on the quality of *Bombay halwa* is not available. Starch being the main gelling agent in the product, effect of its physico-chemical characteristics on the texture of the product is important . The present paper deals with studies on the physico-chemical changes that occurred in starch during the preparation of *Bombay halwa*.

Materials and Methods

Maize *(Zea maize* L.), pregelatinized maize and waxy maize starches were procured from M/s Anil Starch Products Limited, Ahmedabad. Wheat starch was prepared from *maida* as follows: *Maida* (100 g) was hand-kneaded into a dough with 50 ml of water. The dough was soaked in water for about 1 h. The gluten was washed repeatedly with water, till the washings were clear. The combined washings were centrifuged and the sediment (starch) collected was used for the preparation of *Bombay halwa*. Dalda brand vanaspati (hydrogenated vegetable fat) was purchased locally. Carmoisine, a food grade colour used was from Bush Boake Allen (India) Limited, Madras. Tartaric acid from Purex Laboratories Limited, Bangalore, was used. Sepharose CL-2B from Pharmacia Fine Chemicals, Sweden, and standard potato amylose from Sigma chemicals, USA, were used. Other chemicals used were of analytical reagent grade.

Preparation of Bombay halwa: Slurries of different starches (maize, wheat, pregelatinised maize, and waxy maize) were prepared by suspending 25 g of starch from each source in 100 ml water. Tartaric acid at 0.9% by weight of starch was added to the starch slurry. In a separate batch, 1.3% of the acid was added to the maize starch slurry to find out the effect of concentration of acid on the properties of starch. Colour (0.4% of weight of starch) was added to the starch slurry and stirred. Sugar (125 g) was taken in a S.S. pan and dissolved in water (50 ml) by heating. The prepared starch slurry was added to the hot sugar syrup and stirred continuously. After the starch was fully gelatinized, 20-25g molten fat was added. Cooking

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and stirring continued till the required consistency (about 10% moisture) assessed by the weight of the final product was reached. Then, the mass was poured into circular moulds (46 mm dia. x 17 mm height), held at ambient temperature (about 25±2°C) overnight and was then taken for analysis. The product without acid addition and by partly replacing sugar with invert sugar (7:5 w/w) were also prepared for comparison.

Separation of starch from Bombay halwa: *Bombay halwa* was macerated using a pestle and mortar and repeatedly triturated with hexane followed by hot 70% ethanol. The complete removal of sugars was confirmed by testing the alcoholic extract with phenol - sulphuric acid (Dubois et al. 1956). Finally, the residue was washed with chloroform/methanol (3:1) to remove the bound lipids, dried under vacuum and stored in a desiccator. The dried starch thus obtained was used for analysis.

The starches separated from the products prepared using maize, wheat and waxy maize starches with 0.9% tartaric acid are denoted as MBH 1, WBH 1 and waxy BH 1, respectively, while the starch separated from the product prepared using maize starch with 1.3% tartaric acid is denoted as MBH 2.

Physico-chemical characteristics: The solubility and swelling power of starch samples were determined at 70, 80 and 85°C (Schoch 1964). The absorption maxima (λmax) of the iodine complex of the solubles was determined by adding I_2 solution (0.2% in 2% KI) to the supernatant. The specific viscosity of 0.5% samples in 1M KOH was determined, using an Ostwald viscometer at 35°C (Kerr 1950). The reducing end groups were determined to find out the degree of hydrolysis of starch, according to Nelson's method (Nelson 1944), using arseno molybdate and was reported as reducibility (% glucose). The degree of polymerization (DP) was calculated as the ratio of total carbohydrates (taken as 100%) to reducibility (Brown and Volenc 1989). The amylose contents of the samples were determined by measuring the absorbance of iodine complex at 630 nm (Sowbhagya and Battacharya 1971). The λmax of the iodine - polysaccharide complex of the starch samples was also determined to find out the degree of hydrolysis of starch, since the λmax lowers as starch hydrolyses. Blue value of the samples at pH 4.8 (acetate buffer) was determined by measuring the absorbance at 680 nm (Takeda et al. 1983). All the absorbance studies were made using UV-240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

The starch samples were fractionated by gel permeation chromatography (GPC) to find out the molecular degradation of starches separated from *Bombay halwa*. About 50 mg (dry weight) of the sample was dissolved in 10 ml of dimethyl sulfoxide and water (4:1), and 2 ml of this solution was loaded on a Sepharose CL-2B column (1.6 x 60 cm) operating at a flow rate of about 28 ml/h using distilled water, containing 0.02% sodium azide as an eluent. Three ml fractions were collected using a fraction collector (Frac-100, Pharmacia LKB, Sweden). Aliquots (2.5 ml) were treated with 0.2 ml I_2 solution (0.2% in 2% KI), and the absorbance at 630 nm and λmax were determined. The remaining 0.5 ml aliquot was treated with 1 ml of 5% phenol and 5 ml sulphuric acid and the absorbance was measured at 490 nm (Dubois et al. 1956). The absorbance was plotted against fraction number and the relative percentage of the individual peaks was calculated from the total absorbance.

Results and Discussion

Traditionally, *Bombay halwa* is prepared using fermented wheat starch slurry obtained after removal of gluten. To simplify the process and to find out the type of starch suitable for its preparation, maize, wheat and waxy maize starches were used initially. The results revealed that the products prepared had tough texture without cohesive property which became more pronounced after 2 days. Since, traditionally the product is prepared with wheat starch after fermentation, acid was added to native starches to simulate traditional conditions. The products prepared were found to have cohesive texture and good eating qualities. Commercial pregelatinized maize starch increased the viscosity of the mass and required large quantity of water during the preparation of the product. *Bombay halwa* prepared using waxy maize starch was found to be flowy without retaining the shape. This may be due to the poor mechanical properties of waxy starch gels (Leloup et al. 1991). However, it is known that acid-modified high amylose starches have been used for purposes in which good gelling properties are of importance (Pessa et al. 1992). To find out the changes occurring during preparation of *Bombay halwa* in the presence of acid, physico-chemical properties of starch separated from *Bombay halwa* were

TABLE 1. SOLUBILITY, SWELLING POWER AND VISCOSITY OF NATIVE STARCHES AND STARCHES SEPARATED FROM *BOMBAY HALWA*

Sample	Solubility % at °C			Swelling power at °C			Specific
	70	80	85	70	80	85	viscosity
Maize starch	6.75	11.22	13.48	6.41	9.54	11.86	0.86
MBH 1*	12.86	18.90	21.34	12.63	13.63	15.09	0.52
MBH 2**	26.92	27.80	28.79	14.37	17.45	19.17	0.40
Pregelatinised maize starch	19.70	21.10	22.30	13.00	15.30	18.30	0.97
Wheat starch	3.51	3.80	4.90	7.55	8..40	9.43	1.17
WBH 1*	39.46	47.24	50.72	12.22	14.08	14.61	0.58
Waxy maize starch	ND	ND	ND	ND	ND	ND	1.50
Waxy BH 1*	ND	ND	ND	ND	ND	ND	0.50

*MBH 1, WBH 1 & waxy BH 1 : Starches separated from *Bombay halwa* perepared using maize, wheat and waxy maize starches with 0.9% tartaric acid (on starch basis) respectively.

**MBH 2: Starch separated from *Bombay halwa* prepared using maize starch with 1.3% tartaric acid.

ND: not determined

analysed and compared with those of native starches.

The results in Table 1 show that the solubility and swelling power of the starches separated from *Bombay halwa*, (MBH 1, MBH 2 and WBH 1) are higher, when compared to those of the corresponding native starches. Also, there was a decrease in the absorption maxima (λmax) of the iodine complex of the water solubles of these starches (from 620 nm to 595-590 nm). There was a marked decrease in the specific viscosity of the starches separated from the product as compared to the corresponding native starches (Table 1). These results revealed that the starch had undergone degradation to some extent during the preparation of *Bombay halwa*. This is in agreement with Pessa et al (1992), who reported an increase in solubility and swelling power and decrease in viscosity on acid (HCl) hydrolysis of maize starch.

There was an increase in solubility and swelling power of pregelatinized maize starch, compared to native maize starch (Table 1). The solubles of the starch showed a λmax of 630 nm. This may be due to leaching out of more amylose during pregelatinzation and subsequent heating during determination. The solubility and swelling power of waxy maize starch could not be determined, as it formed a coloidal suspension and the supernatant was not clear.

The macromolecular degradation of the starch was further confirmed by the decrease in average DP and the λmax of iodine complexes of the starches separated from the products (MBH 1, MBH 2 and WBH 1), compared to those of the corresponding native starches (Table 2). Singh and Ali (1987) reported decreases in molecular weight of various starches upon acid hydrolysis. The decrease in molecular weight was attributed to the degradation of starch molecule into lower molecular weight components (Mercier 1986). GPC of all starches except waxy maize and waxy BH 1 showed two distinct peaks (Fig 1.), corresponding to a

TABLE 2. PHYSICO-CHEMICAL PROPERTIES OF NATIVE STARCHES AND STARCHES SEPARATED FROM *BOMBAY HALWA*.

Sample	Reducibility, % glucose	DP	λmax, nm	Relative percentage of fraction I of GPC		Amylose content, %	Blue value
				(a)	(b)		
Maize starch	0.053	1887	590	61.2	83.3	32.0	0.286
MBH 1*	0.110	909	560	28.9	61.8	23.9	0.152
MBH 2*	0.145	691	560	9.4	41.7	22.1	0.136
Pre-gelatinised maize starch	0.077	1299	590	50.5	78.6	33.0	0.312
Wheat starch	0.085	1177	595	55.5	75.1	31.5	0.266
WBH 1*	0.119	840	560	29.4	62.3	18.8	0.136
Waxy maize starch	0.022	4546	535	--	--	7.8	0.040
Waxy BH 1*	0.092	1087	535	--	--	6.0	0.020

(a) calculated from absorbance of iodine complexes of the fractions at 630 nm.

(b) calculated from absorbance of the total carbohydrates of the fractions.

*refer Table 1.

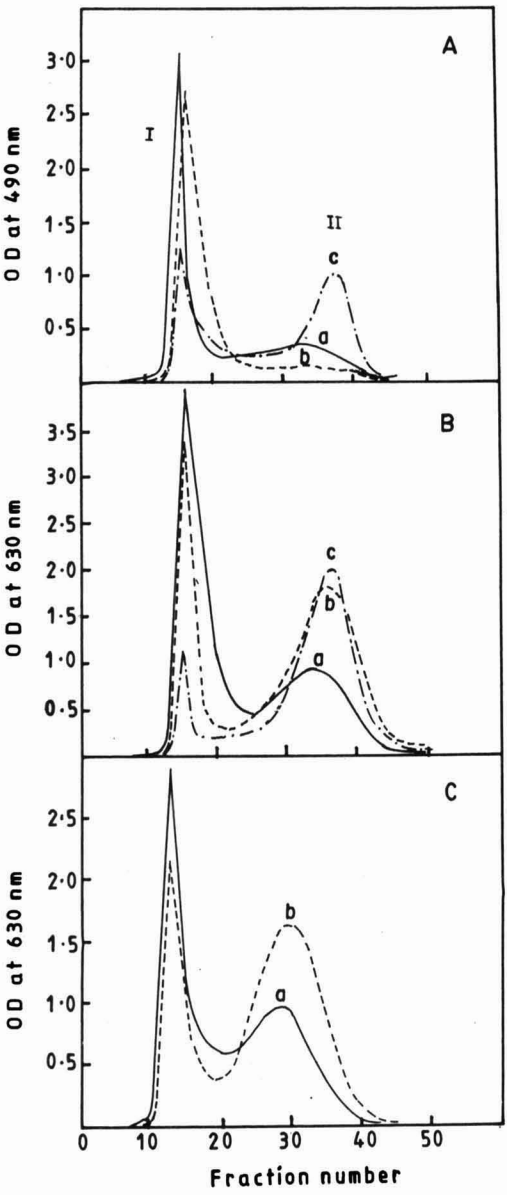

Fig1. Gel permeation chromatography on sepharose CL - 2B of maize starch (A & B) and wheat starches (C). a: native starches, b & c: starches separated from *Bombay halwa* prepared with 0.9% and 1.3% tartaric acid (on starch basis), respectively.

higher molecular weight fraction (fraction I) and a lower molecular weight fraction (fraction II), generally referred as amylopectin and amylose, respectively (Mahanta and Bhattacharya 1989; Chinnaswamy and Hanna 1990). The relative percentage of fraction I of starches separated from *Bombay halwa*, (MBH 1, MBH 2 and WBH 1) decreased as compared to the corresponding native starches, indicating the degradation of amylopectin (Table 2). Pessa et al (1992) reported that the hydrolysis with HCl started in the amylopectin moiety of starch.

There was no additional peak (s) corresponding to the degraded fractions in GPC indicating the absence of dextrins (Fig.1 A). However, there was an increase in the relative percentage of fraction II of starches separated from *Bombay halwa* (MBH 1, MBH 2 and WBH 1). These results revealed that the degraded amylopectin fraction eluted along with amylose. The degree of degradation was higher, when the concentration of acid was increased from 0.9 to 1.3% during the preparation of *Bombay halwa* (Table 2).

It was of interest to observe that the iodine complexes of GPC fractions corresponding to amylopectin showed a significant absorbance at 630 nm also (Fig. 1 B & C). The contribution of amylopectin for the absorbance at 630 nm probably can explain the higher amylose content observed for the samples (Table 2). Similar observation in some rich varieties with high amylopectin has been reported earlier (Chinnaswamy and Bhattacharya 1986).

The decrease in amylose content and blue value in the starches separated from *Bombay halwa* compared to native starches (Table 2) indicates reduction in the iodine binding capacity. These results show that even the linear molecules which are responsible for iodine complex formation had undergone degradation to a certain extent during the preparation of the product. This was further confirmed by the decrease in λmax of the GPC fractions, corresponding to fraction II (amylose). For instance, the fractions of maize starch had λmax of 600-645 nm whereas those from MBH 1 and MBH 2 showed λmax of 580-620 nm and 540-620 nm, respectively. The decrease in amylose content, blue value and λmax on acid hydrolysis of legume and maize starches have been reported earlier (Biliaderis et al. 1981: Inouchi et al. 1987).

These results revealed that amylopectin and the linear molecules responsible for iodine complexing degraded during the preparation of *Bombay halwa* in the presence of acid. This degradation was required in getting a desirable texture to the product and probably was responsible for the differences in physico-chemical properties of the starches. This is in agreement with the

observations of Pessa et al (1992) that the gel formation of acid modified maize starches depends on the extent of amylose leaching and on the amount of amylopectin degradation products, which in turn, depend on the degree of modification.

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Distribution of Collagen in the Muscle Tissue of Commercially Important Tropical Fishes

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Stroma proteins refer to slightly soluble/insoluble proteins obtained after removing water and salt soluble proteins in the muscle. The main constituent of stroma protein is collagen. The collagen contents of a few species of Indian fishes were determined. In the present study, an attempt was made to classify twenty species of commercially important fishes based on the percentage of total collagen on total proteins into three categories - low, medium and high collagen content species. Elasmobranchs were found to have a high content of collagen compared to teleosts.

Keywords : Connective tissue, Acid soluble collagen, Insoluble collagen, Proximate composition, Analogue.

Muscle can be regarded as a two component system, i.e., the muscle fibres and the intramuscular connective tissue (Rowe 1974). The content of these two components in any animal meat is important in deciding the organoleptic quality of the meat. As a result, the role of these components in determining the overall texture of the meat has been studied for years (Dyer et al. 1950; Hashimoto et al. 1979 ; Hatae et al. 1986; Katoh et al. 1989).

The connective tissue proteins contribute only a small fraction of the total protein content in fish meat and are involved in holding together the muscle bundles (myotomes) of fish and thus contribute to the overall texture of the meat. Among connective tissue proteins, collagen is found to be the major protein and is seen to influence the texture of fish meat and also properties such as gelling, emulsification, elasticity, etc.

The content of collagen has been estimated by different methods (Lowry et al. 1951; Baker 1954; Adams et al. 1960; Kubota and Kimura 1967; Grand and Stainsby 1975; Culler et al. 1978; Sato et al. 1986a). Of these, the most widely used method is estimation of collagen on the basis of hydroxyproline content in fish muscle. However, the exact content of collagen cannot be determined in the case of sample in which the hydroxyproline content of collagen is unknown.

In India, no systematic study has been done so far to classify fishes according to their collagen contents. The present paper describes the collagen contents in the muscle tissues of twenty species of fishes of freshwater, brackishwater and marine origin. An attempt has also been made to group these commercially important fishes on the basis of their collagen contents.

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Materials and Methods

For the study, freshwater, brackishwater and marine fishes were used. The freshwater fishes were collected from M/s Pookkot Fish Farm, Trichur, the brackishwater fishes from the 'Matsyafed' Fish Farm at Narakkal, Kochi and the marine fishes from Cochin Fisheries Harbour, Kochi, India. The fishes caught were immediately chilled in ice and brought to the laboratory for analysis.

The fishes were skinned and then filleted. The white muscle from the dorsal part of the trunk was used for determining the proximate composition and estimating the collagen content.

The total proteins, lipid and ash contents of the samples were determined as reported in AOAC (1984) methods. The content of soluble and insoluble collagen of fish muscle was determined as described by Sato et al (1986 b). A weighed quantity of fish muscle was homogenised with five times its volume of distilled water, using a Waring blender at 4°C.The suspension was centrifuged at 10,000 rpm for 20 min. The supernatant, the water soluble fraction was discarded. The residue was extracted overnight with 0.1 N NaOH with constant stirring at 4°C. The alkali extraction was repeated three more times. The residue was extracted with 0.5M acetic acid for three days at 4°C. The acid extraction were repeated twice. The protein solubilized by the acid extraction was used as the acid soluble collagen fraction. The residue was heated with distilled water in an autoclave at 120°C for 1h. The proteins solubilized were used as hot water soluble fraction (insoluble collagen). The acid soluble collagen and the insoluble collagen were determined by estimating protein contents in the supernatants by micro-Kjeldahl method.

Results and Discussion

The proximate composition and collagen content of the fishes studied are given in Table 1. It was ensured that the same part of the fish (white muscle from the dorsal) was analysed, as the composition differs markedly from head to tail of true fish and depends particularly upon the relative amounts of skin, dark muscle and white muscle that are in the sample. Of the fishes studied, the moisture content was maximum for anchovy (80.54%) and minimum for pomfret (70.86%). The protein content was maximum for whiting (22.9%) and minimum for tilapia (17.92%). The fat content was maximum for mackeral (7.5%) and minimum for shark (0.09%). The ash content was found to be high in sole (3.14%) and low in rohu (0.9%).

Content of collagen or stroma protein in teleost muscle is usually in the range of 1-4% of total proteins (Dyer et al. 1950; Hashimoto et al. 1979). In the present study, the total collagen content of teleost was in the range of 1.58-9.08% of total protein and for elasmobranchs 13.11-13.39% of total protein (Fig. 1). This is in agreement with the findings of Sato et al (1986b). These workers have fractionated total collagen into soluble collagen and insoluble collagen and found that the values vary between 0.16-1.08 g/100g wet meat and 0.18-1.27

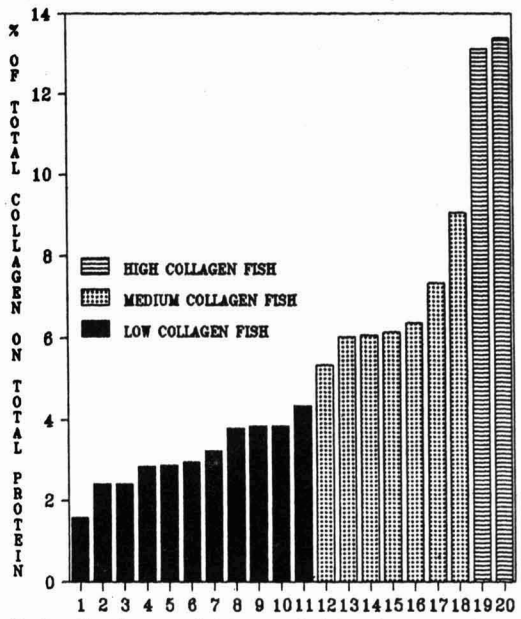

Fig. 1. Classification of fishes studied based on its collagen content

FISH SPECIES

1. *P. argenteus* 2. *R. kanagurta* 3.*S. longiceps* 4. *T. savala* 5. *Caranx* spp. 6. *C.semifasciatus* 7. *C. carpio* 8.*Anchoviella* 9. *O. mossambicus* 10 *L. rohita* 11. *M. cyprinoides* 12.*Barbus* spp. 13.*M. cephalus* 14 *K.kowal* 15.*N.japonicus* 16. *C. catla* 17.*E.affinis* 18. *S. sihama* 19.*S. sorrakowa* 20. *Himantura*

TABLE 1. PROXIMATE COMPOSITION AND COLLAGEN CONTENT OF WHITE MUSCLE OF FISHES STUDIED

Common name	Scientific name	Moisture, %	Protein, %	Fat, %	Ash, %	Acid soluble collagen, %	Insoluble collagen, %	Total collagen %
White Pomfret	*Pampus argenteus*	70.96	18.98	2.80	3.00	0.19	0.11	0.30
Sardine	*Sardinella longiceps*	77.36	18.57	3.20	1.60	0.36	0.09	0.45
Mackeral	*Rastrelliger kanagurta*	74.70	19.53	7.50	1.65	0.38	0.09	0.47
Sole	*Cynoglossus semifasciatus*	72.38	19.48	4.70	3.14	0.40	0.18	0.58
Vatta	*Caranx* spp.	75.80	20.07	0.50	2.01	0.50	0.08	0.58
Ribbon fish	*Trichurus savala*	75.10	21.98	0.68	1.99	0.12	0.51	0.63
White bait	*Anchoviella*	80.54	18.08	1.02	1.58	0.09	0.60	0.69
Tilapia	*Oreochromis mossabicus*	77.60	17.92	2.90	1.10	0.47	0.22	0.69
Common carp	*Cyprinus carpio*	75.20	21.52	3.50	1.17	0.49	0.21	0.70
Rohu	*Labeo rohita*	76.10	18.90	0.80	0.90	0.28	0.46	0.74
Palankanni	*Megalops cyprinoides*	75.90	18.10	1.10	1.00	0.38	0.41	0.79
Paral	*Barbus* spp.	76.50	18.10	2.12	1.40	0.66	0.31	0.97
Veloori	*Kowala koval*	79.84	18.12	0.69	1.38	0.66	0.62	1.22
Kilimeen	*Nempterus japonicus*	78.14	19.32	2.86	1.58	009	1.01	1.10
Mullet	*Mugil cephalus*	75.77	20.22	2.45	1.62	0.94	0.25	1.19
Catla	*Catla catla*	76.00	19.00	1.30	0.90	0.71	0.55	1.26
Tuna	*Euthynnus affinis*	74.85	19.74	3.98	1.50	1.06	0.39	1.45
Whiting	*Sillago sihama*	75.02	22.90	0.30	1.73	1.00	1.08	1.08
Ray	*Himantura*	75.25	20.91	0.50	1.24	2.30	0.50	2.80
Shark	*Scoliodon sorrakowah*	72.00	22.80	0.09	1.50	2.13	0.86	2.99

g/100g wet meat, respectively. In the present study, corresponding values ranged between 0.09-2.30 g/100g wet meat and 0.09-1.08 g/100g wet meat, respectively (Table 1). Sato et al (1986b) found that the total collagen content varied between 0.34-2.11 g/100g wet meat and 1.6-12.4% total proteins.

The solubility of the muscle collagen varies significantly among the various species of fish. The difference in solubility of collagen observed in the present study may reflect the difference in the degree and properties of intra and inter-molecular cross-linking of collagen. A total collagen content in the range of 0.30-2.99% of wet tissue with a corresponding range of 1.58-13.39% of the total proteins was observed in the present study (Table 1). The variation in total collagen content among the species, when expressed as percentage on total proteins (1.58-13.39%) was more distinct, when compared to the total collagen content on wet weight basis. Accordingly, the fishes having total collagen contents upto 5% of total proteins were grouped as low collagen fishes, from 5-10% as medium collagen and above 10% as high collagen fishes (Fig. 1).

During frozen storage of fish muscle alterations in fish myofibrillar proteins (MFP) have been largely accepted as the principal cause of loss of protein functional properties (Shenouda 1980). The retention of these functional properties, namely gel forming ability and water holding capacity of actomyosin are essential for manufacturing surimi-based analogues (Lee 1984). MFPs are responsible for the water holding capacity and other technological properties of the meat, such as emulsifying capacity and tenderness (Lee 1986). Connective tissue is also associated with the texture of the meat. Hence, data reported in the present paper on connective tissue contents of commercially important species of fish provide useful information, regarding texture of product and preparation of products acceptable to consumers.

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Effect of Supplementation of *Spirulina* on Hypercholesterolemic Patients

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The effect of *Spirulina* on hypercholesterolemic patients was carried out in Coimbatore city. Thirty ischaemic heart disease patients without any complications of the disease and with blood cholesterol levels above 250mg/dl were selected for the study and divided into three groups of 10 each for supplementation of *Spirulina*. Subjects in groups A and B received 2g and 4g *Spirulina* per day, respectively for three months. Group C served as control. The study has revealed that *Spirulina* plays a key role in weight reduction, lowering the blood cholesterol levels and improving the lipid profile of patients.

Keywords: *Spirulina fusiformis*, Hypercholesterolemia, Lipid profile, Blue green algae.

Spirulina fusiformis is a multicellular filamentous blue green microalgae (cyanobacteria) known for its potential to bring about a nutritional revolution in the developing countries, where it grows naturally in highly alkaline lakes. (Muratee 1993). Several countries have started growing it commercially. It is gaining popularity in recent years, as a food supplement, because of its remarkable ability to synthesize high quality concentrated food more efficiently than any other algae (Challem 1981; Umesh and Seshagiri 1984). It is an excellent food source, providing the highest amount of proteins (65-71%) with all the essential and non-essential amino acids as well as various vitamins and minerals, including the B complex and chelated minerals in a balanced proportion along with the pigments (MCRC brochure 1990; Venkataraman 1993).

Studies have shown that it has high levels of beta carotene, gamma linolenic acid, iron and vitamin B_{12} (Seshadri and Umesh 1992; Venkataraman 1992). Furthermore, it has a thin cell wall and its digestion and absorption are easy (Iwata et al. 1990).

Extensive research on toxicological aspects has been carried out under the UNIDO (1980) programme in Mexico for a ten year period. This study has proved conclusively that *Spirulina* is a safe food with absolutely no side effects. Other long term and short term toxicological tests on animals and human beings have indicated similar results (Kumari et al. 1981; NIN 1988).

Recently, *Spirulina* has made an important breakthrough in tackling the problem of hypercholesterolemia and in animal experiments, it has proved that *spirulina* had positive effects in reducing serum total cholesterol and elevating high density lipoprotein (HDL) cholesterol levels (Chen et al. 1981; Devi and Venkataraman 1983; Kato et al. 1984; Chokkukannan et al. 1993). The present study was carried out to determine whether *Spirulina* could exert these effects on human subjects.

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Materials and Methods

Thirty patients who were overweight with the blood cholesterol levels ranging from 250-400 mg/dl in the age group of 40-60 years attending the government hospitals, Coimbatore city formed the subjects for this study. From all the 30 subjects, the background informations like socio-economic pattern, dietary practices and health details were elicited through a detailed questionnaire. The data from this questionnaire revealed that the occurrence of ischemic heart disease was more (21 out of 30 subjects) in the age group of 46 to 55 years with sedentary life style and in non-vegetarians with high saturated fat intake.

They were divided into three groups of 10 subjects each for supplementation of *Spirulina*. Subjects in group A received 2g of *Spirulina* per day and those in group B received 4g of *Spirulina* per day for three months, while the group C served as control. The tablets commercially available as *Multinal* supplied by the "New Ambadi Estates Private Limited" (a member of Murugappa Group), Madras were used in the study.

The subjects in group A were administered two tablets twice a day and the subjects in group B received two tablets, four times a day for a period of three months.

The biochemical parameters monitored in this study were serum cholesterol, triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), body weights and the final levels of liver enzymes such as serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). All the above estimations were carried out initially and after three months supplementation for all the 30 subjects. But the blood cholesterol level was estimated three times, viz., at initial, intermediate and final points.

Blood cholesterol level was determined by Zak's method (Varley et al. 1980), and measurement of TG and HDL cholesterol were made by the enzymatic method, LDL cholesterol and VLDL cholesterol were calculated by Friedewald formula (Friedewald 1972)and the final levels of the liver enzymes SGOT and SGPT were estimated by using the method of Rietman and Frankel (Wooton 1964).

Results were statistically analysed as per the methods of Snedecor and Cochran (1967) and Gupta (1981).

Results and Discussion

Body weight

Mean initial weights of subjects from groups A, B and C were 62.7, 62.1 and 61.0 kg, respectively and at the end of three months, the respective mean weights were 60.5, 59.9 and 60.3 kg. There were no significant differences between the reductions observed in two experimental groups, but the reductions were highly significant, when compared to the control group (Table 1).

Serum cholesterol

The mean initial serum cholesterol levels of groups A, B and C were found to be 282.1, 309.8, 280.8 mg/dl, respectively. Clear declining trends of the cholesterol levels were observed in the experimental groups during the end of second month. At the end of third month, the values were 218.8, 206.1 and 269.0 mg/dl, respectively for the three groups. It was noted that the subjects receiving four grams supplementation of *Spirulina* recorded the maximum reduction of serum cholesterol, which was statistically highly significant (Table 2). The differences between the groups B and C as well as A and C were also highly significant ($P \leq 0.01$).

TABLE 1. BODY WEIGHT CHANGES IN THE SUBJECTS (n = 10)

Groups	Body weight, kg Initial	Final	Difference	Groups compared	't' test
A	62.7± 5.39	60.5± 5.0	2.2± 0.87	A vs B	0^{NS}
B	62.1± 5.56	59.9± 5.30	2.2± 0.87	A vs C	4.48**
C	61.0± 5.22	60.3± 5.18	0.7± 0.60	B vs C	4.48**

** - highly significant ($p \leq 0.01$)
NS - Not significant ($p > 0.05$)

TABLE 2. CHANGES IN THE SERUM CHOLESTEROL LEVELS OF THE SUBJECTS (n=10)

Groups	Serum cholesterol, mg/dl Initial	Inter-mediate	Final	Diffe-rence	Groups com-pared	't' test (between initial & final)
A	282.1± 16.59	242.7± 16.49	218.8± 14.78	63.3± 7.58	A vs B	10.11**
B	309.8± 28.70	239.9± 25.33	206.1± 27.36	103.7± 10.11	A vs C	18.21**
C	280.8± 21.07	275.0± 22.45	269.0± 22.08	11.8± 4.75	B vs C	26.02**

** - highly significant ($p \leq 0.01$)

TABLE 3. CHANGES IN THE SERUM TRIGLYCERIDE LEVELS OF THE SUBJECTS (n =10)

Groups	Serum triglycerides, mg/dl Initial	Final	Difference	Groups compared	't' test
A	220.1± 34.20	171.9± 35.94	48.2± 5.62	A vs B	0.86^{NS}
B	222.5± 33.57	171.8± 33.56	50.7± 7.36	A vs C	22.02**
C	218.1± 31.72	213.2± 33.20	4.9± 2.66	B vs C	18.49**

** - highly significant ($p \leq 0.01$)
NS - Not significant ($p > 0.05$)

Serum triglycerides

The mean initial serum triglyceride level observed in groups A, B and C were 220.1, 222.5 and 218.1 mg/dl respectively and at the end of three months, the triglyceride levels were reduced to 171.9, 171.8 and 213.2 mg/dl, respectively. The differences observed between the two experimental groups were not statistically significant among themselves, but both the experimental groups registered highly significant differences, when compared to the control group (Table 3).

High density lipoprotein (HDL) cholesterol

The mean initial serum HDL cholesterol levels in groups A, B and C were 46.1, 44.6 and 45.5 mg/dl and at the end of third month, the values

increased to 51.4, 50.3 and 47.6 mg/dl, respectively. The differences between the two experimental groups were not statistically significant, but the differences were highly significant, when compared to the control group (Table 4).

Low density lipoprotein (LDL) cholesterol

Mean initial serum low density lipoprotein (LDL) cholesterol levels in groups A, B and C were 192.0, 220.5 and 191.8 mg/dl, respectively and at the end of three months, the levels were reduced to 132.8, 121.7 and 178.7 mg/dl, respectively. The highest reduction in LDL cholesterol was observed in the group supplemented with four grams of *Spirulina*. All the comparisons made among the three groups showed highly significant differences ($P \leq 0.01$) (Table 5).

Very low density lipoprotein (VLDL) cholesterol

The mean initial levels of the VLDL cholesterol for the three groups were almost similar viz., 44.0, 44.7 and 43.5 mg/dl, respective;ly. All the three mean values were above the normal levels of 35 mg/dl. It was encouraging to find that *Spirulina* supplementation had brought down the VLDL cholesterol levels to 34.4 mg in group A and 34.3 mg in group B, while a very slight reduction of 0.8 mg was recorded in group C. The differences

TABLE 4. CHANGES IN THE HDL CHOLESTEROL LEVELS OF THE SUBJECTS (n = 10)

Groups	HDL cholesterol, mg/dl Initial	Final	Difference	Groups compared	't' test
A	46.1±	51.4±	5.3±	A vs B	0.63^{NS}
	4.95	4.48	1.42		
B	44.6±	50.3±	5.7±	A vs C	5.57**
	3.61	3.20	1.41		
C	45.5±	47.6±	2.1±	B vs C	6.27**
	3.91	4.25	1.14		

** - highly significant ($p \leq 0.01$)
NS - Not significant ($p > 0.05$)

TABLE 5. CHANGES IN THE LDL CHOLESTEROL LEVELS OF THE SUBJECTS (n = 10)

Groups	LDL cholesterol, mg/dl Initial	Final	Difference	Groups compared	't' test
A	192.10±	132.8±	59.2±	A vs B	10.81**
	15.90	14.50	6.63		
B	220.5±	121.7±	98.8±	A vs C	16.94**
	27.19	25.39	9.51		
C	191.8±	178.7±	13.1±	B vs C	24.69**
	20.18	21.32	5.49		

** - highly significant ($p < 0.01$)

TABLE 6. CHANGES IN THE VLDL CHOLESTEROL LEVELS OF THE SUBJECTS (n = 10)

Groups	VLDL cholesterol, mg/dl Initial	Final	Difference	Groups compared	't' test
A	44.0±	34.4±	9.6±	A vs B	1.04^{NS}
	6.86	10.92	1.91		
B	44.7±	34.3±	10.4±	A vs C	13.91**
	6.75	6.75	1.50		
C	43.5±	42.7±	0.8±	B vs C	18.83**
	6.41	6.65	0.54		

** - highly significant ($p \leq 0.01$)
NS - Not significant ($p > 0.05$)

observed between the two experimental groups were not statistically significant, while both the experimental groups registered high siginificant differences in VLDL cholesterol reduction, when compared to the control group (Table 6).

Serum glutamic oxalo acetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT)

The mean final SGOT and SGPT levels observed among the three groups of subjects were found to be normal (Table 7), showing *Spirulina* supplementation did not cause any damage to the heart and the liver.

The results of the present study reveal that *Spirulina* plays a very impressive role in weight reduction, in lowering the blood cholesterol levels and in improving the lipid profiles of individuals. *Spirulina* contains 5 to 6% essential fatty acids of which gamma linolenic acid and linolenic acid account for approximately 30% (Grattan 1989). It can prevent fat and cholesterol accumulation. Gamma linolenic acid is approximately 170-fold more effective in lowering the plasma cholesterol level than linolenic acid (Nichols and Wood 1986).

Gamma linolenic acid is not present very much in the diet. However, linolenic acid can be converted to gamma linolenic acid in the presence of an enzyme Delta-6-desaturase. This enzyme is not active in some individuals. Moreover, it is inhibited by a high saturated fat in the diet. But, *Spirulina* has got preformed gamma linolenic acid and linolenic acid (Seshadri and Seshagiri 1986; Richmond 1988; Hendrickson 1989).

TABLE 7. ACTIVITY OF LIVER ENZYMES

Groups	SGOT, units/dl	SGPT, units/dl
A	21.1	16.7
B	19.4	15.5
C	21.1	18.6

It can be concluded that supplementation of *Spirulina* tablets resulted in very significant beneficial effects including weight reduction, fall in the levels of blood cholesterol, TG, LDL, VLDL cholesterol and elevation of HDL cholesterol. Four grams of *Spirulina* supplementation had exerted more beneficial effects than the two grams, in reducing serum cholesterol and LDL cholesterol levels.

In addition, *Spirulina* has a higher content of all essential and non-essential amino acids including L-phenylalanine and L-tyrosine, which directly influence the levels of neurotransmitters (norepinephrine and dopamine) in the brain, which control appetite (Challem 1981; Lee 1984). This might be one of the reasons for weight reduction, resulting from *Spirulina* supplementation. The polyunsaturated fatty acids that are present in *Spirulina* help to reduce serum cholesterol levels on a long term basis. By virtue of this three-fold effect, weight and cholesterol reduction could be achieved (Seshadri and Seshagiri 1986).

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Influence of Vitamin E Supplementation of Dietary Fish Oils on Lipid Profile and Blood Glucose Levels in Albino Rats

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Vitamin E supplementation of dietary sardine oil and shark liver oil was found to enhance the hypocholesterolemic properties and other favourable effects like reduction in serum triglycerides and increase in the percentage of HDL cholesterol in albino rats. The effect of diets having these oils with or without added vitamin E (1.5 IU/g) was compared to a sunflower oil diet and a commercial feed. The increase in the blood sugar levels in rats fed with polyunsaturated fatty acid (PUFA)-rich fish oil was insignificant. Influence of dietary fatty acids on the fatty acid composition of the lipids of heart, liver and adipose tissue of the rats fed on these diets is also reported.

Keywords : Albino rats, Dietary fish oils, Vitamin E supplementation, Lipid profile, Blood sugar levels, Fatty acids, Organ lipids.

Beneficial effects of dietary fish oils rich in PUFA of the n-3 series in patients, suffering from cardiovascular problems are well documented (Harris et al. 1981; Lorentz et al. 1983). Mehta et al (1987) reported the effects of fish oils on serum triglycerides, blood pressure, and blood viscosity. Administration of PUFA in clinical trials is known to decrease the levels of vitamin E in the body (Bjornebe et al. 1989; Stam et al. 1989). Vitamin E is an important biological antioxidant, which along with other agents like vitamin C, glutathione peroxidase, β-carotene scavenge peroxy radicals, besides acting as a chain breaking antioxidant. (Diplock 1983; Willson 1983; Wefers and Sies 1988). Haglund et al (1991) confirmed that vitamin E exerted favourable effect on the levels of serum triglycerides, cholesterol, fibrinogen and malonaldehyde in human beings. However, studies on diabetic patients fed on fish oils reported an unexplained increase in plasma glucose levels. (Popp-Snijders et al. 1987; Glauber et al. 1988; Vessby 1989). The effects of vitamin E supplementation of dietary fish oils in the diet of albino rats on the levels of serum cholesterol, HDL cholesterol, blood sugar and on lipids of liver, heart and adipose tissue are reported in this paper.

Materials and Methods

Five groups of 6 weeks old Wistar strain male albino rats (5 numbers in each group weighing each around 100g) were fed diets identical in all respects, except the oil source. Refined sunflower oil (SFO), shark liver oil (SLO), shark liver oil with added vitamin E (1.5 IU/g), (SLO + vit E), sardine oil (SO), sardine oil with vitamin E (SO + vit E) were incorporated in the diet at 10% level. SFO, SLO and SO were purchased from the market. The other ingredients in the diet were fat-free casein to provide 10% proteins, 4% USP salt mixture, 5% cellulose, 1% vitamin mixture (Chapman et al. 1959), and 1% methionine. The diet was made up to 100% with corn starch. One group was fed on a commercial rat feed (CF). CF had 23% proteins, 6% fat, 7% ash, 50% carbohydrates and 1.85% fibre. Each rat was individually housed in a polypropylene cage, having metallic grill cover with provisions for giving feed and water. After an adaptation period of 4 days, their weights were noted and regular feeding started. The rats were fed *ad libitum* for 90 days. Individual food intakes and weekly body weights were recorded. At the end of the feeding period, the rats were starved overnight and sacrificed. Samples of blood, adipose tissue and vital organs (heart and liver) were collected from all the rats. Serum was separated and stored in the freezer along with the organs, pending analysis. Blood sugar in the serum was estimated by the method of Folin and Wu, as cited by Hawk et al (1954) and cholesterol by the method of Rudel and Morris (1973). HDL cholesterol was estimated by the same method after precipitating VLDL + LDL using heparin/manganous chloride (Warnick and Albers 1978). Serum samples of rats fed SLO and SLO + vit. E were fractionated into triglycerides, complex lipids, free fatty acids and cholesteryl esters, using TLC-FID technique in an Iatroscan.

Lipids from the tissues were extracted with chloroform - methanol mixture of 2:1 ratio (Folch et al. 1957). The fatty acid methyl esters of the

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lipids were prepared (Matcalfe et al. 1966) and analysed in a Varian gas chromatograph, equipped with a Vista 402 data processor. Ten per cent Silar 5 CP column with Flame Ionisation Detector was used. Injector and detector temperatures were 220°C and 230°C, respectively. The initial column temperature of 120°C was programmed to increase at the rate of 5°C/min. to 200°C. Nitrogen was used as the carrier gas at a flow rate of 40 ml/min.

Results and Discussion

The fatty acid composition of the three oils used in the diet is given in Table 1. SO was richer in PUFA, while SFO had a high content (74%) of mono and di-unsaturated fatty acids. SLO had almost a balanced distribution of these fatty acids. The n-3 fatty acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were higher in fish oils. (Bruckner et al. 1984).

Relative size of liver, (weight of liver expressed as percentage of body weight) was found to be the highest in the CF group and lowest in the vitamin E-added SO-fed group. Statistical analysis (one way ANOVA) showed the variations to be significant ($P<0.001$). Heart size also showed significant variations ($P<0.01$). Halminski et al (1991) found that feeding of fish oil increased the liver weight and relative liver size. Gilles et al (1988) observed that the heart weights were not significantly different for the rats on different diets. Nevertheless, no correlation was found between the fatty acid composition, or addition of vitamin E on the liver and heart size (Table 2).

TABLE 1. FATTY ACID COMPOSITION OF OILS (AS AREA %)

Fatty acid	SFO	SO	SLO	CF
C12	ND	0.29	ND	15.78
C14	0.14	8.07	4.31	ND
C16	15.75	20.03	23.21	ND
C16 :1	ND	8.95	8.20	ND
C18	3.05	5.60	8.30	27.50
C18 :1	20.48	8.64	16.09	31.54
C18 :2	46.61	1.40	2.22	1.64
C18 :3	ND	1.17	0.89	1.34
C20	0.19	0.91	0.36	ND
C20 :1	ND	1.44	1.91	ND
C20 :2	0.82	ND	1.04	ND
C20 :3	ND	ND	ND	3.43
C20 :4	ND	3.96	3.90	ND
C20 :5	ND	12.17	4.55	1.95
C22	0.58	ND	ND	0.97
C22 :1	3.30	ND	ND	ND
C22 :6	ND	13.40	12.85	ND
C24	ND	ND	ND	1.27
C24 :1	2.71	ND	ND	ND

ND : Not detected

TABLE 2. SIZE OF HEART AND LIVER

Feed	Liver size**	Heart size*
SFO	2.44 ± 0.05	0.24 ± 0.02
CF	3.24 ± 0.08	0.27 ± 0.02
SLO	2.52 ± 0.22	0.24 ± 0.01
SLO+E	2.68 ± 0.10	0.23 ± 0.02
SO	2.52 ± 0.13	0.26 ± 0.02
SO+E	2.26 ± 0.06	0.28 ± 0.01

** Liver size : wt of liver x 100 / wt of rat; $P < 0.001$

* $P< 0.01$

Values are average of five estimations

The levels of total and HDL cholesterol, glucose and artherogenic index (total cholesterol-HDL cholesterol/HDL cholesterol) are given in Table 3. SO in diet decreased the serum cholesterol level and addition of vitamin E to this oil was found to reduce the cholesterol level further. Cholesterol lowering effect of SO has already been established (Sen et al. 1977; Nair et al. 1985). The values for total cholesterol and HDL cholesterol were statistically significant ($P<0.001$ and $P<0.01$, respectively. When the SO and SO + vitamin E groups were omitted, the values for total cholesterol were not statistically significant. SLO did not lower serum cholesterol significantly, in spite of its high PUFA content and a good percentage of mono and di-unsaturated fatty acids. The higher cholesterol content of SLO (1.12g/100g) compared to that of SO (0.56g/100g), might have offset its hypocholesterolemic effect. SLO fed rats had the highest HDL cholesterol content. However, the proportion of HDL cholesterol as percentage of total cholesterol was highest for SO + vitamin E group, followed by the SLO + vitamin E group and almost the same for SLO and SO-fed groups. The artherogenic indices of the different diets showed that SFO was more artherogenic than the other oils used in this study and that vitamin E supplementation of SO and SLO further reduced their artherogenic index. Though blood sugar levels were found to be higher in the rats fed SO and SLO diets compared to the rats fed other diets, the increase was not significant ($P<0.5$). Fish oils were reported to increase plasma glucose levels in human beings (Haglund et al. 1991). Addition of vitamin E to this oil was not found to alter the sugar level.

TABLE 3. SERUM CHOLESTEROL AND GLUCOSE LEVELS IN RATS FED DIFFERENT DIETS

Feed	Total ** cholesterol, mg/100ml	HDL cholesterol, mg/100ml	HDL cholesterol as % of total cholesterol	Artherogenic index	Glucose#, mg/100ml
SFO	90.29±5.80	22.90±1.60	25.40	2.94	116.10±6.70
CF	69.36±2.40	25.00	36.00	1.77	112.20±6.00
SLO	84.60±8.60	31.55±2.63	37.30	1.71	121.60±7.30
SLO+E	74.40±5.01	29.50±0.42	39.70	1.52	129.80±9.10
SO	55.88±2.00	20.78±1.05	37.20	1.69	129.50±7.00
SO+E	47.06±2.64	21.47±1.90	45.60	1.19	128.00±12.70

** : P <0.001

* : P <0.01

: Not significant

Values are average of five estimations

Fractionation of serum lipids of rats fed SLO and SLO + vitamin E into different classes showed that vitamin E supplementation of these oils reduced the content of free fatty acids, triglycerides and also the complex lipids (Table 4). Gilles et al (1988) reported the effect of dietary salmon oil in lowering free and esterified cholesterol, triglycerides and complex lipids in serum. The n-3 fatty acids, responsible for this reduction, are protected from *in vivo* peroxidation by vitamin E. Triglycerides are now recognised as an independent risk factor in artherogenesis (Carrisson et al. 1979), and thus, this finding assumes special significance.

The fatty acid composition of different organ lipids varied with the type of dietary lipids. In liver (Table 5), major changes were noticed in the C18:2, C20:4 and C 22:6 (DHA). In the SFO and the CF- fed groups, oleic and C18:2 each contributed to about 15% of the total fatty acids. Similar observations were made by Charnock (1984) and Charnock et al (1986). In animals fed with SO and SLO, the C18:1 assimilation almost doubled with a marked concomitant decrease in C18:2 and C20:4. Higher assimilation of DHA was noticed with SO/SLO (high PUFA) diet fed groups. It is known that EPA depletes C20:4, by inhibiting the enzyme Δ 6 desaturase activity, thereby preventing the first step in the conversion of C18:2 to C20:4 (Crofe 1984).

In adipose tissue (Table 6), the major storage (about 50%) was contributed by C16 and C18:1.

TABLE 4. SERUM LIPID PROFILE (mg/100 ml SERUM)

Feed	Cholesterol esters	Triglyceride	Fatty acid	Complex lipids
SLO	58.68	55.06	13.31	324.05
SLO+E	55.91	38.47	11.93	273.55

Values are average of five estimations

C16 assimilation was more in SO,SLO and SLO+vit E- fed groups, followed by CF and SFO. C18 was assimilated more or less to the same level in all the diet groups, while higher assimilations of C18:1 (33-43%) in SFO-fed animals and of C18:2 (about 4 times in comparison) were noticed in SFO and CF groups. Small amounts of C20:1, C20:3, C22:3 and C24:1 were also seen in the SFO-fed groups. SO/SLO groups effected small level assimilation of DHA and EPA into the fat. The conversion to C20:4 is not that effective, in spite of the assimilation of C18:2. Hence, the fatty acid composition of the organ lipid appears to depend on the role played

TABLE 5. FATTY ACID COMPOSITION OF LIVER LIPIDS OF RATS FED ON DIFFERENT DIETS (AREA %)

Fatty acid	Source of lipid in the diet					
	SFO	CF	SO	SO+E	SLO	SLO+E
C12	ND	ND	ND	ND	ND	0.30
C13	ND	ND	ND	0.25	ND	ND
C14	ND	2.38	0.81	0.58	ND	ND
C15	ND	ND	ND	ND	0.60	ND
C16	15.96	15.59	21.17	27.52	29.80	31.50
C18	16.79	11.08	10.91	9.22	10.60	9.50
C18:1	13.52	10.07	27.14	18.41	22.70	25.40
C18:2	17.22	18.02	6.98	9.54	6.50	7.40
C18:3	ND	0.21	ND	ND	ND	ND
C20	ND	0.47	ND	0.47	ND	0.60
C20:1	0.79	0.77	ND	ND	0.34	ND
C20:3	ND	19.18	ND	ND	ND	ND
C20:4	27.27	ND	12.03	13.60	13.40	10.90
C20:5	ND	1.70	ND	ND	ND	ND
C22	0.89	ND	ND	ND	1.71	1.70
C22:1	ND	ND	8.52	8.06	ND	0.40
C22:2	0.96	ND	ND	0.51	0.50	0.50
C22:3	ND	ND	ND	ND	0.73	ND
C22:6	ND	5.31	10.32	9.71	9.40	8.50
C24	1.10	ND	ND	0.53	0.53	0.50

ND : Not detected

TABLE 6. FATTY ACID COMPOSITION OF ADIPOSE TISSUE LIPID OF RATS FED ON DIFFERENT DIETS (AREA %)

Fatty acid	Source of lipid in the diet					
	SFO	CF	SO	SO+E	SLO	SLO+E
C12	0.15	0.29	0.24	ND	ND	ND
C14	1.08	1.05	6.85	0.64	2.91	2.78
C16	15.57	19.88	29.95	14.68	29.95	27.56
C16:1	2.67	2.47	12.29	1.08	9.50	8.72
C18	3.12	3.77	4.90	10.25	5.06	5.50
C18:1	43.23	34.17	24.07	8.21	33.83	34.18
C18:2	16.37	18.57	2.75	3.99	3.11	3.94
C18:3	ND	0.22	0.73	0.69	ND	1.00
C20	ND	0.20	0.56	0.54	0.20	0.27
C20:1	0.22	ND	1.95	0.54	ND	2.11
C20:2	ND	1.81	ND	6.23	ND	ND
C20:3	0.67	0.99	ND	2.62	0.62	0.87
C20:5	ND	ND	1.83	4.47	0.99	1.20
C22	1.43	0.81	0.75	7.30	ND	1.14
C22:1	ND	4.58	ND	ND	ND	ND
C22:3	0.60	ND	1.60	4.95	0.96	1.16
C22:6	ND	1.03	ND	4.44	1.27	1.75
C24:1	1.30	ND	ND	ND	ND	ND

ND:Not detected

by the concerned tissue. There was enrichment of the lipid with n-3 fatty acid (EPA and DHA) in fish oil, supplemented animals as also observed earlier (Awad et al. 1990). Awad et al (1990) further stated

TABLE 7. FATTY ACID COMPOSITION OF HEART LIPIDS OF RATS FED ON DIFFERENT DIETS (AREA %)

Fatty acid	Source of lipid in the diet					
	SFO	CF	SO	SO+E	SLO	SLO+E
C12	1.33	0.34	1.54	0.19	0.44	0.68
C14	1.85	0.66	3.22	2.65	0.86	1.36
C16	14.22	10.72	18.66	16.48	9.80	9.62
C16:1	1.71	ND	5.35	4.88	ND	2.84
C18	16.59	11.16	18.42	14.76	10.26	10.11
C18:1	19.00	6.64	14.91	14.44	11.70	11.22
C18:2	2.29	2.66	2.81	6.27	7.07	6.51
C18:3	ND	0.60	0.31	0.76	ND	ND
C20	1.50	ND	ND	0.41	0.29	ND
C20:1	6.78	6.40	1.87	0.69	1.28	ND
C20:2	ND	ND	4.31	0.21	ND	ND
C20:4	5.64	2.67	1.87	12.50	ND	13.28
C20:5	4.01	6.31	3.95	ND	10.41	ND
C22	ND	ND	ND	2.54	5.43	5.63
C22:1	ND	ND	ND	ND	ND	1.13
C22:3	ND	ND	ND	0.69	ND	ND
C22:4	ND	ND	ND	0.30	3.25	ND
C24:6	2.32	4.25	2.29	14.61	7.74	8.62
C24	3.55	ND	ND	ND	ND	ND

ND:Not detected

that other than the changes noticed in the fatty acid composition, the variation in the lipid source did not play a role in the body composition or fat metabolism in adipose tissue of mature rats.

In the fatty acid composition of the cardiac lipid (Table 7), the C16 and C18 assimilation was almost the same, irrespective of the dietary lipid source. In all the groups other than CF, the C18:1 content was higher. More of 20:1 was noticed in SFO/CF groups. C20:4 content was higher in SO + vitamin E and SLO + vitamin E groups and this observation is identical to that noticed in rats fed on sheep kidney fat incorporated diet (Charnock, 1984). The higher assimilation of C18:2 noticed in the lipids of the rats fed on SO/SLO diet, may be due to the peroxisomal retroconversion of DHA, as reported earlier (Hagve and Christopherson 1986). The data in the present study tend to support the view (Diplock 1983; Kagan 1989) that vitamin E in addition to protecting unsaturated fatty acids from oxidation stabilizes the membrane phospholipids by a controlling influence on the C18:2 and C20:4 residues.

The PUFA assimilation in the organ lipids of the rat fed with diet containing vitamin E were slightly higher (Tables 5, 6 and 7), signifying the antioxidant activity of the vitamin.

This study, thus, confirms the beneficial effects of vitamin E supplemented dietary fish oils in reducing triglycerides and cholesterol. At the same time, the increase in blood sugar levels in rats fed with dietary fish oil was insignificant.

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Deep-Fat-Frying Characteristics of *Urd Vada* in a Model System

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Moisture loss and oil uptake during deep-fat-frying of Indian traditional snack, *urd vada* made from *vada* mix, were studied. Moisture migration was dependent on frying temperature, initial moisture content of the dough and frying time. The oil content of the product increased slightly with increase in frying time and initial moisture content. Little quantity of the oil was absorbed by the core of the product. Surface heat transfer coefficient was estimated to be 939±54 W m^{-2} K^{-1}. A proposed empirical model, relating the moisture content of the product with frying temperature, time of frying and initial moisture content of the dough, correlated well (r= 0.916) with the experimental values.

Keywords: Deep-fat-frying, *Urd vada*, Moisture and oil migration, Heat transfer coefficient.

Like many food processing operations, frying of foods involves simultaneous heat and mass transfer (Mittelman et al. 1983). Apart from physico-chemical changes, the loss of moisture from the product and entrance of oil into the product (Gamble and Rice 1987) are of importance during frying, as these determine the product quality, process standardization and energy expenditure (Blumenthal, 1991). Process parameters of importance, during deep-fat-frying, include temperature of frying, initial moisture content of the raw material and time of frying (Mittelman et al (1983). A study of these parameters and of the temperature profile of the product during the operation, gives an insight into the heat and mass transfer characteristics of the process and product (Pravisani and Calvelo 1986).

Extensive work has been done on deep-fat-frying characteristics of potato chips, french fries and meat patties. For example, Gamble and Rice (1987) reported the relationship between oil uptake and moisture loss during frying of potato slices. Pravisani and Calvelo (1986) estimated the cooking time required for potato strip frying. Mittelman et al (1983) developed a mathematical model for moisture loss during potato slice frying and computed the heat transfer coefficient of the oil film for a solid copper sphere to be 1800 m^{-2} K^{-1}. Rice and Gamble (1989) also developed a mathematical model for moisture loss during potato slice frying. Dagerskog and Bengtson (1974) reported the heat transfer coefficient as 500 ± 200 W m^{-2} K^{-1} for a disc shaped product, such as meat patties at a medium temperature of 160°C. Rene et al (1986) reported that the heat transfer coefficient was about 900 W m^{-2} K^{-1}, at oil side during potato slice frying.

No information on deep-fat-frying characteristics of traditional Indian foods is available. The effect of frying time, frying temperature and initial moisture content of the dough on moisture loss and oil uptake was studied in case of deep-fat-frying of *urd vada*. Also, time temperature profile of the product during frying and estimation of heat transfer coefficient were investigated.

Materials and Methods

Urd, i.e., blackgram *(Phaseolus mungo)*, *vada mix*, commercially available and refined groundnut oil were procured from local market.

Dough preparation for frying: Known quantity of water was added to dry mix and mixed thoroughly. The ratio of dry mix to water taken was 2:1-1.8 in order to get the dough moisture in the range 60-90% (dry basis). Spherical dough balls of about 38 mm dia were made and the weight of each ball was noted. These balls were allowed to rest for 15 min for moisture to equilibrate.

Frying of vada: Refined groundnut oil (1 kg) was heated in a frying pan to the desired frying temperature, by using an electrical immersion heating coil (1 kw). The spherical dough ball (one at a time) was then dropped into the hot oil and frying was carried out at the set temperature, the oil being constantly stirred during frying by a laboratory mixer to get uniform temperature. All frying experiments were conducted for upto 5 min and each frying experiment was repeated thrice.

Temperature during frying: The temperature of the frying oil and the product were monitored, using metallic temperature probes (1.5 mm dia) and a temperature data logger (Century Instruments, Chandigarh, India). Frying was carried at five diffe-rent temperatures (155, 165, 172, 182 and 192°C).

Heat transfer coefficient: The temperature profile in the inner portion of the product indicated that, upon frying for about 7 min, a steady state was reached at all the positions inside the product and at all the frying temperatures studied, whereas, steady state was reached much earlier in the portion closer to the surface of the product. Assuming a linear boundary condition of the third kind or the convective boundary condition at the surface at the sphere (Ozisik 1987), i.e., at x = s

$$-k \frac{dT}{dx_{x=s}} = h \{T_s - T_\infty\} \quad \ldots\ (1)$$

The above equation was used to determine the heat transfer coefficient (h). The thermal conductivity of the surface layer (k) was estimated from its proximate composition using the empirical parallel model (Lewis 1987) given by $k = v_s k_s + v_w k_w$, where v_s and v_w denote the volume fraction of solids and that of water and k_s and k_w, the thermal conductivities of solids and that of water. The thermal conductivities of the solid fractions are as given by Miles et al (1983). The surface temperature (T_s) was estimated from the time-temperature profile. T_∞ is the frying oil temperature. Surface heat transfer coefficient, h, was determined at four different temperatures, viz., 192, 185, 176 and 173°C at an initial moisture content of 73.5, 89.8, 80 and 64.8% (dry basis), respectively. In the representative sample, $k = 0.288\ W\ m^{-2} K^{-1}$, $T_\infty = 192°C$ and $T_s = 188.85°C$ were used.

Moisture and oil content : After frying all samples were cooled for 2 min and then subjected to moisture and oil content estimation, in triplicate according to AOAC (1980) methods.

Statistical analysis: The results were statistically analyzed by determining mean ± standard deviation (SD), and compared in terms of coefficient of variation (cv), where cv = SD X 100÷Mean. Regression equations were obtained by Linear regression techniques using the method of least squares (Snedecor and Cochran 1968).

Results and discussion:

Migration of moisture and oil during frying: The *vada* mix consisted of (%): moisture 10.6, protein (N*X 6.25) 20, total ash 5.4, carbohydrates (by difference) 60 and fat 4%. Fig. 1 and 2 show the effect of frying temperatures (155-192°C) on moisture losses and oil uptake, respectively. At all frying temperatures, as frying progressed, moisture content of the product decreased, but the rate of moisture loss was high during initial stages of frying (<2.5 min) (cv=0.5 - 4.5% for moisture values and 0.5-15% for oil values). In case of very low moisture content, such as 60%, gradient in moisture loss is still higher even after 3 min (Fig. 3), which may be due to the formation of a thinner crust because of low initial moisture (Pravisani and

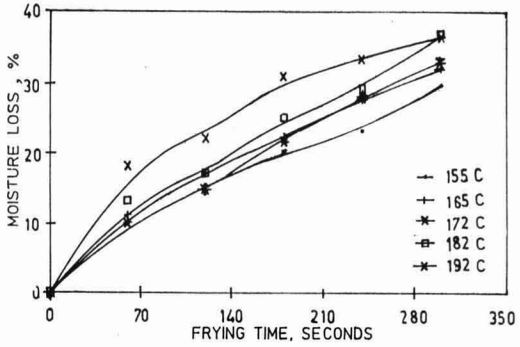

Fig.1. Moisture loss at different frying temperatures, when the initial moisture content was 68% (db).

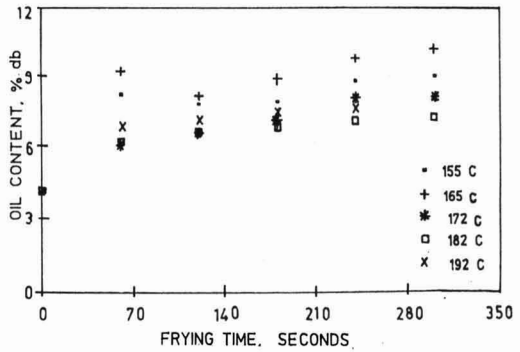

Fig.2. Oil content of product during frying at different frying temperatures, when initial dough moisture was 68%.

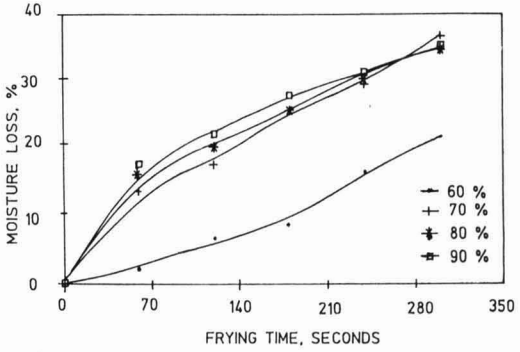

Fig.3. Effect of initial dough moisture on moisture loss during frying at 185°C.

Calvelo, 1986). As frying proceeds, the rate of moisture loss decreases due to the formation of hard crust on the product surface (Mittelman et al. 1983). The thickness of the crust increased with increase in frying temperature and also initial moisture. The thickness being 1.5-2.5 mm at 155°C, 2.5-3 mm at 170°C and 3-4 mm for 182 and 192°C oil temperatures after 3 min of frying. The observation on crust formation agrees with those of Mittleman et al (1983) and Pravisani and Calvelo (1986), reported in case of frying of potato strips.

The oil content of the product increases gradually with frying time at all temperatures. At lower frying temperatures (155 and 165°C), the oil content was slightly higher as compared to that at higher frying temperatures (172-192°C). Furthermore, at frying temperatures of 182 and 192°C, the oil was noticed to be just on the surface of the product. This was visually noticed, but the oil content was determined experimentally. Analysis of the crust showed around 12% (w/w) oil, whereas hardly any extra oil had been absorbed by the core, which was found to be around 5% (dry basis) and closer to the oil content of the raw material (ready mix) taken for frying. For a similar crust forming product, Guillaumin (1988) observed a similar phenomenon in potato strips; at the end of frying, these absorbed only 10% fat, all located on the surface of the fried food.

Figs. 3 and 4 show the effect of initial dough moisture (70-90%) on moisture loss and oil content of the product, respectively, during frying at 185°C. At low initial moisture content, such as 60%, the moisture loss was markedly lower than at higher (70-90%) moisture levels. Thus, as the initial moisture content of the dough increased, the rate

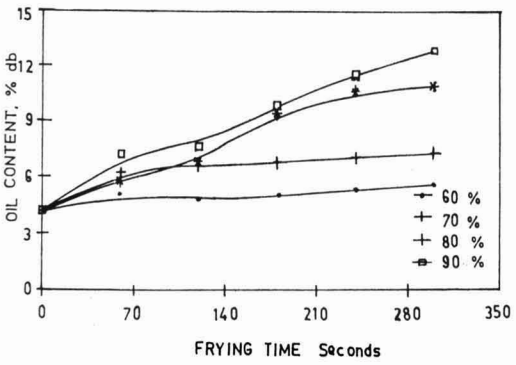

Fig.4. Oil content of the product during frying at 185°C with different initial dough moisture

of moisture loss upon frying also increased (cv=0.5-5.8%). The moisture loss values were not different in doughs, having 70-90% initial moisture. At any given frying time, the oil content of the product increased, in general, as the initial dough moisture level increased (Fig. 4) (cv=0.5-14%). The rate of oil pickup was rather low at lower (60-70%) initial dough moistures, as compared to higher dough moistures (80-90% db). Though there was a definite increase in all pickup from initial 4% to 10% in the product with time of frying. It varied little with frying temperature, and increased with increase in initial moisture. As the oil picked up by the product after frying was negligible (Fig. 2), the effect of oil content was considered insignificant (Indira 1992) and hence has not been included in the empirical model.

The product moisture (M) could be related to the frying time (Θ) at any frying temperature with an exponential equation (Eq 2) of the form:

$$M=A\ e^{-B\Theta} \qquad \ldots (2)$$

where A and B are empirical constants. In the present experiment, for the entire range of initial dough moisture and frying temperature studied, the correlation coefficient was 0.975, where A was found to be roughly constant with respect to frying temperature, but varied with initial dough moisture. Hence, the effect of initial dough moisture on the constant A was studied and found to be linearly related (r= 0.985). On the other hand, B was found to vary linearly with frying oil temperature and initial dough moisture as given below:

$$\ln M = a+\Theta(b+C*T_\infty)$$

where $a = -1.1333+1.0724*M_i$ (r=0.985)

$b = -0.0085+0.01303*M_i$ (r=0.955)

$c = -4.64*10^{-5} - 8*10^{-5}*M_i$ (r=0.996)

Hence, the general empirical equation relating product moisture (M), frying temperature (T_∞), frying time (Θ) and dough moisture (M_i) could be given by:

$$\ln (M)=(a_o+a_1M_i)+e[\{b_o+b_1M_i\}+T_\infty\ (c_o+c_1M_i)\} \qquad \ldots (3)$$

where a_o, a_1, b_o, b_1, c_o and c_1 are empirical constants given by: a_o=-1.1333, a_1=1.0724, b_o=-0.0085, b_1=0.01303, c_o=4.64E-05 and c_1=-8E-05.

The calculated moisture content (M) of the product correlated well with the experimental values (r=0.916) in the entire range of initial dough moisture, frying oil temperature and the time of frying. A plot of the calculated values of the product moisture, using Eq (3), against the experimental

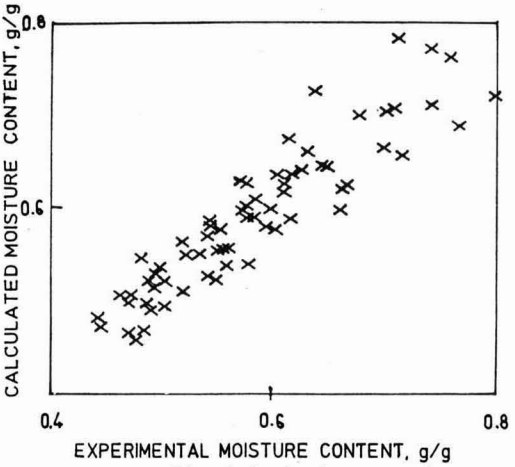

Fig. 5. Comparison of the calculated and experimental moisture content of the product during frying.

data is shown in Fig. 5. The percentage error in the moisture values from the calculated to the experimental was found to lie between -14 and +10.

Time-temperature profile and heat transfer coefficient: Fig. 6 shows the time-temperature profile at six different locations in the product during frying at an oil temperature of 192°C with an initial moisture level of 73.5%. The temperatures at different locations in the product attained steady state at different times, depending upon their distance from the surface of the product (Pravisani and Calvelo 1986). Heat transfer coefficient (h) was calculated by extrapolating the temperature profile to the surface so as to yield the surface temperature of the product. Three oil temperatures were used for finding h in this study and include 176, 173 and 185°C at an initial moisture of 80, 64.8 and

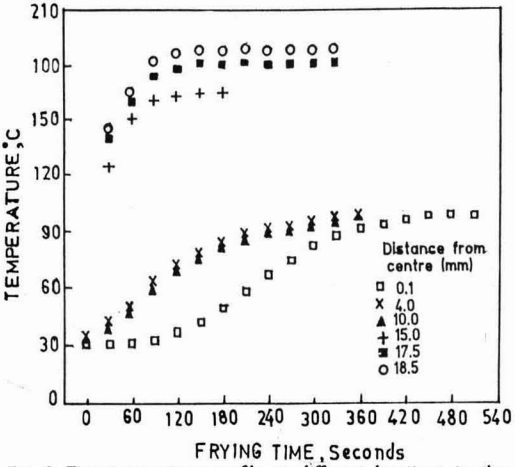

Fig. 6. Time-temperature profile at different locations in the product.

89.8 % (dry basis), respectively. Literature reports show wide variations from 300 to 1800 W m^2 k-1 in the values of heat transfer coefficients during frying. However, the heat transfer coefficient observed in present studies was 939 ± 54 W m^2 k-1. It was not clear, if the value of h varied with initial moisture in the range studied.

In conclusion, the present investigation indicates that moisture migration is sensitive to frying process variable, such as, frying oil temperature, initial moisture content of the dough and frying time. The thickness of the crust formation increases with the oil temperature and also initial moisture. The moisture content at any frying temperature is exponentially related to frying time.

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Nutrient Composition of Used Coffee Grounds and Their Supplementary Effects

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Analysis of used coffee grounds and raw coffee seeds indicated that the protein and fat contents were nearly the same.Unsaturated fatty acids constituted nearly 50%, while polyunsaturated fatty acids accounted for one third of the total fatty acid content of the seed fat. During roasting of coffee seeds and extraction of soluble coffee, some of the amino acids were destroyed. Tryptophan was the limiting amino acid of raw coffee seed proteins. Increasing the used coffee grounds in the diets from 0 to 15% had no effect on the food intake of animals. However, as the level of used coffee grounds increased, gain in body weight, protein efficiency ratio, net protein utilization, protein and dry matter digestibilities decreased significantly, the former being significantly negatively correlated to all the later attributes.

Keywords : Used coffee grounds, Raw coffee seeds, Nutrient composition, Amino acid composition, Fatty acid profile, Protein efficiency ratio, Net protein utilization.

India occupies sixth position in coffee seed production in the world (Anon 1994). It was estimated that about 210,000 tonnes of coffee seeds were produced in the country during 1991-92 (Anon 1994). Out of this, 60,000 tonnes were consumed domestically and the remaining quantity was exported (Anon 1995). India earns 300 crores of rupees by coffee export (Anon 1995). Instant or soluble coffee production in India is increasing gradually. During 1990-91, soluble coffee production was 2904 tonnes and it increased to 3307 tonnes during 1992-93 (Joseph 1993).

As a by-product of soluble coffee industry, around 10,000 tonnes of used coffee grounds are available in the country (Joseph 1993). There are no avenues for its use at present, except as a fertilizer (Joseph 1993). But, information on the analysis of used coffee grounds is scanty. Natarajan and Gopalakrishna Rao (1968) and Gopalakrishna Rao and Natarajan (1974) have analysed used coffee grounds for their proximate composition and fatty acid profile. Hence, used coffee grounds were analysed for their chemical composition, fatty acid profile and amino acid contents. Finally, an attempt was made to evaluate the protein quality of used coffee grounds in rats. Raw coffee seeds were studied as control.

Chemical composition : Samples of 'c.robusta' coffee grounds were obtained from Brooke Bond India Ltd., Ghatkesar plant. Used coffee grounds employed in this study were from 'c.robusta' seeds only, but not from coffee chicory blends. These were dried at 100°C overnight, ground initially in a coffee grinder followed by in SHEMIL to pass through 60 mesh and pooled. Raw coffee seeds were purchased in the local market, cleaned and ground before analysis. Protein (N x 6.25) was estimated by Kjeldhal method while fat, ash, fibre and minerals were determined by AOAC methods (1990). Trace elements were estimated, using an atomic absorption spectrophotometer (Varian Techtron, model AAS 1000) (AOAC 1990).

Fatty acid composition : Fat, extracted with solvent ether from used coffee grounds and raw coffee seeds, was subjected to methanolysis and transesterification to yield methyl esters of fatty acids. The pattern of distribution of fatty acids as methylesters was monitored, using GLC (Varian model 3700), equipped with flame ionisation detector (Kishimoto and Hoshi 1972). The stationary phase consisted of 10% Silar 10 C adsorbed on chromosorb W and was packed in 10 ft column of 1/8" dia. The column temperature was maintained at 180°C. The temperature of injector and that of detector were 210°C and 230°C, respectively.

Amino acid analysis: Hundred mg each of used coffee grounds and raw coffee seed powder were hydrolysed in 6 N constant boiling hydrochloric acid at 110°C for 20 h in evacuated sealed ampoules. After hydrolysis, excess acid was removed by flash evaporation under reduced pressure. Amino acid analyses were carried out by ion exchange chromatography in an automatic amino acid analyser (Moore et al. 1958). Tryptophan content was estimated in an alkaline hydrolysate by microbiological method, using *Leuconostoc mesenteroids* P-60 according to the method of Barton-Wright (1946).

Biological evaluation: Two animal experiments were carried out in this study. In the first experiment, used coffee grounds were sole the source of proteins, while casein-based diets were supplemented either with used coffee grounds or raw coffee seeds

TABLE 1 COMPOSITION OF EXPERIMENTSL DIETS %

	Experiment 1		Experiment 2						
	III	IV	III	IV	V	II	VII	VIII	IX
	Used Coffee Grounds								
	Raw diet	Cooked diet	Casein + 5% UCG	Casein + 10% UCG	Casein + 15% UCG	RCS	Casein + 5% RCS	Casein + 10% RCS	Casein + 15% RCS
Casein	-	-	11.6	10.7	9.8	-	11.6	10.7	9.8
Used coffee grounds	70	70	5	10	15	-	-	-	-
Raw coffee seeds	-	-	-	-	-	70	5	10	15
Starch	14	14	67.4	63.3	59.2	14	67.4	63.3	59.2

In both the experiments, animals belonging to group 1 received protein-free and group 2 animals 10% casein control diets. All the above diets contained in addition to the nutrients mentioned in the table, 1% vitamin mixture, 1% of 50:50 of choline chloride and starch mixture, 4% salt mixture as well as 10% oil. UCG = Used coffee grounds, RCS = Raw coffee seeds

at 0,5,10 and 15% levels in the second experiment. All the diets were adjusted to contain 10 g% proteins, except the protein-free group.

In the first experiment, 24 weanling 'Wistar' male rats, 21 days old, weighing around 40 g were randomly distributed into 4 groups of six each. Composition of experimental diets is given in Table 1. All the diets were complete with respect to all the nutrients, except diet 1, which was protein-free diet. Animals were kept individually in raised bottom cages. Food and water were given *ad lib.* Records of daily food intake and weekly body weight changes of individual animals were recorded. Animals receiving cooked or uncooked used coffee ground diets, died during the second week of the experiment and the experiment was terminated. Weanling mice and one day old chicks fed cooked or uncooked used coffee ground diet also died between 10 and 15 days of the experiment.

In the second experiment, 54 weanling male rats were randomly distributed into 9 groups of six each (Table 1). Experimental diets were fed for a period of 28 days. Other details were as given in experiment No. 1. During the last 3 days of the experiment, faecal samples of individual animals were collected and their nitrogen contents were estimated. From the data, dry matter and protein digestibilities were calculated (Campbell 1963). At the end of the experiment, all the animals were sacrificed and the carcasses were hydrolyzed in 6N hydrochloric acid at 1.05 kg/sq cm pressure. Net protein utilization was calculated according to Miller (1963). Diet No. VI was also fed to weanling mice and one-day old chicks. Data were analysed for statistical significance by Duncan's multiple range test.

Protein and fat contents of used coffee grounds and raw coffee seeds were found to be similar (Table 2). Ash content of used coffee ground was much lower than that of raw coffee seeds. This may be due to leaching of minerals, during soluble coffee preparation and this is also true for calcium, phosphorus and magnesium contents of used coffee grounds. Crude fibre contents of used coffee grounds were very high.

Fatty acid levels of used coffee grounds and raw coffee seeds were similar (Table 2). Unsaturated fatty acids constituted nearly 50%, while polyunsaturated fatty acids accounted for 1/3 of the total fatty acid contents of these fats. Coffee seed fat is a rich source of linoleic acid. Subramanyam and Achaya (1957) reported similar fatty acid profile for raw coffee seed fat and roasting did not affect the composition of the seed fat.

Coffee seed protein is a good source of lysine, cystine and methionine. But, it is deficient in tryptophan. Due to roasting of coffee seeds, losses of amino acids occurred in used coffee grounds protein. This may be due to pyrolysis of amino acids, due to high temperature used for roasting of coffee seeds. Maillard products formed during roasting of coffee seeds were shown to be mutagenic (Nagao 1979).

Weanling rats and mice as well as one-day old chicks, fed on diets (before and after cooking), containing used coffee grounds and raw coffee seeds as a sole source of proteins died during the second week of experiment. Food intake of animals on these diets was very low (1-2 g/day/animal). Cooking of diet did not improve the food intake in the animals. The reduced food intake by the animals may probably be due to unpalatability of diets, which in turn, caused the death of animals.

Increasing the used coffee grounds content in the diet from 0 to 15% had no effect on the food intake of animals (Table 3). However, as the used coffee grounds content increased in diet, the

TABLE 2. NUTRIENT COMPOSITION OF USED COFFEE GROUNDS AND RAW COFFEE SEEDS

	Used coffee grounds	Raw coffee seeds		Used coffee grounds	Raw coffee seeds
Constituent			**Amino Acid (g/16gN)**		
Moisture, g %	8.7	6.3	Lysine	4.5	1.9
Protein, g %	13.9	14.2	Histidine	1.7	1.1
Fat, g %	10.3	10.7	Arginine	4.6	N.D
Ash, g %	2.5	4.8	Aspartic acid	5.8	1.9
Crude fibre, g %	44.5	N.E	Threonine	2.7	2.2
Phosphorus, mg/100g	66.0	168.0	Serine	4.0	1.3
Calcium, mg/100g	45.0	86.0	Glutamic acid	15.8	7.4
Magnesium, mg/100g	32.0	70.0	Proline	3.8	N.D
Zinc, mg/100g	2.1	1.7	Glycine	5.2	3.0
Copper, mg/100g	2.9	2.0	Alanine	3.6	3.3
Manganese, mg/100g	2.9	2.9	Cystine	2.6	N.D
Chromium, mg/100g	0.3	0.2	Valine	3.5	3.5
			Methionine	2.4	N.D
Fatty acid composition			Isoleucine	5.1	2.7
			Leucine	8.2	6.6
16:0, %	46	40	Tyrosine	2.1	2.0
18:0, %	7	4	Phenylalanine	4.2	3.7
18:1, %	16	16	Tryptophan	0.4	N.D
18:2, %	31	36	Chemical score	26.0	
18:3, %	Tr	4			
Total saturates, %	53	44			
Total unsaturates, %	47	56			
Poly unsaturates, %	31	40			

N.E. = Not estimated
N.D. = Not detected.

parameters such as gain in body weight, protein efficiency ratio, net protein utilization, protein and dry matter digestibilities decreased gradually. Even though food intake of animals receiving diets containing 0-15% used coffee grounds was not different, gain in body weight of rats decreased progressively as the used coffee ground content, increased from 0 to 15%. This may be due to reduced dry matter and protein digestibilities of these diets observed in this study.

Crude fibre content of used coffee grounds was high. As the used coffee grounds content increased in the diet, the dietary fibre content of the diets increased concomitantly. During roasting of coffee seeds, some of the amino acids were destroyed due to pyrolysis. This may partly explain the decreased

TABLE 3. SUPPLEMENTARY EFFECT OF USED COFFEE GROUNDS AND RAW COFFEE SEEDS TO CASEIN-BASED DIETS

	II	III	IV	V	VI	VII	VIII	IX
	Casein	Casein + 5% UCG	Casein + 10% UCG	Casein + 15% UCG	Casein	Casein + 5% RCS	Casein + 10% RCS	Caseon + 15% RCS
Food intake, g/4 weeks	324±4.5^{a}	313±4.5^{a}	328±5.3^{a}	314±2.8^{a}	324±4.5^{a}	272±5.4^{b}	229±3.6^{c}	207±6.9^{d}
Gain in body weight, g/4 weeks,	112±3.5^{a}	98±4.9^{b}	96±0.7^{b}	84±1.4^{c}	112±3.5^{a}	81±2.9^{b}	53±1.3^{c}	44±3.4^{d}
Protein efficiency ratio	3.424± 0.0576^{a}	3.221± 01154^{a}	2.865± 0.0580^{b}	2.765± 0.0928^{b}	3.424± 0.0576^{a}	3.066± 0.0859^{b}	2.271± 0.0584^{c}	2.179± 0.1225^{c}
Dry matter digestibility, %	95±2.2^{a}	91±2.6^{b}	86±1.7^{c}	80±0.8^{d}	95±2.2^{a}	92±0.6^{b}	90±0.6^{c}	88±0.2^{d}
Protein digestibility, %	86±0.5^{a}	81±0.5^{b}	77±0.4^{c}	67±1.6^{d}	86±0.5^{a}	84±0.1^{a}	80±3.5^{b}	77±0.3^{c}
Net protein utilisation	68±1.1^{a}	63±2.0^{b}	56±1.1^{c}	55±0.8^{c}	68±1.1^{a}	63±1.4^{b}	50±1.3^{c}	43±1.6^{d}

Values with the same superscript are not different, UGC= Used coffee grounds, RCS=Raw coffee seeds

dry matter and protein digestibilities of diets, as the used coffee grounds content increased progressively from 0 to 15% in the diets. As the used coffee grounds content increased in the diet from 0 to 15%, the available energy and amino acids were reduced, due to low dry matter and protein digestibilities as well as due to pyrolysis of amino acids during roasting of coffee seeds and due to high temperature and pressure used for the extraction of soluble coffee. Increasing the raw coffee seeds content of diets from 0 to 15% brought about a progressive decrease in food intake, gain in body weight, protein efficiency ratio, net protein utilization, dry matter and protein digestibilities (Table 3).

Significant negative correlations were observed between % used coffee grounds and raw coffee seeds in diets and gain in body weight (r = -0.7905, -0.9460), protein efficiency ratio (r = -0.8195, -0.9031), dry matter digestibility (r = -0.9773, -0.8464), protein digestibility (r = -0.9446, -0.8751) and net protein utilization (r = -0.8373, -0.9462). In the case of food intake, used coffee grounds had no effect, but a significant negative correlation existed between % raw coffee seeds in the diet and food intake of the animals (r = -0.9514). On the other hand, significant positive correlations existed between gain in body weight and protein efficiency ratio (r = 0.8919) dry matter digestibility (r = 0.7784), protein digestibility (r = 0.7642) and net protein utilization (r = 0.8136) in case of used coffee grounds containing diets. Similar significant positive correlations were also observed in animals receiving raw coffee seed diets between food intake and gain in body weight (r = 0.9836), protein efficiency ratio (r = 0.9235), dry matter digestibility (r = 0.8993), protein digestibility (r = 0.8410) and net protein utilization (r = 0.9005).

Carew Jr et al (1967) observed even at 2.5%, deoiled raw coffee meal decreased feed intake and growth rate of chicks. With 10% deoiled coffee meal, the mortality was 14/50 and at 20% it was 43/50. Addition of used coffee grounds even at 5% level caused significant reduction in weight gain by the rats in the present study. Hence, used coffee grounds may not be suitable as an ingredient of livestock feed.

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Effect of Soy Milk Supplementation on the Microbiological Quality of Cheese Made with Microbial Rennet *(Mucor miehei)*

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Cheddar cheeses were prepared from cow milk (using calf and microbial rennets) and blends of cow milk and soy milk (95:5, 90:10, 85:15, 80:20 and 75:25, using microbial rennet) and ripened at 8+1°C (Equilibrium Relative Humidity 75-80%) for 240 days. An increase in microbiological counts (log_{10} cfu/g cheese) were observed in cheese samples made from increasing proportions of soy milk in the blend. All microbiological counts initially increased during ripening followed by decreasing trend till end of the ripening.

Keywords : Soy cheese, Cow milk, Microbial rennet, Ripening period, Microbiological counts.

The total production of milk in India is estimated to be 57.10 million tonnes per annum, of which very small part is converted into cheese. Food and Agriculture Organization estimated that more than 40% of the total world milk production is converted into cheese (FAO 1991). Cheese production is considered to be a way of conserving selective constituents of milk. Cheddar, a medium hard cheese, is the most popular variety because of its keeping quality, transportability, consumer acceptability and mild flavour (Scott 1979). Compositional and biochemical changes in cheese manufactured from cow milk and soy milk blends using calf and microbial rennets were studied by Meenakshi Rani and Verma (1995). Soybean, with its 38-42% proteins (Schroder et al. 1973) makes it an excellent and economic source of proteins. Soy proteins are unique among plant proteins by virtue of their relatively high biological value, and essential amino acid contents (Schroder et al. 1973). Hence, soy milk has the potential for replacing cow milk, at least partially, in the production of cheddar type cheese (Del Valle et al. 1984). Present investigations were conducted to study the microbiological changes due to substitution of cow milk, with soy milk using microbial rennet in cheddar cheese preparation and ripening.

Materials : Cow milk and mature soybeans, (Variety 'PK-262'), were obtained from the Livestock Research Centre and University Farm, respectively. Pure culture of *Streptococcus lactis* was obtained from the National Dairy Research Institute, Karnal. Bulk culture was prepared by using skim milk (Scott 1981). Modilase, double strength microbial rennet, produced from *Mucor miehei* was supplied gratis by Chr. Hansens Laboratory Pvt. Ltd., Adelaide, Australia.

Preparation of soy milk and cheese: Soy milk was prepared according to the method described by Nelson et al (1976). Cow milk was pasteurized to 63°C for 30 min and soy milk heated to 100°C for 5 min, was added to it in different proportions (95:5,m 90:10, 85:15, 80:20 and 75:25). The casein to fat ratio in all blends was adjusted to 0.70. Bulk starter culture (1.5%, v/v) was added to milk at 30°C, and incubated for 30 min. Calf and microbial rennets were added to different blends of cow milk and soy milk at the rate of 0.0015% and 0.001%, respectively, at 30°C. The resultant curd was cut with cheese knives and scalded for 50-60 min at 1°C rise/min upto 37-39°C. After scalding, whey was drained off followed by cheddaring until acidity reached 0.55%. The curd was milled, salted at 2% level (w/w), and pressed. Green cheese samples were paraffined,and ripened at 8±1°C (equilibrium relative humidity 75-80%) for 240 days and analysed for various microbiological counts at an interval of one month.

Analytical method: The cheese samples were analysed for various microbiological changes according to the method described by Speck (1976). 1 g cheese was asceptically weighed and ground in pestle and mortar. Dilution blanks for various microbiological counts were prepared. For determination of various counts the specific media was sterilized (temp=121°C, time=20 min) and cooled to 45°C, inoculated with different samples dilutions (1 ml), incubated for suitable periods, temperatures and then counted using colony counter.

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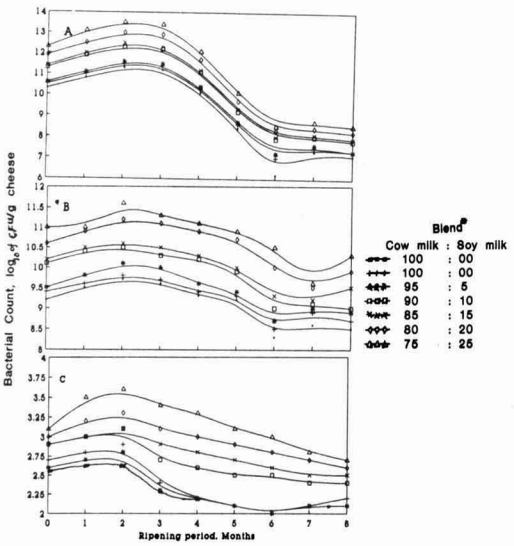

Fig. 1a. Effect of soy milk and ripening period on microbial growth in cheese. Each value is mean of six determinations.

* Calf and microbial rennets were used for clotting cow milk, while microbial rennet alone was used for clotting cow milk:soy milk blends.

A= Total viable count, B=Lactic acid bacteria count, C=Yeast and mould count.

Composition of cow milk, soy milk and the cheese made from their blends reported by Meenakshi Rani and Verma (1994). It is apparent from Fig. 1a that the initial values for total viable, lactic acid bacteria, yeast and mold counts in cheese made with calf and microbial rennets were

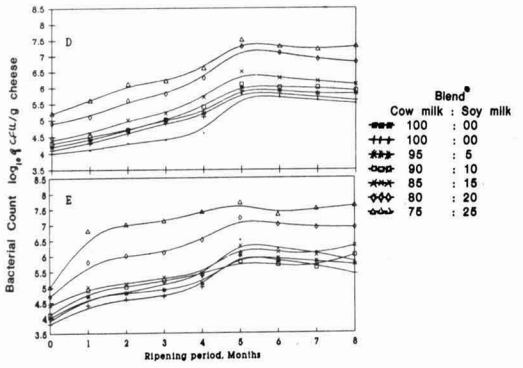

Fig. 1b. Effect of soy milk and ripening period on microbial growth in cheese. Each value is mean of six determinations.

* Calf and microbial rennets were used for clotting cow milk, while microbial rennet alone was used for clotting cow milk:soy milk blends.

D=Proteolytic count, E=Lipolytic count.

10.3, 9.2, 2.6 and 10.5, 9.4, 2.7 $\log_{10}$ cfu/g cheese, respectively. At 240 days these three counts were 7.1, 8.5, 2.1 and 7.3, 8.7, 2.2 log10 cfu/g cheese made with calf and microbial rennets, respectively. These three microbiological counts in green cheese made from cow milk to soy milk ratio of 95:5 and 75:25 were 10.6, 9.5, 2.6 and 12.3, 11.0, 3.1 $\log_{10}$ cfu/g cheese, respectively. The total viable, lactic acid bacteria, yeast and mold counts showed an increasingly trend upto 60 days, thereafter, a decreasing trend was observed (240 days). At 240 days, the above three counts in soy cheese made from 95:5 and 75:25 cow:soy milk blends were 7.3, 8.9, 2..1 and 8.5, 10.3, 2.7 $\log_{10}$ cfu/g, respectively.

The increase in total viable, lactic acid bacteria, yeast and mold counts upto the two months was probably due to prolific growth of lactobacilli and thereafter, the decreasing trend was probably due to the depletion of energy source and increase in acidity (John and Cole 1959; Franklin and Sharpe 1963; Prasad et al. 1983).

The results of proteolytic and lipolytic counts of cheese made from cow milk and cow:soy milk blends using calf and microbial rennets are shown in Fig. 1b. The initial proteolytic and lipolytic counts for cheese made with calf rennet were 4.0, 3.9 $\log_{10}$ cfu/g cheese, respectively. The proteolytic and lipolytic counts in green soy cheese ranged from 4.2 to 5.2 and 4.0 to 5.0 $\log_{10}$ cfu/g cheese, being lowest and highest in soy cheese made from cow milk to soy milk ratio of 95:5 and 75:25, respectively. The proteolytic and lipolytic counts showed an increasing trend till 150 days, thereafter, a decreasing trend was observed till end of the ripening period in all the samples. The observed decrease in proteolytic count after 150 days was probably due to depletion of nutrients, high acidity and accumulation of metabolites which were mainly detrimental for the growth of organisms (Davis 1965; Reddy 1982). Our results agrees with the findings of Green and Foster (1974); Prentice and Brown (1983); Tewari and Chakraborty (1987); Mabbitt et al (1987); Gooda et al (1988) who observed similar growth trend of various bacterial population during ripening of cheese. The decline in growth of lipolytic bacteria in the later stages of cheese ripening, could be attributed to increased amounts of lactic acid, free fatty acids (Reddy 1982). The observed increase in various counts with increased proportions of soy milk could be attributed to the higher moisture and soluble nitrogen contents in cheese made from various cow:soy milk blends.

It is concluded that cheese samples having more proteolytic and lipolytic counts were due to higher proteolytic and lipolytic activities of microbial and calf rennet, respectively. However, microbiological counts in cheese made from the blends containing 15% soy milk were found to be negligible.

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Bamboo Parts and Seeds for Additional Source of Nutrition

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Different edible parts of seven bamboo species were analyzed for their nutrient contents viz., total carbohydrates, proteins,vitamin C and minerals. It was found that among all the edible parts, total carbohydrate content was highest in the seeds of *Bambusa arundinacea* (38.0%), proteins in the seeds of *Dendrocalamus strictus* (13.54%) and vitamin C in the seeds of *Bambusa arundinacea* (50 mg/100g). The nutrient contents compared favourably with those of *Cajanus cajan* (arhar) and *Triticum aestivum* (wheat), which constitute the staple diet of the majority of Indians.

Keywords: Bamboos, Edible parts, Nutrition, Nutrients, Supplement diet.

Young shoots of both running and clump forming bamboos are eaten. The tender shoots of *Dendrocalamus giganteus, D. hamiltonii, D. membranaceous* and *D. strictus* are consumed either as vegetables or as pickles. *Bambusa polymorpha* is considered as one of the best in the world for producing quality edible shoots, which have a distinct sweet taste in the raw state (Kennard and Freyre 1957). The tender shoots of *B. balcooa* are cooked and eaten, but are also generally preserved after fermenting and drying. For the preparation of edible products, the tender portions are cut into rings or pieces of suitable size and then steamed to remove hydrocyanic acid. The shoots are then ready to be consumed in various forms.

In India, the seeds of *B. arundinacea, Cephalostachyum pergracile* and *D. strictus* are extensively eaten by the poor during famines. The seeds are pickled and candied and used for making beer (Raizada and Chatterjee 1956). The leaves of a number of bamboo species are much valued as fodder, particularly during scarcity. Young bamboo leaves and twigs are a favourite fodder of elephants and cattle.

Reports of analyses of nutrient contents of edible bamboos are rather limited. Therefore, the authors have endeavoured to probe further the potential of edible bamboos in supplementing the nutrient deficient diets of the tribals and other populace, deriving sustenance from forests. The edible shoots of two species of bamboo viz., *Bambusa vulgaris* and *Melocanna baccifera* and seeds of *Bambusa arundinacea, B. nutans* and *Dendrocalamus strictus* have been analysed for their vitamin C, carbohydrates, protein and mineral contents. The leaves and shoots of *B. arundinacea, B. polymorpha, D. longispathus* and *Melocanna baccifera* have also been analysed with a view to supplementing animal feed.

Succulent shoots and leaves of edible bamboos were collected from the TFRI campus. Seeds were obtained from the seed bank of the Institute. Vitamin C, proteins, carbohydrates, minerals and moisture contents were analysed. Vitamin C was determined by the method of Sadasivam and Manickam (1992). Protein was analysed by the method of Lowry et al (1951). Na and K were determined by Flame photometry, Ca and Mg volumetrically and phosphorus by colorimetry (Jackson 1973). Total carbohydrate content was estimated by Anthrone method (Hegde and Hofreiter 1962).

Results and analysis of proteins, vitamin C, total carbohydrates, minerals and moisture contents of edible shoots, leaves and shoots and seeds of seven bamboo species are given in Table 1. The highest content of vitamin C (mg/100g) was found in the seeds of *Bambusa arundinacea* (50), and *D. strictus* (28) followed by the leaves and shoots of *D. longispathus* (23), as compared to arhar (25) (Wealth of India 1992). Total carbohydrates were highest in the seeds of *B. arundinacea* (38%) and *B. nutans* (36%) (arhar 16.9% and wheat 87.9%). The seeds of *D. strictus* (13.54%), leaves and shoots of *B. polymorpha* (7.46%) and edible shoots of *Melocanna baccifera* (7.36%) had appreciable protein contents (arhar 9.8% and wheat 8.8%-9.8%). The minerals estimated were sodium, potassium, calcium, magnesium and phosphorus. Sodium content ranged from 0.02 to 0.40%, potassium from 0.22 to 1.4%, calcium from 0.32 to 0.56%, magnesium from 0.05 to 0.19% and phosphorus from 0.08 to 0.22%.The above results indicate that edible bamboo parts can be a good dietary supplement to both humans and animals.

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TABLE 1. NUTRIENT ANALYSIS OF EDIBLE BAMBOOS PARTS

Species	Plant part	Moisture, %	Protein, g %	Total carbo-hydrates, g %	Vitamin C, mg/100g	Minerals, %				
						Na	K	Ca	Mg	P
Bambusa vulgaris	Shoot	77.0	4.16	5.0	13.7	0.40	0.92	0.32	0.10	0.22
Melocanna baccifera	Shoot	84.0	7.36	2.0	12.5	0.02	0.24	0.40	0.05	0.14
	Leaves and shoot	54.0	5.86	21.0	15.0	0.10	0.46	0.48	0.19	0.08
Bambusa polymorpha	Leaves and shoot	74.0	7.46	8.0	17.5	0.12	0.56	0.56	0.14	0.10
Dendrocalamus longispathus	Leaves and shoot	19.0	3.20	ND	23.0	0.16	1.30	0.56	0.09	0.19
Bambusa arundinacea	Leaves and shoot	54.0	5.33	10.0	7.5	0.14	1.40	0.40	0.05	0.15
	Seed	ND	7.52	38.0	50.0	0.18	0.22	0.40	0.14	0.19
Bambusa nutans	Seed	ND	4.48	36.0	30.0	0.18	0.32	0.48	0.09	0.21
Dendrocalamus strictus	Seed	ND	13.54	26.0	28.0	0.09	0.22	0.48	0.09	0.19

The value of bamboo shoot as food is based not only on its total fresh weight, but also on the edible portion, which amounts to about 27%. A toxic constituent, taxiphyllin has been isolated from the shoots of *B. guadua, B vulgaris, B. arundinacea, D. giganteus* and *D. hamiltonii.* However, this can be removed by several changes of water during cooking or by presoaking for a long time in several changes of a solution of 2% salt. Bamboo shoots also contain cyanogenic glycosides, which on endogenic hydrolysis, produce toxic hydrocyanic acid, varying from 0.05 to 0.3 %. Another constituent of the bamboo shoot known as homogentisic acid is responsible for the disagreeable pungent taste of bamboo shoots.

The edible shoots were found to contain appreciable quantities of vitamin C, carbohydrates, proteins and mineral contents. Thus, this easily available source of nutrients from the forests should be consumed for increasing the nutrition as well as safeguarding against possible deficiencies. The succulent leaves and shoots can be most nutritious, when consumed by animals. A study to continue with the present work is proposed to evaluate the seasonal variations in contents so that the leaves could be collected at the stage of highest nutritive value for conversion to a meal for feeding animals.

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Inhibitory Activity of *Lactobacillus acidophilus* Against Different Pathogens in Milk

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Lactobacillus acidophilus-301 exhibited inhibitory activity against *Salmonella typhi, Escherichia coli, Proteus vulgaris, Yersinia enterocolitica* and *Staphylococcus aureus*, when grown in milk as a mixed culture. The antagonism shown by this organism against different pathogens was not due to acid production alone. This activity may also be attributed to elaboration of antibiotic-like compounds by this organism.

Keywords: *L. acidophilus*, Antagonistic activity, Enteric pathogens, Food-borne pathogens, Antibiotic-like compounds, Lactic acid bacteria.

Inhibitory activity of *Lactobacillus acidophilus* against pathogenic species is an important criterion for its use as a dietary adjunct (Gilliland 1989; Mital and Garg 1992). Milk is an essential component of diet. Hence, it has been used to develop products to facilitate ingestion of this organism. Some such products are: sweet acidophilus milk, aco-yoghurt, biogarde, biolact, A-38, bioghurt, dietary yoghurt (Kurmann et al. 1992). Contamination of milk and milk products with pathogenic organisms during their processing is a recurring problem. There are many reports about the inhibitory activity of *L. acidophilus* against several Gram positive and Gram negative organisms (Gilliland and Speck 1977; Mehta et al. 1983; Appella et al. 1992). However, information on antagonistic activity of this organism in milk and milk products is scanty. The objective of this study was to determine the inhibitory activity of *L. acidophilus* against common intestinal and food-borne pathogens, when grown simultaneously in milk.

Fresh cow milk (fat 4.3%; T.S. 14.0%) obtained from Livestock Research Centre of the University was used. *Lactobacillus acidophilus*-301 was obtained from National Dairy Research Institute, Karnal, India. It was maintained by bi-weekly transfers in sterile litmus milk at 37°C for 16 h and was held at 4 ± 1°C between transfers. The sources of pathogenic species of bacteria were:Indian Veterinary Research Institute, Izatnagar, India for *Salmonella typhi*-83, *Escherichia coli*-6 and *Proteus vulgaris*-204; Wisconsin University, Madison, Wisconsin, USA for *Staphylococcus aureus* C2-T10 and National Institute of Public Health, Geitmyrsveien, Oslo, Norway, for *Yersinia enterocolitica*-03. The stock cultures of these organisms were grown on nutrient agar slants at 37°C for 16-24 h and maintained at 4 ± 1°C between quarterly transfers. Brain heart infusion (BHI) broth was used to propagate these organisms in liquid medium. All the cultures were sub-cultured 2-3 times prior to use and routinely checked for their purity.

Sterilized milk (200 ml) was brought to incubation temperature (37°C), and inoculated individually with 0.2 ml of test pathogen culture grown in BH1 broth, (cell density 3.0-6.0 x 10^8/ml), followed by active *L. acidophilus* culture @ 2.5%, grown in sterile skim milk (cell density 4.5 x 10^9/ml). The control consisted of sterilized milk inoculated only with the test pathogen in the same manner as the experimental. Acidified milk samples were prepared by inoculating milk with the test pathogen and adjusting its pH equivalent to that of experimental samples at two hour intervals, using 20% sterilized lactic acid. The inoculated samples were incubated at 37°C for pre-determined periods and the population of the test pathogen was estimated according to APHA (1972) procedures, using selective medium. The selective media used were:brilliant green agar for *S. typhi*; mannitol salt agar for *Staph. aureus;* violet red bile agar for *E. coli* and McConkey's agar for *P. vulgaris* and *Y. enterocolitica*. The changes in pH were measured by using a digital pH meter (Century Instrument, Chandigarh). Percent inhibition of the test pathogen at selected intervals was estimated according to the following equation as suggested by Gilliland and Speck (1977).

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$$\% \text{ Inhibition} = \frac{(\text{cfu/ml in control sample}) - (\text{cfu/ml in experimental sample})}{(\text{cfu/ml in control sample})} \times 100$$

The growth of different pathogens in milk as a mixed culture with *L. acidophilus* is shown in Fig. 1. Fermenting organisms produce acid during their growth in milk. Therefore, to assess the effect of acid, antagonistic activity of acidified milk against different pathogens was also investigated. All the pathogens showed increases in their population in the control milk sample throughout the incubation period. In acidified milk, the growth of all the pathogens was more or less the same, as observed in their respective controls upto 4 h of incubation. Thereafter, the inhibitory effect of acid manifested itself and population of all the pathogens tested declined. In milk inoculated with individual pathogen and *L. acidophilus*, the population of the pathogens, increased but at a much slower rate than observed for the corresponding controls and acidified milk samples. After 8 h, the population of all the pathogens in mixed culture samples showed a steep decline. Table 1 shows the percent inhibition of different pathogens when grown in acidified milk and as a mixed culture with *L. acidophilus*. In acidified milk, no inhibition of *Staph. aureus*, *P. vulgaris*, *E. coli* and *Y. enterocolitica* was recorded upto 4 h, whereas *S. typhi* showed 8.0% inhibition. In contrast, different pathogens showed 85 to 97% inhibition when grown with *L. acidophilus* during the same period of incubation. In 8 h of incubation, significant degree of inhibition, was observed in both acidified (61.0-89.0%) and mixed cultured milk (92-99%) samples. Earlier researchers have also made similar observations about antagonistic activity of *L. acidophilus* (Gilliland and Speck, 1977; Mehta et al. 1983; Apella et al. 1992). Attaie et al (1987) compared inhibition of *Staph. aureus* during production of acidophilus yoghurt, acidified milk and regular yoghurt and noted much greater inhibition of this organism in the former than in the latter two. Apella et al (1992) found that inhibition of *Shigella sonnei* began at 6 h of incubation and its death phase at 9 h, when this organism was grown with *L. acidophilus* as a mixed culture.

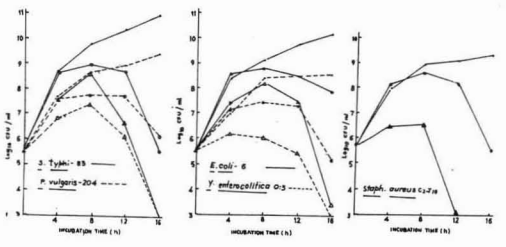

Fig 1. Growth of different pathogens with *Lactobacillus acidophilus*-301 in mixed cultures (●——● Control; o——o Acidified milk; Δ——Δ Milk + *Lactobacillus acidophilus*)

TABLE 1. INHIBITION OF DIFFERENT PATHOGENS GROWN IN ACIDIFIED MILK AND AS MIXED CULTURES WITH *L. ACIDOPHILUS*-301.

Pathogen	4 h[1]		8 h[1]	
	Acidified milk	Mixed cultured milk	Acidified milk	Mixed cultured milk
	% Inhibition			
S. typhi-83	8.0	91.0	89.0	95.0
Staph. aureus- C2-T10	0.0	97.0	61.0	99.0
P. vulgaris-204	0.0	85.0	87.0	94.0
E. coli-6	0.0	91.0	67.0	92.0
Y. enterocolitica-03	0.0	86.0	89.0	99.0

[1]The pH values of acidified and mixed cultured milk samples were 5.8 ± 0.2 and 4.8 ± 0.2 at 4 and 8 h, respectively.

The pH values of both acidified milk and mixed cultured milk samples were 5.8 ± 0.2 at 4 h, and 4.8 ± 0.2 at 8 h of incubation. Although the pH of both use more or less same at any given time, greater inhibition was recorded in mixed cultured milk samples than in acidified milk samples. Therefore, it can be concluded that antagonism produced by *L. acidophilus* towards different pathogens is not due to acid alone. *L. acidophilus* has been reported to produce antibiotic-like compounds, such as acidolin (Hamdan and Mikolajcik 1974), acidophilin (Shahani et al. 1977) and lactocidin (Vincent et al. 1959). *L. acidophilus* has also been reported to produce another antibiotic-like compound (Mehta et al. 1983; Silva et al. 1987). Therefore, enhanced inhibition of different pathogens observed in this investigation in mixed cultures than in acidified milk may be attributed to the activity of such compounds produced by *L. acidophilus*.

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Effect of Processing on the Functional Properties of Some Local Varieties of Horsegram (*Dolichos biflorus* L.) in Madhya Pradesh

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The functional properties of raw, heat processed and germinated horsegram flour in three varieties, differing in seed colour namely, red, brown and black, were studied. Autoclaving reduced fat absorption in black and red varieties, while roasting decreased it in the black variety. It was maximally measured in germinated brown variety. Water absorption capacity increased in processed samples, whereas emulsification capacity drastically reduced. Heat processing reduced foam capacity and foam stability. Foam capacity stimulated in germinated flour, whilst foam stability remained unaffected.

Keywords: Functional properties, Heat Treatments, Germination, Horsegram.

Horsegram (Kulthi, *Dolichos biflorus* L.) is an important source of proteins and has been identified as one of the potential food sources by the National Academy of Sciences of the United States of America (NAS 1979). The use of dry seeds of horsegram as human food is limited due to its poor cooking quality. They are, however, consumed as sprouts in many parts of India (Ghorpade et al. 1986). Improvement in the nutritional quality of horsegram subjected to heat treatment has previously been largely advocated (Ghorpade et al. 1986; Khader and Rao 1987). There has been an increasing interest in recent years on the functional properties of plant proteins or their isolates for food use. Thus, information on the changes in the functional properties of horsegram, as influenced by processing is needed.

Horsegram seeds of three varieties were procured the local market of Chhatisgarh, Madhya Pradesh, India. These were distinguished on the basis of the colour of the seed coats viz., black, red and brown and were subjected to different heat treatments and germination. The seeds were either roasted at 210°C for 1 min in sand, after adjusting their moisture content to 17.0 ± 0.5% or autoclaved in steam at 15 lbs pressure for 30 min. Prior to germination, the seeds were soaked overnight (24 h) in distilled water. Wet seeds were allowed to germinate in muslin cloth at room temperature so as to achieve the plumule length to 3/4 or 1 inch. Germinated and autoclaved seeds were oven-dried (50°C, 24 h). The processed seeds were finely powdered and passed through a 60 mesh size sieve.

Water absorption (Sosulski 1962) and fat absorption capacity (Sosulski et al. 1976) of kulthi flour were measured, using 1-2 gm flour sample. Per cent absorption capacity was expressed as the amount of water or oil retained by 100 g flour, as the case may be. Foam capacity and stability were also determined and expressed in per cent increase in volume (Huffman et al. 1975). Foam was allowed to settle down for 15 min and the difference in the initial and final volumes represented foam stability. Emulsification capacity was determined, using 2 gm sample blended for 30 sec. in 25 ml distilled water. It was expressed in ml of groundnut oil emulsified per g of protein or sample (Beuchat et al. 1975). The data were subjected to analysis of variance and Duncan's multiple range test (Steel and Torrie, 1980), after calculating their mean ± standard deviation, using conventional formulae.

The fat absorption property was more or less equally depicted by black and red varieties of horsegram (Table 1). The absorption ability decreased slightly, but significantly in these varieties after autoclave treatment (Table 1). Roasting retarded its ability in black variety only, whilst germination elevated it significantly in the brown variety (Table 1). Heat processing is reported to increase the fat absorption capacity of winged bean (Narayana and Rao 1982), moth bean (Pawar and Ingle 1988) and cowpea flour (Giami 1993). However, in the present study, both the heat processes could not increase it, as was also observed for faba bean (Shroti 1993).

Germination of the seeds, however, improved the stability of the flour to bind and retain oil, though significantly in brown variety only (Table 1). It is possible that solubilization and ultimate dissociation of the proteins into its sub-units on germination, may unmask the non-polar residues

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TABLE 1. FUNCTIONAL PROPERTIES OF THREE VARIETIES OF RAW AND PROCESSED HORSEGRAM *(DOLICHOS BIFLORUS* L.)

Treatments	Fat absorption, ml/100 gm			Water absorption, ml/100 gm		
	Black	Red	Brown	Black	Red	Brown
Raw	100.000 ±0.000[a]	100.000 ±3.227[a]	87.500 ±3.227[b]	92.417 ±2.089[b]	92.383 ±2.438[b]	114.667 ±1.733[b]
Autoclaved	93.750 ±2.795[b]	83.333 ±2.635[b]	87.500 ±3.227[b]	145.280 ±5.143[c]	162.633 ±2.469[d]	140.850 ±0.449[c]
Roasted	93.570 ±2.795[b]	95.833 ±4.167[a]	87.500 ±0.000[b]	186.730 ±1.485[a]	174.530 ±1.146[a]	200.800 ±1.855[a]
Germinated	104.167 ±2.635[a]	102.083 ±2.083[a]	104.167 ±2.635[a]	88.030 ±2.211[b]	98.350 ±1.314[c]	118.850 ±1.135[b]

Values are mean + SEM of six observations along with their mean differences.

The means having similar alphabets as superscript are not significantly different from one another ($p<0.05$)

from the interior of the protein molecule, which may ultimately lead to increase in oil absorption.

The water absorption capacity was similarly expressed by black and red varieties, whilst brown variety depicted its higher level (Table 1). This may possibly be due to the differences in the number and types of polar groups present in it (Kunitz 1971) and is attributed to their protein contents. Autoclaving or roasting improved the water absorption capacity of horsegram flour. Germination failed to do so, except in the red variety (Table 1).

Heat treatment has been reported to increase the water absorption capacity of mung bean (del Rosario and Flores 1981), winged bean (Narayana and Narasinga Rao 1982), cowpea (Giami 1993), soybean (Wu and Inglett 1974) and sunflower flour proteins (Lin et al. 1974). During heat treatment, major proteins are dissociated into sub-units that might have more water binding sites than the native or oligomeric proteins (Catsimpoolas et al. 1970). Gelatinization of carbohydrates and swelling of the crude fibre may also occur, leading to increased water absorption.

All the three varieties exhibited more or less similar foam capacity. Germination increased it, but both the heat treatments decreased it markedly. Roasting of seeds affected the capacity of flour more than autoclaving process (Table 2). A similar effect of protein isolates from moth bean (Pawar and Ingle 1988), yellow pea, faba bean and lentil (Hsu et al. 1982), cowpea (Giami 1993) and peanut flour (Rahma and Mustafa 1988) was previously reported.

Foamability is assumed to be dependent on the configuration of the protein molecules. Flexible protein molecules give good foamability, but highly ordered globular molecules give low foamability, because they cannot reduce the surface tension of the air water interface (Grahm and Philips 1976). Heat processing diminished the nitrogen solubility of proteins by denaturation and thus, also reduced their foam capacity (Yasumatsu et al. 1972).

Foam stability was slightly reduced in heat processed horsegrams flours, whilst germination failed to alter this ability in them (Table 2). The findings are in agreement with those reported by Rahma and Mostafa (1988) in peanut flour and was

TABLE 2. FUNCTIONAL PROPERTIES OF THREE VARIETIES OF RAW AND PROCESSED HORSEGRAM *(DOLICHOS BIFLORUS L.)*

Treatments	Foam capacity, ml/100 gm			Foam stability, % after 15 min			Emulsification capacity, ml/100 gm		
	Black	Red	Brown	Black	Red	Brown	Black	Red	Brown
Raw	49.833 ±1.424[d]	50.333 ±0.333[d]	47.000 ±2.294[d]	91.003 ±0.632[ac]	92.900 ±0.334[a]	92.045 ±0.577[a]	31.716 ±0.427[a]	29..580 ±0.417[a]	28.750 ±0.854[a]
Autoclaved	36.330 ±1.201[c]	39.667 ±0.615[c]	24.000 ±0.516[c]	89.718 ±0.386[c]	89.978 ±0.496[bc]	90.053 ±0.236[b]	9.333 ±0.105[c]	10.000 ±0.129[c]	10.000 ±0.000[c]
Roasted	29.667 ±0.615[b]	16.333 ±0.955[b]	16.667 ±0.422[b]	84.963 ±0.420[b]	90.247 ±0.384[c]	91.705 ±0.539[a]	5.000 ±0.000[b]	5.830 ±0.167[b]	5.667 ±0.247[b]
Germinated	57.330 ±0.667a	55.330 ±0.422[a]	52.667 ±1.333[a]	91.735 ±0.307[a]	92.268 ±0.367[a]	92.333 ±0.342[a]	14.883 ±0.238[d]	11.000 ±0.183[d]	15.500 ±0.129[d]

Values are mean + SEM of six observations along with their mean differences.

The means having similar alphabets as superscript are not significantly different from one another ($p<0.05$)

attributed to the denaturation of the protein on heating that became less soluble. The reduction of foam volume is mainly due to collapsing and bursting of the formed air bubbles during whipping. Foam stability has been suggested to be related to the amount of native protein (Lin et al. 1974), being considerably low in the denatured protein (Yasumatsu et al. 1972).

Emulsifying capacity was more or less similarly depicted by the three varieties tested (Table 2). This refers to their similar capabilities of absorbing the oil by a protein before the emulsion breaks and is linked to their soluble protein contents (Yasumatsu et al. 1972; Volkeart and Klein 1979). Heat processing markedly decreased their capacities (Table 2) and are consistent with the findings observed for winged bean (Dev and Mukherjee 1986), moth bean (Pawar and Ingle 1988), soybean and peanut flour proteins (McWatters and Holmes 1979). It may be due to decreased solubility of proteins during heating. Moreover, heating time was the primary determinant in the reduction of nitrogen solubility and emulsification capacity (McWatters and Holmes 1979).

On germination too, the emulsification capacity was markedly reduced in all the three varieties of horsegram (Table 2). Increased solubility of the proteins on germination (Giami 1993) may effect the equilibrium between its hydrophillic and lipophillic groups and thus, may influence the emulsification capacity of the legumes (Nakai 1983). Other food components such as carbohydrates and lipids may equally contribute through proteins, carbohydrates and/or protein-lipid interactions (Pawar and Ingle 1988).

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Effects of Blanching of Pearl Millet Seeds on Nutritional Composition and Shelf-life of Its Meal

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Pearl millet cultivars exhibited a wide variation in the fat acidity both in fresh and stored meals. Blanching of seeds at 98°C for 10 sec in boiling water before milling effectively retarded the development of fat acidity in the meal and enhanced its shelf life upto 25 days.The blanching treatment of seeds did not exhibit any adverse effects on nutritional constituents and *in vitro* digestibilities of starch or proteins.

Keywords: Pearl millet, Blanching, Storage, Fat acidity, Nutrients, Shelf-life.

A rapid development of fat acidity in the pearl millet meal mainly due to the action of lipase (Lai and Varriano-Marston 1980) causes bitterness and makes the meal unacceptable within 5 to 8 days after milling of millet (Kaced et al. 1984). Use of antioxidants, defatting and salting of flour or heat treatments to the meal (Kapoor and Kapoor 1990) have been attempted to retard the lipolytic and oxidative spoilage of pearl millet meal and to improve its storage life.

Recently, a simple boiling water blanching treatment to the seeds before milling has been standardized to effectively retard the lipolytic spoilage of the meal during storage (Chavan and Kachare 1994; Kadlag et al. 1995). The treatment consists of blanching of dry seeds in boiling water at 98°C for 10 sec, followed by drying and subsequent milling. The meal of blanched seeds could be stored at ambient conditions upto 25 days without any appreciable increase in the fat acidity. This communication reports the results of varietal variations in the development of fat acidity, effectiveness of blanching treatment for different cultivars and influence of blanching treatment of seeds and storage of meals on the nutritional constituents and sensory properties of *roti*.

The seeds of 8 pearl millet cultivars, 200g each, were loosely tied in muslin cloth and dipped in boiling water (1:3 w/v) at 98°C±2 for 10 sec, drained and dried at 40°C for 2 h to initial weight. Both blanched and control seeds were pulverized in Braun make laboratory grinder to about 60 mesh. The meals were packed in cloth bags and stored in an incubator at 27°C±2 with 70 to 80% relative humidity. The extent of lipid hydrolysis in the meal was monitored by estimating the fat acidity (AOAC 1980) at 0, 10, 20 and 30 days of storage. The seeds (1 kg) of one popular cultivar 'RHRBH-8609' were separately subjected to blanching treatment and the meals from both blanched and control seeds were stored upto 30 days as described earlier. The changes in crude fat, acid value, crude proteins (AOAC 1980), free amino acids (Rosen 1957), total sugars (Ranganna 1977), *in vitro* protein digestibility (Singh et al. 1982) were monitored during storage. The *rotis* were prepared from the meals at an interval of 5 days of storage by the procedure of Subramanian et al (1986) and evaluated for taste and flavour properties by a panel of 5 semi-trained judges using a 9-point Hedonic scale.

The fat acidity ranged from 16.8 to 53.3 mg KOH/100g in the fresh meals of untreated seeds, while it varied from 213.2 to 611.6 mg KOH/100g meal after 30 days of storage (Table 1). The increase in fat acidity during storage was 12 to 19-fold in different cultivars. In the meals of blanched seeds, the increase in fat acidity during 30-day storage was less than 2-fold in all the cultivars. These results clearly indicated a wide variability in the fat acidity levels among the different cultivars and that boiling water blanching of seeds was quite effective in retarding the formation of fat acidity during storage of millet meals. Storage of meal upto 30 days did not affect the contents of crude fat, crude proteins, free amino acids, *in vitro* protein or starch digestibility, but there was a marginal increase in total sugars and a significant increment in the acid value of oil, extracted from the stored meal (Table 2). A blanching treatment to the seeds before milling resulted in a slight decrease in the crude lipids in fresh meal and a significant

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TABLE 1. EFFECTS OF HOT WATER BLANCHING OF SEEDS ON DEVELOPMENT OF FAT ACIDITY IN THE MEALS OF PROMISING PEARL MILLET CULTIVAR

Cultivar	Fat acidity, mg KOH/100 g meal							
	Untreated seeds				Blanched seeds			
	Meal storage, days							
	0	10	20	30	0	10	20	30
'ICTP-8203'	16.8±1.25	78.6±5.77	157.1±06.73	213.2±06.7	16.8+±.25	22.4±1.24	28.1±1.10	33.6±3.84
'RHRBH-8609'	16.8±1.25	89.8±±.58	179.6±06.53	274.9±07.80	15.2±0.49	20.4±1.20	22.4±1.14	28.1±1.80
'RHRB-890'	19.6±1.10	112.2±7.66	241.3±07.91	308.6±11.04	16.8±1.25	22.4±1.14	22.4±1.14	28.1±1.80
'ICMV-87901'	19.6±1.10	142.8±9.39	252.6±08.87	370.8±08.61	16.8±1.25	19.6±1.10	22.4±1.14	28.1±1.80
'RHRBH-8804'	22.4±1.14	101.0±6.79	185.2±07.14	230.1±07.06	16.8±1.25	22.4+±.14	28.1±1.80	28.1+±.80
'MH-169'	25.3±1.06	162.7±8.52	258.1±08.47	359.1±08.11	16.8±1.25	25.5±1.08	28.1±1.80	39.3±4.00
"BK=560'	28.1±1.80	173.9±8.61	263.7±10.10	381.6±09.34	16.8±1.25	22.4±1.14	33.7±3.86	39.3±4.00
"MLBH-104'	53.3±1.14	263.7±8.64	493.8±09.14	611.6±09.79	44.9±4.90	61.7±5.30	89.8±6.58	95.5±6.05

TABLE 2. EFFECTS OF HOT WATER BLANCHING OF SEEDS ON NUTRITIONAL QUALITY OF PEARL MILLET MEAL DURING STORAGE (CV, 'RHRBH-8609')

	Treatment	Meal storage, days			
		0	10	20	30
Crude fat, %	Control	6.80 ± 0.70	6.70 ± 0.68	6.60 ±0.67	6.80 ±0.70
	Blanched	6.40 ± 0.66	6.40 ± 0.66	6.60 ±0.66	6.50 ±0.67
Acid value, mg/KOH/g oil	Control	3.17 ± 0.12	19.66 ± 1.10	33.33 ±2.57	40.43 ±4.67
	Blanched	1.66 ± 0.08	5.72 ± 0.38	8.03 ±0.76	8.25 ±0.57
Crude protein, %	Control	10.20 ± 0.90	10.20 ± 0.90	9.90 ±0.90	9.80 ±0.90
	Blanched	10.10 ± 0.80	9.90 ± 1.00	10.10 ±0.80	10.00 ±0.80
Free amino acids, mg/100 g meal	Control	0.18 ± 0.03	0.19 ± 0.04	0.29 ±0.06	0.29 ±0.06
	Blanced	0.19 ± 0.04	0.18 ± 0.03	0.30 ±0.07	0.28 ±0.05
Total sugars, %	Control	1.93 ± 0.22	2.02 ± 0.06	2.15 ±0.10	2.20 ±0.09
	Blanched	1.90 ± 0.15	2.14 ± 0.11	2.20 ±0.09	2.25 ±0.07
Protein digestibility (*in vitro*), %	Control	80.40 ± 6.32	-	-	78.90 ±7.00
	Blanched	81.40 ± 6.32	-	-	77.00 ±6.10
Starch digestibility, (*in vitro*)	Control	159.30 ± 11.4	-	-	150.50 ±9.20
mg maltose/2h/g meal	Blanched	151.20 ± 10.3	-	-	151.90 ±10.1

retardation in the increment of acid value with a little effect on other nutritional constituents. The consumers prefer roasted flavour and sweet taste for pearl millet *roti* and the *roti* scoring more than 7 points were considered acceptable. The *roti* of control meal showed acceptable flavour and taste only upto 10 days of meal storage whereas *roti* prepared from blanched seed meal exhibited acceptibility upto 25 days of storage (Table 3).

TABLE 3. EFFECTS OF HOT WATER BLANCHING OF SEEDS AND STORAGE OF MEAL ON CHANGES IN TASTE AND FLAVOUR PROPERTIES OF PEARL MILLET *ROTI*[a]

Meal storage,	Taste of *roti*[b]		Flavour of *roti*[b]	
days	Control	Blanched	Control	Blanched
0	8.0±0.7	8.0±0.7	8.0±0.7	8.0±0.7
5	8.0±0.7	8.0±0.7	8.0±0.7	8.0±0.7
10	8.0±0.7	8.0±0.7	7.6±0.5	8.0±0.7
15	6.2±0.3	8.0±0.7	6.7±0.4	8.0±0.4
20	4.0±0.2	7.7±0.5	5.0±0.3	7.7±0.7
25	4.0±0.2	7.1±0.5	4.0±0.3	7.3±0.6
30	4.0±0.2	6.8±0.6	4.0±0.3	6.5±0.4

[a] The colour and texture of *roti* remained unaffected due to storage of meal

[b] Sensory score : 9 excellent; 8 very good; 7 good; 5-6 poor and >5 very poor.

A rapid increase in fat acidity of the meal during storage can be attributed to the action of lipase located in the germ and surface layers of the seeds, which get mixed with the meal during, milling and the meal lipids, get decomposed into free fatty acids (Kaced et al. 1984). A wide variation observed in the initial fat acidity levels in the meals of different cultivars provide opportunities to select desirable cultivars for longer shelflife of millet meal. The results obtained on different chemical constituents in the present study concur with the literature reports (Lai and Varriano-Marston 1980; Subramanian et al. 1981; Chavan and Kachare 1994; Kadlag et al. 1995). In the stored meal, total

lipids remain almost constant, while only the free fatty acids increase (Kaced et al. 1984). Similarly, there was no significant change in total protein content of the meal due to storage, but it affected adversely the feed intake by the experimental animals, biological value and digestibility of meal proteins (Kapoor and Kapoor 1990). The loss in protein quality was attributed to the formation of complexes between proteins and lipid degradation products and to the lower feed intake due to rancid taste and flavour of the stored meal. In the present studies, both storage and blanching treatment did not adversely affect the *in vitro* digestibility of starch or proteins. Thus, a short time blanching treatment of seeds is severe enough to retard lipolytic spoilage and extend the shelf-life of pearl millet meal without having any adverse effect on the nutritional properties related to starch and protein utilization.

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Mechanical Deoiling of Soybean

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Application of mechanical deoiling of soybean, which is commercially not practised was investigated for its possible application at decentralized rural level. This communication reports the soy oil recoveries (at different moisture contents) obtained, using indigenous technology available for mechanical deoiling of other oilseeds. Soybean oil can be extracted by using a table oil expeller to the extent of 69.5% by adjusting the moisture to 8.5%.

Keywords: Mechanical deoiling, Pretreatment, Gravitational settling, Soybean deoiling, Expeller.

Soybeans are directly solvent-extracted for oil, primarily due to their low oil content. Mechanical deoiling has not been commercially practised and consequently very limited literature is available. Singh and Agrawal (1988) have critically reviewed the literature on mechanical deoiling of soybean. They have professed that solvent extraction alone is not suitable under Indian conditions and mechanical deoiling at decentralized rural level should be practised. They have proposed a possible technological approach and have indicated an urgent need to develop a mechanical deoiling technology for soybean. Suggested mechanical deoiling system to be investigated includes cleaning, dehulling, flaking, grinding, steam conditioning/ cooking of oilseeds before expelling. These involve optimization of several unit operations, before an effective technology is evolved. In addition, mechanical deoiling equipments available are meant for seeds with high oil content and would require redesigning for soybean, which has low oil content.

It was felt that if the existing indigenous expelling technology for other oilseeds with minimum simple pretreatment could be used for soybean, it would be useful to farmers, small entrepreneurs for deoiling in oil milling at present, till a more effective technology is evolved. Therefore, the objective of this study was to investigate the soybean oil recoveries, using the indigenous mechanical deoiling technology available for other oilseeds. The approach was to use a table oil expeller on dehulled and split soybeans with moisture adjustment only. The process could easily be adopted at decentralized rural level.

Bragg variety of soybean was used in this study. The cleaned dehulled and split soybean had a moisture content of about 6% w.b. The other moisture levels were adjusted by adding moisture and tempering for 72 h. Dehulled split soybean was expelled through table oil expeller at five moisture levels over a range of about 6-14% (w.b.).

Choke opening was fixed at a value determined through preliminary trials. The suspended particles in the expelled oil were separated by gravitational settling for 24 h. Oil content of split soybean was determined by soxhlet apparatus, using AOCs Ba3-18 method. The sample size in each experimental run was 3 kg. The table oil expeller used in the study was super deluxe model of S.P. Engineering Co. Kanpur with the following specifications:

Length of the machine	1.14 mts.
Width of the machine	55 "
Height of the machine round	96 "
Weight of the machine	255 Kg
No. of cage bars	22
Diameter of puller	36 cm
Capacity/h. (crushing)	50 kg
Horse power	5 hp.
Diameter of worm	80 mm
RPM	450

Choke opening was fixed by trial and error at 4 screw threads outside. Preliminary trials indicated that the reduced choke opening resulted in choked expeller, whereas increased opening did not expel oil. The speed of the driving pulley was maintained at 460 rpm.

The trials on soybean was to run the expeller idle by recirculating a small amount of soybean over and over again, until the expeller was heated up and the oil started being expelled. It was observed that the time for idle running varied with moisture content of the raw materials. However, it was generally around $1^1/2$ to 2 h.

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TABLE 1. OIL RECOVERY AT DIFFERENT MOISTURE LEVELS FROM SOYBEAN SPLITS.

Moisture content soy splits, % w.b.	Cake obtained,		Suspended solids separated,		Oil recovered,		Material loss,	
	kg	% of total sample	kg	% of total sample	kg.oil recovery	% of weight of soya	kg.	% of total sample
5.84	2.10	70.0	0.349	11.6	0.285	9.5	0.267	8.9
	2.00	66.7	0.198	6.5	0.331	11.0	0.473	15.8
	2.40	80.0	0.372	12.4	0.212	7.1	0.016	5.0
Average	2.17	72.3	0.306	10.2	0.276	9.2	0.248	8.3
8.54	2.00	66.7	0.034	2.8	0.405	13.5	0.511	17.0
	2.25	75.0	0.121	4.0	0.466	15.5	0.614	5.5
	2.15	71.7	0.072	2.4	0.414	13.8	0.364	12.1
Average	2.13	71.0	0.092	3.1	0.428	14.3	0.350	11.7
10.25	2.10	70.0	0.234	7.8	0.213	7.1	0.453	15.1
	2.40	80.0	0.164	5.6	0.409	13.6	0.023	7.7
	2.15	72.0	0.174	5.8	0.363	12.1	0.313	10.0
Average	2.22	74.0	0.192	6.4	0.328	10.9	0.260	8.7
10.98	2.55	85.0	0.180	6.0	0.340	11.3	-0.070	-2.3
	2.35	78.3	0.114	3.8	0.377	12.6	0.159	5.3
	2.40	80.0	0.096	3.2	0.405	13.5	0.099	3.3
Average	2.43	81.0	0.130	4.3	0.374	12.4	0.063	2.1
13.85	2.40	80.0	0.120	4.0	0.202	6.7	0.278	9.3
	2.50	83.3	0.096	3.2	0.331	11.0	0.073	2.4
	2.45	82.7	0.102	3.4	0.354	11.8	0.094	3.1
Average	2.45	81.7	0.106	3.5	0.296	9.8	0.148	4.9

The data on expelling at the different moisture levels are given in Table 1. The oil recovered is plotted in Fig. 1, as percent of total sample against moisture content. It was observed that, in general, the oil recovery first increased with the increasing moisture content and then decreased. The maximum oil recovery was 14.3% as weight of the sample and was obtained at a moisture content of 8.5%. The oil content of soya split was determined to be 21.7% as weight of the sample. Thus, a maximum of 65.9% of the total oil present in soya split could be recovered. The cake and the suspended solids obtained at this moisture level were observed to be 71.9% and 3.1%, respectively, as weight of the sample. Analysis of variance was performed on the data to establish whether or not the differences are within the replication and the effect of moisture content was significant. The ANOVA results are shown in Table 2. It was observed that the calculated F value for replications was lower than the table F value at 95% confidence level, indicating that the variations within replications were not significant, The effect of moisture content on the oil recovery was found to be statistically significant at 95% confidence level, since the calculated F value was higher than the table value.

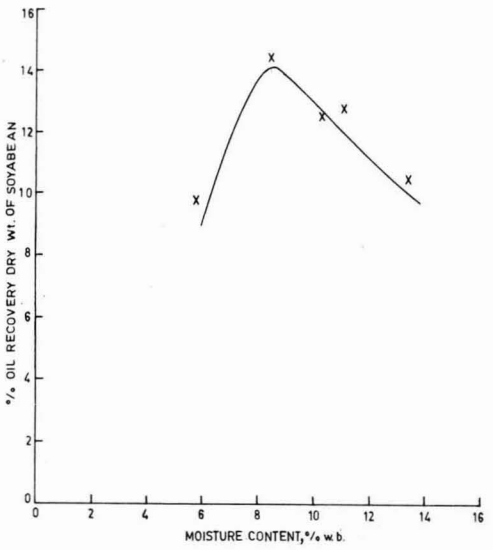

Fig. 1. Oil recovery as affected by moisture content of soya splits

Soybean can be mechanically deoiled, using the indigenous technology available for other oilseeds by only dehulling, splitting and moisture adjustment. The moisture content of soya split should be adjusted to 8.5% w.b. for expelling through a table

TABLE 2. ANOVA RESULTS

Source	Degree of freedom	Sum of square	Mean sum of square	F cal	F table 5%
Moisture content	4	53323.0006	13330.90015	3.84325	3.54
Replication	2	26648.1340	13324.067	3.84128	4.46 5%
Error	8	27749.1958	3468.64985	-	3.11 10%

oil expeller. Prior to expelling, the expeller should run idle by recycling a small sample, until the expeller is heated up to the extent that the oil starts coming out Choke opening should be set by keeping 4 screw through outside, and oil recovery of 65.9% of the total oil present can be achieved. Encouraged with the results, one expeller has been modified with heating device and further work is under way to evaluate the performance of the expeller.

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Studies on Utilisation of *Rajmah (Phaseolus Vulgaris)* Flour in Some Indian Traditional Products

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Red variety of *Rajmah dhal* was analyzed for nutrient composition-protein content was 21.2%, calcium 260 mg% and iron 5.1 mg%. The *dhal* recovery on milling was 70%. Pressure cooking method was found to be the best for cooking *dhal*. *Rajmah* flour was used in the preparation of composite flour in combination with wheat flour (25:75). The products made with *Rajmah dhal*, flour and composite flour were well accepted. Composite flour stored well for 12 weeks.

Keywords: Composite flour, Subjective and objective methods, Alcoholic acidity, Lipase activity, Dietary fibre.

Pulses have been traditionally recognised as an important constituent of the Indian diet and a variety of pulses are produced and consumed. *Rajmah (Phaseolus vulgaris)* is also known as kidney bean, garden bean, field bean and French bean (Wyte 1966). This legume is widely grown in Southern Mexico and Central America for its edible bean and pods (Chatterjee and Bhattacharya 1986). *Rajmah* is a good source of protein (22.9%), energy (346 Kcal) and minerals (3.29g) (Gopalan 1989). It is also high in cellulose, non-cellulosic polysaccharides, lignin and so has been reported to have hypocholesterolemic and hypoglycemic effects comparable to *Bengal*gram *(Cicer arietinum)* and fenugreek seeds *(T. foenum graeaum)* (Sharma 1986; Mathur et al. 1964). *Rajmah* is traditionally consumed as whole legume in Northern parts of India. The legume is usually soaked overnight, drained, pressure-cooked and incorporated in many curry preparations. But, use of *Rajmah* as a split *dhal* is not common in Northern parts. Owing to the high cost of legumes in the present day, there is a need for alternate sources to replace legumes. Though *rajmah* is grown largely in recent years, the consumption, is not common in the State of Andhra Pradesh. This study was designed to process *rajmah dhal* by dehulling and splitting, incorporating *dhal* and flour to meal and snack products and develop composite flour. The experiment also was aimed at assessing the shelf-life and acceptability of composite flour.

Dehulling and milling of Rajmah : Red variety of *rajmah* was purchased from the local market, Hyderabad. *Rajmah* was sun-dried for two days to facilitate seed coat removal. The legume was, then, dehulled and split to *dhal* in a mechanical agrohuller (APAU model). Dehulling time, percent *dhal* recovery and other milling yields were observed. Dehusked *dhal* was milled to a fine flour (60 mesh).

Preparation of composite flour : Wheat was purchased from one of the super markets in Hyderabad and milled to a fine flour (60 mesh). Composite flour was prepared by mixing whole wheat flour and *rajmah* flour in the ratio of 75: 25.

Preparation of products : Several main meal and snack items, using *rajmah dhal*, flour and composite flour were prepared and tested for acceptability by subjective and objective methods. The recipes prepared with *dhal* were *dosa, vada*, fried nuts, curries with greens/vegetables and those with flour included *sev, muruku, bajji, bonda* and *pakoda, Pulka, puri* and *chapathi* were prepared with composite flour. Sensory evaluation of products was carried out with the help of a trained panel of judges (10 to 12) for various sensory attributes (appearance, texture/consistency, taste, flavour and overall acceptability), using a 5 point scale (highly acceptable-5, moderately acceptable 4, Neutral 3, slightly acceptable 2 and not acceptable 1.

Storage of composite flour: Composite flour was stored at room temperature of 28-30°C upto 12 weeks, packed and sealed in polythene sachets (20 μ / LDPE) and aluminium containers. The composite flour was analyzed initially, after 6 and 12 weeks of storage for moisture (AOAC 1984) and chemical components - protein (AOAC 1984), lipase activity (Kantharaj Urs et al. 1962), alcoholic acidity (IS 1009, 1968), total sugars (Dubois et al. 1956), reducing sugars (Hodge and Hofreiter 1962) and total dietary fibre (Asp et al. 1983). Sensory evaluation of the products made with fresh and stored composite flour was done at 0.6 and 12 weeks of storage. Dough making, rolling and *roti-*

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making and the assessment of textural qualities of the products made from composite flour was carried out thrice in the laboratory and the mean values were calculated (Desikachar and Chandrasekhar 1981).

When *rajmah* was milled, the *dhal* recovery was 70%, husk 10%, brokens 4% and undehulled grain 16%. Dehulled *rajmah dhal* is creamy white in appearance and is hard in texture. *Rajmah dhal* cooked in a pressure cooker took only 20 min in contrast to 55 min for the *dhal* cooked in an open vessel. The increase in weight and volume of *dhal* upon cooking was ten times more than its initial weight and volume. Flour obtained from *rajmah dhal* was of fine quality (60 mesh). *Rajmah dhal* had a characteristic beany flavour and therefore the nuts deep-fried in oil scored less for flavour (3.8/5.0), compared to control (4.0/5.0). While the scores obtained for *vada* made with blackgram *dhal* and *rajmah* (1:1) was more (4.1/5.0) than vada prepared with 100% *rajmah* (4.0/5.0). *Vada* prepared with blackgram *dhal* (*Phaseolus mungo* roxb) scored maximum (5.0/5.0).*Dosa* made with a combination of blackgram *dhal*, *rajmah* and rice (15:15:70) tasted fair (3.1/5.0), compared to control (5.0/5.0). *Pooran poli* was moderately accepted. *Sev* and *muruku* prepared with rice flour and *rajmah* flour (3:1) and *pakoda* made with 100% *rajmah* flour were given similar scores (4.0/5.0) for acceptability (Table 1). Dough making quality of composite flour was similar to that of wheat flour and dough was soft and elastic and could be rolled easily (21.5 cm for composite flour and 20 cm for wheat flour). The textural qualities of *rotis*, as judged by chewing and eating, were similar to wheat *roti*. The *rotis* were soft and did not break even after 5 h of preparation.

TABLE 1. MEAN SCORES OF SENSORY EVALUATION OF *RAJMAH* PRODUCTS

Product	Appearance	Flavour	Taste	Texture	Overall acceptability
Dhal					
Nuts	4.2	3.8	4.2	4.5	4.2
	(4.7)	(4.0)	(5.0)	(4.7)	(4.9)
Vada					
(100% *rajmah*)	4.3	4.0	4.0	4.3	4.1
	(4.6)	(5.0)	(4.9)	(4.9)	(4.9)
Vada (50% *rajmah*					
50% blackgram)	4.1	4.1	4.1	4.6	4.6
Dosa (rice 75%					
rajmah 25%)	3.5	3.3	3.5	3.3	3.5
	(4.7)	(4.8)	(5.0)	(4.8)	(4.8)
Dosa (70% rice,					
15% *rajmah*					
15% blackgram)	4.0	3.0	3.1	3.3	3.0
Pooranpoli					
(100% *rajmah*)	3.9	4.1	4.3	3.4	3.9
	(4.5)	(4.5)	(4.5)	(4.5)	(4.5)
Flour					
Sev.(75% rice,					
25% *rajmah*)	4.2	3.6	4.0	4.0	4.0
	(4.6)	(4.5)	(4.5)	(4.5)	(4.5)
Muruku					
(75% rice,					
25% *rajmah*)	4.0	4.0	4.0	4.0	4.0
	(4.5)	(4.5)	(4.5)	(4.5)	(4.5)
Pakoda					
(100% *rajmah*)	4.0	3.8	3.8	3.8	4.0
	(4.6)	(4.5)	(4.5)	(4.5)	(4.5)
Composite flour					
Pulka	4.4	3.8	3.8	4.2	4.4
	(4.3)	(4.2)	(3.8)	($.5)	(4.3)
Poori	4.4	4.2	4.2	4.2	4.4
	(4.5)	(4.8)	(4.8)	(4.5)	(4.5)
Chapati	4.6	4.4	4.6	4.4	4.6
	(4.8)	(4.5)	(4.8)	(5.0)	(4.8)

Figures in parentheses represent sensory scores for control samples. 5 point scale, 5-highly acceptable; 4-moderately acceptable; 3-neutral, 2-slightly acceptable; 1-not acceptable.

The sensory evaluation scores for products made with composite flour, namely *pulka*, *poori*, *chapati*, as compared to wheat flour (Table 1) revealed that the scores for various organoleptic characteristics did not show appreciable difference and all were well accepted.

Composite flour stored in aluminium container and polythene sachets for 12 weeks did not show any insect infestation. Moisture, protein and alcoholic acidity values did not change significantly on storage. Lipase activity started increasing after 6 weeks, but was still within the acceptable limits,

TABLE 2. STORAGE OF COMPOSITE FLOUR : POLYTHENE SACHET VS. ALUMINIUM CONTAINER

Parameters	Storage period, weeks				
	0	6		12	
	Fresh flour	PS	AC	PS	AC
Moisture, g %	10.71	10.9	10.7	11.7	11.2
Proteins, g %	11.81	11.2	11.8	12.2	12.4
Lipase activity, ml of 0.1 N NaOH	2.55	2.70	2.80	5.60	5.80
Alcoholic acidity, %	0.17	0.25	0.22	0.25	0.22
Total sugars, %	2.00	4.00	4.25	4.50	5.00
Reducing sugars, %	0.75	0.45	0.36	0.42	0.52

PS : Polythene sachet AC : Aluminium container

TABLE 3. MEAN SCORES FOR PRODUCTS MADE WITH COMPOSITE FLOUR STORED FOR 12 WEEKS.

Product		Appearance	Flavour	Taste	Texture	Overall-acceptability
Pulka	PS	4.5	4.0	4.0	4.3	4.2
	AC	4.8	4.5	4.3	4.8	4.6
		(4.4)	(3.8)	(3.8)	(4.2)	(4.4)
Poori	PS	4.3	4.3	4.5	4.5	4.4
	AC	4.0	4.0	4.5	4.8	4.5
		(4.2)	(4.2)	(4.2)	(4.2)	(4.4)
Chapati	PS	4.5	4.8	4.5	4.8	4.5
	AC	4.3	4.8	4.5	4.8	4.5
		(4.6)	(4.4)	(4.6)	(4.4)	(4.6)

Figures in parentheses represent sensory scores for fresh samples a maximum score of 5 - highly acceptable; 4-moderately acceptable 3-neutral ; 2-slightly acceptable ; 1-not acceptable.
PS - Polythene sachet; AC - Aluminium container

even after 12 weeks of storage. There was a gradual increase in the quantity of total sugars in both types of storage containers (Table 2).

During sensory evaluation, *pulka, poori and chapati* made with stored composite flour were given higher scores than products made with fresh composite flour. The reason could be reduction in the beany flavour of the composite flour. Thus, composite flour can be safely stored, till 12 weeks in either aluminium containers or polythene sachets (Table 3).

The study has shown that utilisation of *rajmah* can be further increased by popularising its potential use as a substitute for other legumes in traditional products. *Rajmah* flour can be used in the preparation of *rajmah* : wheat composite flours. Such composite flours have higher protein contents and nutritive value than wheat flour alone.

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Quality of Chicken Patties as Influenced by Microwave and Conventional Oven Cooking

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Chicken patties were prepared with addition of different levels of chicken fat as well as other ingredients and subjected to hot air and microwave oven cooking. The product yield, gain in height, pH, fat and protein content differed significantly ($p < 0.05$). However, the shear force values, shrinkage in diameter, moisture and certain sensory attributes were not influenced by the cooking methods. Acceptability was better in hot air oven cooked than mocrowave oven cooked patties, the former having a significantly ($p < 0.05$) higher overall acceptability score.

Keywords: Chicken patties, Microwave cooking, Shrinkage, Shear force value, Sensory attributes.

Cooking, whether at commercial scale or in home, affects the nutrient value of most foods, including poultry meat (Posati 1979). Nutrient changes can occur through heat liability, leaching into cooking liquid, drip losses or absorption of cooking media (Posati 1979). Proteins and amino acids may be lost in small amounts in drip of the cooked meat (Erdman and Erdman 1982), but losses are not of serious concern (Mauron 1982). Fat remains fairly stable during the normal cooking of poultry (Chang and Watts 1952). An apparent increase in lipid content with cooking may be due to moisture loss, transfer of lipids from skin to flesh or uptake of frying oil (Posati 1979). Because poultry meat is always cooked by varieties of ways data comparing the cooking methods may be useful in minimizing nutrient losses. In the present study, an attempt was made to determine the effect of hot air and microwave oven cooking on the quality of chicken patties.

Spent hens were brought from the experimental layer farm of the Institute and dressed scientifically (Mountney 1970). Hot (41.5°C) deboning was made manually. The lean meat was kept in refrigerator at 4± 1°C for 24 h, after processing. Patties were prepared by adding different levels of chicken fat along with spice mixes 1.5% (Majhi and Panda 1973), condiment mixture 5% (onion: ginger: garlic at 3:1:1), sodium tripolyphosphate (0.5%), sodium nitrate (0.01%), sodium nitrite (0.01%), monosodium glutamate (0.05%), *maida* (5%) chilled water (10%) and salts (2%) to the minced meat. These patties were subjected to hot air and microwave oven cooking.

Hot air oven cooking was done by maintaining the oven temperature at 180 ± 5°C for 30 min (20 min for one side and 10 min for another side of the patties). Microwave oven cooking was performed, using the Microwin mx 1100 (Microwin Electronics, Ltd., Mandideep, India) with 4 and 2 min on one and another sides and power level at 7, which were standardized in the Institute. In both of these methods, the core temperature of the cooked patties was 75 ± 1°C. The patties were cooled to room temperature (27°C) by spraying tap water and held at room temperature, so that the sprayed water evaporated.

Proximate composition (moisture, fat and proteins) of the raw and cooked patties were determined as per AOAC (1990). The pH of sample was measured using combined electrode pH meter. Product yield was calculated as % weight of the cooked patties to weight of the raw patties. Shear force values were recorded, using a Warner-Bratzler shear press (GR Elect. Mfc Co., Washington D.C. USA-model No. 81091 313, capacity 50 x 0.1 lb) and converted into kg. The gain in height and shrinkage in diameter were noted by calculating increase in height and decrease in diameter as % of the height and diameter of the raw patties, respectively. Sensory evaluation was done by a semi-trained taste panel, consisting of not less than 8 to 10 members, using a 7 point Hedonic scale (Kramer and Twigg 1973). For each treatment, at least five samples were taken per trial with three trials. The data were analyzed statistically following standard procedure (Snedecor and Cochran 1968). Means were compared by Duncan's multiple range test (Duncan 1955).

The pH values of the cooked patties were higher than those of the raw patties (Table 1). These results agree with the findings of Fogg and Horrisson (1975), who found an increase of 0.3 unit in pH

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value on cooking of the meat. Microwave cooked patties showed higher pH value than the hot air oven cooked patties, thereby indicating the effect of cooking methods on the pH of the patties. The higher pH of the microwave cooked patties may be due to attainment of internal temperature (75 ± 1°C) earlier than that in hot air oven cooked patties (Lewis et al. 1967).

There was reduction in moisture content of cooked patties as compared to raw patties. This moisture loss may be due to loss of water, which escapes as steam (Mountney 1970). An apparent increase in fat and protein contents of the cooked patties may be due to loss of moisture from the product (Keetan 1983). Ground beef patties containing different fat levels (0-15%) also showed increases in protein contents after boiling (Hoelscher et al. 1987). Hot air oven cooked patties were having higher protein and lower fat contents, in comparison to microwave cooked patties. However, in terms of the moisture content, there was no significant difference between the two methods of cooking (Table 1).

The significant difference ($p<0.05$) in product yield of microwave cooked (88.43%) and hot air oven cooked patties (79.97%) may be due to wide difference in time required for cooking in two methods. These results agree well with the findings of Bramblett and Veil (1964), who reported that meat cooked quickly to a given internal temperature showed a lower cooking loss and was more juicy than that cooked slowly at the same temperature. The higher product yield of microwave cooked patties also may be due to their higher pH value in comparison to hot air oven cooked patties (Bouton et al. 1957). The patties cooked by both these methods showed a similar shear force value, thereby indicating that cooking methods had not affected shear force values. The shrinkage (%) in diameter of the cooked patties was not significantly ($p<0.05$) higher in microwave cooked patties, as compared to that in hot air oven cooked patties (Table 1). The overall acceptability score of the patties cooked by hot air oven was significantly ($p<0.05$) higher than the microwave cooked patties on a 7 point Hedonic scale. The cooking methods had not exerted any significant ($p<0.05$) influence on colour, flavour, juiciness, tenderness and stickiness scores of the sensory attributes of the patties.

TABLE 1. EFFECTS OF COOKING METHODS ON THE QUALITY AND ACCEPTABILITY OF CHICKEN PATTIES

Parameters	Raw patties	Microwave cooked patties	Hot air oven patties
Physico-chemical properties			
pH	6.13±0.00	6.31^a±0.01	6.29^b±0.01
Moisture,%	62.20±0.28	60.05^a±0.24	60.16^a±0.37
Fat, %	11.77±0.24	13.06^a±0.26	11.28^b±0.35
Protein, %	17.81±0.18	17.84^a±0.15	18.62^b±0.32
Product yield, %	-	88.43^a±0.33	79.97^b±0.41
Shear force value, kg	-	0.13^a±0.02	0.13^a±0.02
Gain in height, %	-	6.63^a±0.27	6.19^b±0.21
Shrinkage in diameter, %	-	5.97^a±0.20	5.90^a±0.22
Sensory attributes			
Colour	-	5.20^a±0.07	5.32^a±0.07
Juiciness	-	5.42^a±0.08	5.40^a±0.08
Tenderness	-	5.34^a±0.08	5.27^a±0.08
Stickness to mouth	-	2.31^a±0.09	2.35^a±0.09
Overall acceptability	-	5.22^a±0.08	5.40^b±0.08

Means with same superscript in each row do not differ significantly ($P<0.05$), Sensory evaluation scores varies from 7 (Excellent) to 1 (Extremely poor) but for stickiness to mouth, it was reversed.

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ACTIVE FOOD PACKAGING- Edited by N.L. Rooney, Published by Blackie Academic and Professional, An Imprint of Chapman and Hall, Wester Cleddens Roak, Bishopbriggs, Glassgow G64. 2NZ. 1st Edition April 1995, (Available from Chapman and Hall India, R. Seshadri, 32 second Main Road, C.I.T. East, Madras-600 035, India) pp. 260, Price £ 59.

The classic functions of any packaging are to provide protection, containment, information and utility of use or convenience. Active food packaging, in addition to the classic functions mentioned above, plays a significant role in the protection of food from physiological, physico-chemical and microbiological deterioration. Active packaging, therefore, helps in the maintenance of quality, safety and hygiene of foods, performing a role other than providing an inert barrier to external conditions. It may include an oxygen scavenger or an anti-microbial agent or free oxygen absorbers, water absorbers etc. The key focus of active food packaging is to optimise the shelf-life, and enhance the safety of food packaging processes by adding two or more forms of hurdles in active packaging.

Active packaging has tremendously developed in recent years into a series of topics, depending on the type of food product and the type of active packaging system employed, which involves the influence of package on the environment of the food, thus retaining "freshness" and/or preventing microbial or chemical or biochemical deterioration of food. Active packaging technologies have, therefore, wide ranging applications - from preserving fresh horticultural produce to diverse processed foods. Besides, active packaging provides also the opportunities to reduce the cost of packaging materials or packaging processes other than the conventional processing. Active packaging concepts, chosen to respond to various food properties, quite often unrelated (like in the case of horticultural produce and processed foods) are sometimes presented to the food industry, with few supporting results. This leads to a high degree of uncertainty to its potential use.

This book on "**Active food packaging**" introduces as well as consolidates active packaging concepts and is a timely addition to benefit scientists, technologists, students, food industrialists and regulators. The importance of the book lies in the fact that most of the literature in this field presented in this book, consists very largely of patents, technical leaflets and reviews. As such, this book edited by M. L. Rooney, with the individual topics contributed by several international experts, representing food and allied industries, brings out information, which is otherwise not so easily accessible. A brief account of the information presented in this book may be useful.

This book is divided into 11 chapters of which the 1st and the 4th chapters are by the editor. The first chapter gives an overview of active food packaging and in chapter 4 on active packaging in polymeric films, details are given on oxygen scavenging, packaging, moisture control films, removal of taints and food constituents, ingredient (such as antioxidant) release from films, permeability modifications, along with their commercial use, regulatory and environmental impacts.

Chapter 2, by Zagory on ethylene removing packaging systems deals principally with active packaging of post-harvest horticultural produce and the different ethylene scavenging systems. Chapter 3, on the design of modified atmosphere packaging of fresh horticultural produce by Yam and Lee, presents up-to-date information, essential for the designing of modified atmosphere packaging (MAP) of fresh produce.

The authors Cug, Gontard and Guilbert in chapter 5, have updated the information on the three types of edible film formulations or coatings containing polysaccharides, proteins and lipid compounds as active layers in the packaging of fresh as well as processed foods. The role of edible, superficial layers or coatings in providing an essential means to control physiological, microbiological and physico-chemical changes in food products has been brought out well.

The food industry's needs for less energy intensive forms of food preservation than drying, freezing or thermal processing, and consumer needs for convenience foods with extended shelf-life, yet retaining their 'fresh' characteristics, resulted in MAP technology and interactive packaging involving "Sachet" technology. Smith, Hoshino and Abe have detailed in chapter 6, the significant role that interactive packaging plays in the preservation of food, which includes antimicrobials and antioxidant films, ethylene absorbing sachets and temperature control indicators, oxygen control and atmosphere modification systems. The authors have presented the recent developments in (i) oxygen absorbents and their application in shelf-

life extension of foods and (ii) ethanol vapour generators, as antimicrobial agents in providing viable alternative or supplement to gas packaging in the preservation of packaged foods. The use of gas absorbents and ethanol vapour generators is one of the most exciting interactive packaging technologies available to the food industry.

The role of enzymes accelerating chemical reactions in biological systems has been well recognised, but their incorporation into active packaging system is a relatively new concept. Brody and Bundy have presented in chapter 7, the available information on incorporation of enzyme in active packaging systems. Relatively, a small number of enzyme incorporations into package structures have actually been achieved on a practical basis, which include - oxygen removal by means of glucose oxidase plus catalase, lactose by lactase and cholesterol from whole milk by the enzyme cholesterol reductase. The trials on incorporation of enzymes into active packaging are few because of their inherent vulnerability to variations in temperature, RH etc., which necessitates special processes or techniques of incorporation into packaging materials.

Chapter 8, on the history of oxygen scavenger bottle closures by Teumac opens up a new frontier in the application of oxygen scavenging crown closures, successfully employed in beer industry, for other beverage products, especially for those beverages based on natural products such as fruit juices, thereby avoiding the use of additives.

In chapter 9, Sacharow has discussed in detail a number of commercial applications of active packaging in food industry in North America. The time-temperature indicators for monitoring the useful life of packaged perishable food products, based on product intergrity, quality and authenticity are highlighted in chapter 10 by Selman. Out of the over 100 patents extant for such indicators based on a number of physico-chemical principles, only a few have found commercial use. This shows that there is a need for new indicators, which are much more precisely designed to meet the needs of the food industry. However, the history of the developments, described in this chapter, will certainly lead to exciting developments in the coming years.

The last and the 11th chapter on "Safety considerations in active packaging" by Hotchkiss decidedly the most important one from the point of view of commercial acceptance of active packaging technologies, gives an in- depth analysis on the status of the problem. Active packaging systems, which improve quality or reduce environmental impact, will also be required to maintain high level of safety and should conform to governmental regulatory standards, is stressed.

This book written excellently by several experts goes beyond fulfilling merely the objectives of introducing and consolidating information on active food packaging. It will certainly be a useful book to all concerned with processing, packaging and marketing of food products. Based on my over a decade's R & D experience in the MAP of fresh and processed horticultural produce, I can confidently state that the information provided in this book will certainly augur well for further developments in active food packaging in the years to come.

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FOOD POLYSACHARIDES AND THEIR APPLICATIONS: Edited by A. M. Stephen; Published by Marcel Dekker Inc., 270, Madison Avenue, New York, 10016, 1995; pp. 654; price; not mentioned.

This book is an excellent compendium of literature, past and present, on various aspects of Food Polysaccharides. It is very nicely edited by Prof. A. M. Stephen of University of Cape Town, South Africa and it is presented in 19 individual chapters, written by several authoritative researchers in the area of carbohydrates. Some of the chapters are complimentary to one another in that they provide information on various facets of a set of food polysaccharides and their applications.

Each chapter is classified according to the molecular structure of the respective food polysaccharides, both starch and non-starch, covering their source, physico-chemical and rheological characteristics, some structural details and their functions in processed foods. Also, included are chapters on various types of polymer-polymer interactions with special emphasis on the ralationship between polysaccharide rheology and sensoty attributes, the nutritional importance of dietary fibre components, some analytical approaches to food polysacharide analysis and finally some thoughts on regulatory aspects.

Starch and cellusose are the most versatile and abundant polysaccharides known so far. In chapters 2-4, a detailed description of starch chemistry, modification and production and utilization of starch hydrolysates are all highlighted with considerable detail. The type of starch modification employed dictates the final characteristics of the processed foods. In fact, the modified starches can actually be tailor-made to match specific requirements. High fructose corn syrup is an important product derived from starch modification, which finds applications in a variety of foods including confectionery. Properties such as sweetness, hygroscopicity and viscosity that govern the usefulness of starch syrups in the food industry are discussed.

In chapter 5 are reviewed the chemistry, modification and properties of cellulose and cellulose derivatives. Being the principal cell wall component of plants, its use as such in food is limited because of its insolubility (in water) and non-biodegradability. However, the various chemically modified celluloses are valuable food additives. Surface activity, water-binding capacity and the tendency to thermal or solute-induced gelation of modified celluloses are of great value in the food industry. The greatest proportion of polysaccharides consumed in the earth's biosphere are cellulose and hemi-celluloses and they generally constitute the essential components of animal nutrition. Also, discussed in here is the biological and structural role of xyloglucans, which are also known as amyloids, because of their positive starch-iodine blue colour reaction. The polysaccharide of tamarind kernel is an amyloid of considerable technological utility.

The seed galactomannans are important hydrocolloids (chapter 6) of commerce. They dissolve in water giving solutions of very high viscosity, a property which is made use of in various food and non-food uses. Guar and locust bean gums are commercially useful plant polysaccharides. The latter polysaccharide is of significance, as it interacts effectively with other polysaccharides to form gels (later described in chapter 14)

The succeeding three chapters (7 to 9) are concerned with the study of seaweed polysaccharides, such as agar, carrageenans and alginates. The first two are polysaccharides of galactose and its derivative. They have widespread applications in the food industry, because of their ability to form thermoreversible gels. Cation binding of carageenans (kappa-and iota-) leads to the formation of elastic gels, the molecular basis of such transformations and their general mechanisms are discussed nicely in these chapters.

Chapter 10 offers an extremely wide coverage of the chemistry and functional characteristics of pectic polysaccharides, which are universally present in plant cell wall matrices. The gelling behaviour of pectins, dependent on the degree of esterification of galacturonic acid carboxyls, is the most vital property. The importance of pectins in the manufacture of confectionery items, jams and jellies can hardly be over estimated. Included in this section are distribution of pectic substances in plant cell walls, methods of isolation and analysis, molecular and conformational structure, viscosity and gelling properties and their uses in various food systems.

The chemistry of gums and mucilages is dealt with in chapter 12. Essentially, these are polysaccharides having excellent rheological characteristics, combining a relatively low viscosity with stabilizing and emulsifying properties. Their complex, molecular structures are discussed in full. Acacia gums are highly branched arabino-galactans with L-Rhap and uronic acid substituents, whereas gum tragacanth is a pectin-like polysaccharide.

Chitin and chitosan (de-N-acetylated derivative of chitin) are abundantly available basic polysaccharides having very few applications in food processing sector (chapter 13). They are linear polysaccharides of β(1->4)-linked D-G1cn and have innumerable properties such as texturising, emulsifying, foaming and gelling, in addition to several beneficial effects, such as antimicrobial, hypocholesterolemic and hypolipedemic, that attract their possible usage in food processing technologies. Especially chitosan has the unique property of complexing with heavy metals, which may be of use in the waste water management.

In comparison with plant polysaccharides, microbial polysaccharides have the special advantage of uninterrupted production and also of reproducible chemical structure. Presently, xanthan gum, having FDA approval, is the only polysaccharide, enjoying full potential of its multitude of functions - applications. Gellan gum, though having FDA clearance, is not that widely used. The thixotropy shown by xanthan dispersions and the broad range of gels formed by gellan under various conditions are properties most valued by the food technologists. All these aspects are very lucidly covered by VJ Morris in chapter 11.

Macromolecular interactions are of great significance in improving the sensory properties, such as mouth-feel and texture, of the prepared foods. In chapter 14 is given an account of mixed polysaccharide systems, by availing the data generated by x-ray diffraction, ^{13}C-NMR and ESR spectroscopy and differential scanning calorimetric studies. These techniques are of growing importance in the mechanistic study of synergistic interaction of different polysaccharides. Notable amongst them are the locust bean gum and tara gum, the konjac glycomannan with the galactan derivatives agarose, carrageena, etc., and the more recently discovered interactions between alginates of high guluronic acid content and highly esterified pectins. Whereas in chapter 15 are reviewed details about emulsion stabilization by polysaccharide and protein-polysaccharide complexes. Emulsion stability, in particular, is affected by such interactions.

The beneficial effects of dietary fibre in the diet in alleviating the onset of modern civilization diseases affecting the GI tract, such as coloretal cancer and inflammatory bowel disorders, diabetes, atherosclerosis, etc. are well presented in chapter 17. The chemical structure of these unavailable - non-starch carbohydrates, their nutritional status and physiological functions, and their method of analyses are all very comprehensively reviewed by the author.

In chapter 18 is summarized a brief description of the various analytical approaches for the study of food carbohydrates. Included are GLC, IR and NMR technical details as well as the recently developed ELISA technique.

Finally in the last chapter (19), some information on the regulatory aspects for the use of polysaccharides in the food industry is presented. There is an increasing demand for incorporating various polysaccharide additives in food formulations for specific end uses, such as their use as low-calorie fat substitutes, health or functional foods, etc. A list of abbreviations and symbols used in the text is provided at the end.

In summary, all the chapters are well written and are accessible even to a layman. The whole coverage is as up-to-date as can be expected of any field that is subject to rapid progress. The subjects covered in this book will appeal to a broad spectrum of researchers from the food technological, medical, biological and biochemical communities. All the chapters are adequately illustrated and provided with the latest cross references, which I consider very useful. The get up of the book is really attractive and only a few typographical errors are seen here and there. One specific comment is that the types of prints used are too small to allow one interested for a continuous reading.

Thus, the book"Food Polysaccharides and Their Applications" provides an invaluable account of the field and will certainly be a welcome addition to the bookshelf of any chemist, biochemist, food scientist and technologist.

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CHLOROFORM : IPCS ENVIRONMENTAL HEALTH CRITERIA 163, Published under Joint Sponsorship of United Nations Environmental Programme, the Inernational Labour Organisation and World Health Organisation (WHO), as a part of the IPCS (International Programme on Chemical Safety), WHO- Geneva, 1994. WHO-Regional Office for South-East Asia World Health House, Indraprastha Estate, M. G. Road, New Delhi-110 002, pp. 174, Price SW Fr 18.90/ in developing countries.

This report contains the collective views of an International group of experts, mainly concerned with the chemistry, human and environmentsl exposure, environmental transport, distribution and transformation and toxicology of chloroform. This monograph contains 12 chapters with the following; Section 1. summary 2. chemistry and analytical methods; 3. sources of human and environmental exposure; 4. environmental transport, distribution and transformation, 5. environmental levels and human exposure, 6. kinetics in laboratory animals and humans; 7 effects on laboratory mammals and *in vitro* test system; 8. effects on humans, 9. effects on other organisms in the laboratory and field 10. evaluation of human health risks and effects on the environment, 11. further research and 12. previous evaluation by International bodies. The monograph also contains a brief resume and exhasative citation of references.

Chloroform is a clear, colourless, volatile liquid with a characteristic odour and sweet taste. The folowing significant points on chloroform are covered in the monograph. Chapter 1 summarizes the overall contents of the monograph. In chapter 2, detailed physical and chemical properties and analytical methods are

discussed. The sampling of air, water and biological samples for chloroform estimation is well covered, highlighting the different instrumental techniques (GC, GC-MS). The methodology is based on direct column injection, adsorption on activated adsorbent or condensation in a cool trap, then desorption or evaporation by solvent extraction or heating and subsequent GC or GC-MS analysis. Chapter 3 highlights that chloroform exposure is mainly through anthropogenic sources, as it is widely used in industries. In chapter 4, environmental transport and transformation are discussed. It is highlighted that chloroform present in water is transferred to air due to its volatility. Chloroform is found to have a residence time in the atmosphere of several months and is removed from there through chemical trensformation. Chapter 5, covers environmental air, water and food levels of chloroform and human exposure. The total estimated mean intake of chloroform is 2 μg/kg body weight per day. However, it is estimated that the total intake in dwellers is 10 μg/kg body weight per day. In chapter 6, the absorption kinetics of chloroform in animals and humans dependent upon the vehicle of delivery and species-specific metabolic capacities is highlighted. It is indicated that after inhalation exposure in humans, 60-80% of the inhaled quantity is absorbed. It is also indicated that hydration of the skin appears to accelerate absorption of chloroform. In chapter 7, the effects of the chemical on laboratory mammals and *in vitro* test system are discused. In this, there is a detailed discussion on single and cumulative effects; short-term and long-term exposures of chloroform. There is also a detailed information, pertaining to reproductive toxicity, embryotoxicity, teratogenicity, mutagenicity and carcinogenicity of chloroform. The liver is the major target organ for chloroform toxicity. It is also shown that choloroform undergoes oxidative biotransformation to trichloromethanol through cytochrome P-450 mediated pathway. Further, there is little evidence to show that chloroform caused gene mutation or DNA damage. Hence, the toxicity of chloroform is attributed mainly through cytotoxicity mediated cell proliferation. In chapter 8, the effects on humans are discussed with reference to anaesthesia-induced death, due to respiratory and cardiac arrhythmias. Renal tubular necrosis and renal dysfunction have also been observed in humans. The lowest levels at which liver toxicity due to occupational exposure to chloroform has been reported are in the range of 80-160 mg/m^3. In chapter 9, the effects of chloroform on aquatic fauna are discussed. The chemical is toxic to the embrolarval stages of some amphibian and fish species. The lowest IC_{50} value (0.3 mg/L) is reported for larvae of Hylacrucifer. Fishes are not so sensitive to chloroform toxicity. In chapter 10, a detailed discusion is made on evaluation of human health risks and effects on the environment. Based on the data available in chapter 11, further research is recommended with reference to (i) compensatory cell regeneration, (ii) species-specific carcinogenicity, (iii) an inhalation carcinogenicity bioassay (iv) aquatic toxicology and (c) *in vitro* cytotoxicity.

Based on the above studies, drinking-water guideline value of 200 μg/L for an excess life time cancer risk of 10^{-5} has been recommended for chloroform by the World Health Organisation (WHO 1993). The references given at the end are comprehensive. I strongly recommend this book for scientists, public health officials and toxicologists for their reference in designing future plan of work on chloroform.

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MICROBES FOR BETTER LIVING: Proceedings of MICON INTERNATIONAL '94 and 35th Annual Conference of Association of Microbiologists of India held at Mysore from 9-12 November 1994. Edited by Dr. (Mrs). R. Sankaran and Dr. K.S. Manja, Defence Food Research Laboratory, Mysore-570 011, India. pp 653, Price Rs. 400/- or US $40/-

This book covers different branches of Microbiology viz., Agricultre microbiology, Basic microbiology, Environmental microbiology, Food microbiology, Industrial microbiology and Medical microbiology.

The section on agriculture microbiology covers biofertilizers, biocontrol, serodiagnosis of plant pathogens and microbial degradation of pesticides. The basic microbiology section includes microbial genetics, microbial biodiversity, molecular biology and ecology. Environmental microbiology section covers a variety of topics like biomethanation, biosorbents, sewage disposal, biodegradation and geomicrobiology. The section on food microbiology includes microbial spoilage of different kinds of food, preservation of food, food fermentation and microbes as food. Industrial microbiology section covers a variety of topics in microbial biotechnology such as microbial enzymes, single cell protein production of organic acids, polysaccharides, surfactants

and other microbial metabolites. Medical microbiology section is the shortest section with only four papers and covers *Brucellosis* and rapid pathogen typing techniques.

Most of the reviews in the book are well illustrated with tables and protocols, which would be extremely useful to researchers, industrialists, University teachers and post-graduate students interested in microbiology. Reviews on genetic engineering and recent molecular biology techniques such as Tn-5 mutagenesis, RAPD analysis PCR technique, DNA finger printing and their application spread under different sections in the book are very informative.

The getup of the book is very good. The author and subject indices make the book complete. This book should not only find its place on library shelves, but also of individuals.

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METHYL BROMIDE: IPCS ENVIRONMENTAL HEALTH CRITERIA 166: The International Programme on Chemical Safety WHO, Geneva, 1995, pp 324, Sw Fr 47/-

UNEP/ILO/WHO, publish information on the effect of various chemicals on environment, human health and risk evaluation. The data on "Methyl bromide" in this book show that it has more drawbacks than advantages in general. Although occurring widely in nature predominantly in the oceans, in 1984 the world consumption of methyl bromide was 100,000 kg. The compound is easily produced by the action of methanol on HBr and it is usually available as a liquefied gas in stainless steel cylinders for commercial use. Methyl bromide is mainly used as a soil fumigant and also for fumigation of stored foods like cereals, spices, dried fruits, nuts, fruits, vegetables, dairy products etc. Levels of methyl bromide and inorganic bromide in such foods are important in assessing human risk. Bromide levels are found weeks later in crops grown in soils fumigated with methyl bromide. When methyl bromide is used for seed treatment, the seeds show reduced germination activity. Barley seeds are worst affected and barley plants show albinism and stunted growth. Methyl bromide is not as toxic to insects as other fumigants. The use of methyl bromide in glasshouse crops has been questioned.

The chapter on analytical methods of determination of "Methyl bromide" and inorganic bromide is dealt with thoroughly. Methods for residue analyses in air, water, soil, cereal and other foods and in body fluids like blood, serum, plasma and urine are adequately described. Humans run the risk of exposure to methyl bromide both during its manufacture and during application as a fumigant in the solid and for stored foods. Many fatalities have been reported at these risk points, when adequate presentation has not been taken through negligence or ignorance. The gas is mixed with warming lachrimator compounds like chloropicrin.

Methyl bromide and bromide residues in animal feeds lead to either toxic effects in animals and birds or presence of these compounds is seen in fatty foods like milk, cheese, butter, eggs etc.

The books deals very comprehensively on all aspects of methyl bromide; its metabolism, its desiccation in air, water, foods; its effects on insects, nematodes, slugs, fish; its effect on experimental animals - multigeneration studies, its effect on humans, its phyto toxicity, problems with seed and soil treatment and a host of other studies found in one volume. Details of clinical, neurological and non-neurological effects on human and animals exposed to methyl bromide have been dealt with in detail. As with all IPCS programs, this book also deals with epidemiological and laboratory protocols for risk assessment studies.

Those interested in knowing anything and everything on "Methyl bromide" will find this book very informative and suggestions are given for further reading along with literally hundreds of cross-references. According to me, if this book is studied, no further reading in any other suggested book would be necessary - so very comprehensive is the information.

The other interesting and special feature of this book is the information in capsule form given in 52 tables interspersed between a mass of written information. Going through the tables, one would get the idea of all information the book contains.

Having gone through this volume, it makes me wonder the phenomenal mass of information and data available on the hundred and more other chemicals listed on the front and back inside covers of the book.

The book on "Methyl bromide" is a must for all interested in agrochemical - post-harvest quarantine treatment, soil fumigation, seed treatment and all the paraphernalia of information.

The study on a single chemical and the way the systematic information is generated in this book can serve as a guideline for development of newer pesticides.

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SAFETY OF IRRADIATED FOODS: by J.F. Diehl, Second Edition, Revised and Expanded, Published by Marcel Dekker, Inc., New York, 10016, 1995, pp. 454, Price US $175/-

Consequent to the approval for radiation processing of onions, potatoes and spices for internal marketing and consumption by the Ministry of Health and Family Welfare, Government of India in August 1994, food irradiation has been hotly debated in the print media and TV in this country. Some consumer groups have opposed food irradiation on the mistaken notion that irradiated foods are not safe for human consumption. Consumer groups in other countries had also raised similar opposition to sale of irradiated foods, but forty countries have now regulations, approving this process for a variety of food items and many of them are increasingly applying the technology commercially.

The book entitled 'Safety of Irradiated Foods' Second Edition, Revised and Expanded by J.F. Diehl is undoubtedly the most up-to-date and comprehensive and authoritative coverage of the extensive scientific studies carried out internationally during the last three to four decades on the safety, nutritional adequacy and wholesomeness of irradiated foods.

This book containing more than 1050 references explains the basics of radiation technology, chemistry and biology as well as complex issues in physics, microbiology and toxicology in easy-to-understand terms. The first chapter provides a historical perspective of research and development in this new technology in the United Kingdom, U.S.A., France, Canada and Germany and also by the International Project in the Field of Food Irradiation (IFIP) created in 1970 with the active participation of 24 countries under the aegis of FAO, IAEA, OECD and WHO, with the specific aim of sponsoring a worldwide research programme on the wholesomeness and safety of irradiated foods for human consumption. Chapter 2 deals with the types of ionizing radiations and sources used in food irradiation namely, gamma rays from radionuclides, Cobalt-60 and Cesium-137, X-ray and electron beams generated by machines. Interaction of radiation with matter, dosimeters used for the measurement and monitoring of radiation doses absorbed and its distribution in the food and aspect of process control are discussed. Some basic considerations of radiation chemistry and the effect on main food components like water, carbohydrates, proteins, lipids and on food packaging materials are dealt with in chapter 3.

The biological effects of radiation on living plant tissues, bacteria, yeasts, moulds, viruses, protozoa, parasitic helminths and insects causing spoilage as well as foodborne diseases of public health importance are enumerated in chapter 4. A new chapter on "Identification of irradiated foods" has been added in this revised edition to take stock of the progress made in the detection of irradiated foods based on electron spin resonance, thermoluminescence, and changes in chemical properties of food components including DNA.

A major concern of consumers is whether consumption of irradiated food can pose health risk to humans. This aspect has been dealt with very comprehensively in chapter 6. This chapter alone lists 200 references and analyses the results of studies carried out worldwide on the toxicological safety of irradiated foods. The author critically examines the frequently cited report of the National Institute of Nutrition, Hyderabad on the increased incidence of polyploidy in malnourished children and laboratory animals, who had consumed freshly irradiated wheat and the failure to replicate these results by several other laboratories in the world, including IFIP and Bhabha Atomic Research Centre. The overwhelming scientific evidence that food irradiation does not pose any health risk to humans is unequivocally brought out in this chapter.

In chapter 7, questions related to the microbiological safety of irradiated foods are considered in detail to ensure that irradiation does not create microbiological problems basically different from those caused by other methods of processing. Chapter 8 cites 139 references on nutritional value of irradiated foods, which demonstrates that micronutrients are not significantly altered in terms of nutrient value and digestibility, whereas the partial losses of some of the vitamins are not basically different from losses in foods treated by other processes.

The wholesomeness evaluation of irradiated foods, by the FAO/IAEA/WHO Export Committee and other National Advisory Groups is enumerated in chapter 9. The potential and current applications of food irradiation is covered in chapter 10. Government regulations and clearances of irradiated foods in different countries are discussed in chapter 11, while chapter 12 considers acceptance of food irradiation by consumers and consumer organizations. The outlook for commercial food irradiation is briefly dealt in chapter 13. The Codex General Standards for Irradiated Foods and the Recommended International Code of Practice for the Operation of Irradiation Facilities used for the treatment of foods are given in Appendices I and II.

The book is thoroughly readable and informative and given a most valuable perspective gained by the author from nearly a quarter century of intensive involvement in food irradiation. This book is a valuable reference for both scientists and non-scientists, who wish to have a balanced view of this innovative technology. It is essential reading for food scientists, technologists, microbiologists, chemists, dieticians, nutritionists and also to decision makers in the food industry and government officials involved in food and drug administration and public health.

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FRUITS AND VEGETABLES PRESERVATION PRINCIPLES AND PRACTICES: by R.P. Srinivasa and Sanjeev Kumar, International Book Distributing Co., (Publishing Division) Chapman Studio Building, 2nd Floor, Charbagh, Lucknow-226 004. U.P. (India). First Edition 1994. pp 326. Price Rs. 150.00/-

Fruits and vegetables are an important requirement for human beings, as these foods not only meet the quantitive needs to some extent, but also supply vitamins and minerals, which improve the quality of the diet and maintain health. It is, therefore, necessary to make them available for comsumption throughout the year in either fresh or processed/preserved form.

India though the third largest producer of fruits after Brazil and U.S.A. and the second largest producer of vegetables after China, proceses less than 0.5 % of the total production of fruits and vegetables. Most of the procesed products are consumed domestically. In order to improve the nutritional status of the people and also to exploit the export potential of processed products, there is need to increase the production of processed foods in the country.

The present book has been written to improve information on the products prepared from various fruits and vegetables, commercially as well as on home scale.

The authors have taken considerable efforts and interest to write this book. This volume has been divided into 25 chapters. The first two chapters deal with the history and scope for preservation in the developing countries like India.

The 3rd to 23rd chapters present relevant information on enzymes, colours, additives, flavours, plastics, brewing, toxins, adulterants etc., have also been given very clearly and suitably to understand for the better benefit of the processing industries. In each chapter, the authors have presented additional theoretical imformation to understand the basic principles and methodology.

In chapter 24, utilization of fruit and vegetable wastes, which poses the problem of their disposal has been solved to a certain extent by manufacturing by-products, resulting in reducing the cost of production.

Chapter 25 details important methods of analysis of fruits/vegetables and their products. The methods have been given clearly to determine the nutrients present in them, so that the losses of nutrients during processing can be avoided to retain the quality to the maximum extent.

At the end of the text, 16 appendices have been given for the benefit of the readers.

The present volume, no doubt, is more useful to graduates and post-graduates in horticulture, food technology and foods and nutrition disciplines. For the home scale preservation, the iinformation should be in the form of statement to help the housewives. Hence, this can serve as a basic source book for the industry rather than households.

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EVALUATION OF CERTAIN VETERNARY DRUG RESIDUES IN FOOD: WHO Technical Report Series 851, 1995, pp 42, Price Sw fr 10/-

This publication is precise with 4 sections : Introduction, General considerations, Comments on residues of specific veterinary drugs and recommendations; two annexures, acknowledgement and references.

This publication is the outcome of 42nd Joint FAO/WHO Expert Committee meeting on "Food Additives" held in Rome, 1-10 February, 1994. The experts were drawn from related disciplines like veterinary pharmacology and toxicology, food safety, meat environmental chemicals etc. The recommendations are thus a pooled wisdom of scientists and experts.

Problems of drug residues in foods are subtle and not immediately manifested. The manifestations are of varied nature. These vary from a mild to serious ones and generally falling in two categories - allergies and drug resistance in the pathogenic bacteria.

Management of drug residues requires understanding the drug metabolism in two diferent host systems, quantitative estimation of residues *vis-a -vis* quantity of food comsumed i.e., daily intake and the nature of drug residues (i.e., after metabolism in systems of food animals) and the alterations during processing (e.g., heat) of food (e.g., meat), containing residues. Hardly any controlled and such horizontally spread study with chemical is possible. Therefore, deciding the Acceptable Daily Intake of drug residue is not easy. However, it is important to be careful about the recommendation, as it is used in cases of disputes arising from national and internationsl trade. Nevertheless, consumer's safety against drug residues is very important. Ther recommended MRL (annexure-2) with respect to Levamisole, Spectinomycin, Sulfadimidine, Dexamethasone and Diminaxzene arrived at by the Experts would be useful.

More informations are reqired for the rest of the compounds viz., Chloramphenicol, Flumequine, Olaquindox and Ronidazole.

More precise studies on Leramisole metabolism in cattle and other farm animals provided data to enable the committee to revise ADI and MRL recommended at its earlier meeting. The recommendations for Spectinomycin have been made on the basis of information available on rats, dogs, pigs, cattle and poultry. Similar informations about this drug in sheep and goat are also required to be considered. Further, information about plasma-protein binding values of Spectinomycin, its excretion in urine and effect on bone-marrow would be useful.

With respect to Sulphadimidine, the committee considered less susceptibility of primates including human to its anti-thyroid effects compared to those of rats and pigs. It was concluded that (a) MRLs recommended at the 34th meeting should remain and (b) Sulphadimidine should not be used in laying hens as high concentration in eggs was expected.

The glucocorticosteroid, Dexamethasone exhibits its effects on several biochemical pathways and transport systems. It is rapidly absorbed and excreted in urine and faeces. It is quickly eliminated from muscle and cow's milk. Its depletion from liver is slow. Besides these, the committee considered threadbare the teratogenecity, maternal toxicity and many other related data on NOEL before deciding ADI and MRLs in muscle, kidney, liver and milk.

In order to recommend ADI and MRL for Diminazene, a 9 month study on dogs to determine NOEL, other studies in several species of animals and residue anlysis in bovine milk, muscle, liver and kidney were considered. The committee aptly used the quantification limit of the analytical method.

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